METHODS AND KITS FOR DETECTING OR DETERMINING AN AMOUNT OF AN ANTI-B-CORONAVIRUS ANTIBODY IN A SAMPLE

RELATED APPLICATION INFORMATION

[0001] This application claims the benefit of U.S. Provisional Application No. 63/009,079 filed April 13, 2020, U.S. Provisional Application No. 63/009,981, filed April 14, 2020, U.S. Provisional Application No. 63/015,408, filed April 24, 2020, U.S. Provisional Application No. 63/019,088 filed on May 1, 2020, U.S. Provisional Application No. 63/021,313 filed on May 7, 2020, U.S. Provisional Application No. 63/079,906, filed on September 17, 2020, U.S. Provisional Application No. 63/093,735 filed on October 19, 2020, U.S. Provisional Application No. 63/123,673 filed on December 10, 2020, U.S. Provisional Application No. 63/132,143 filed on December 30, 2020, U.S. Provisional Application No. 63/132,138 filed on December 30, 2020, U.S. Provisional Application No. 63/136,007 filed on January 11, 2021, U.S. Provisional Application No. 63/143,591 filed on January 29, 2021, U.S. Provisional Application No. 63/146,854 filed on February 8, 2021, U.S. Provisional Application No. 63/149,694 filed on February 16, 2021, U.S. Provisional Application No. 63/153,239 filed on February 24, 2021, U.S. Provisional Application No. 63/154,505 filed on February 26, 2021, U.S. Provisional Application No. 63/157,393 filed on March 5, 2021, U.S. Provisional Application No. 63/160,615 filed on March 12, 2021, U.S. Provisional Application No. 63/163,908 filed on March 21, 2021, U.S. Provisional Application No. 63/165,567 filed on March 24, 2021, U.S. Provisional Application No. 63/170,259, filed on April 2, 2021, the content of each of the aforementioned applications is herein incorporated by reference in their entirety.

TECHNICAL FIELD

[0001] The present disclosure relates methods, kits, systems, algorithms and improvements for detecting the presence of or determining an amount, quantity, concentration and/or level of an antibody against at least one type of β-coronavirus, such as, for example, an antibody against SARS-CoV or SARS-CoV-2, in one or more samples obtained from a subject. In some aspects, the methods, kits and systems relate to detecting the presence of or determining an amount, quantity, concentration and/or level of at least one type of anti-β-coronavirus antibody, such as an IgG and/or IgM antibody, in one or more samples obtained from a subject. The methods, kits systems, algorithms and improvements can also be used to monitor a subject's response and/or

treatment to a β-coronavirus, determine whether or not a subject will develop or experience a cytokine storm, predict outcome in a subject, determine whether a subject can be administered a vaccine for a β-coronavirus, monitoring antibody response in individuals that have received a β-coronavirus vaccine (such as a SARS-CoV-2 vaccine), and/or determine the immune status of a subject.

BACKGROUND

[0002] Viruses of the family *Coronaviridae* possess a single-strand, positive-sense RNA genome ranging from 26 to 32 kilobases in length (reviewed by Lu et al., *The Lancet*, 395:565-574 (February 22, 2020)). The *Coronaviridae* are further subdivided (initially based on serology but now based on phylogenetic clustering) into four groups, the alpha, beta, gamma and delta coronaviruses. Coronaviruses have been identified in several avian hosts, as well as in various mammals, including camels, bats, masked palm civets, mice, dogs, and cats.

[0003] Among the several coronaviruses that are pathogenic to humans, most are associated with mild clinical symptoms, with three exceptions. Severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) is a novel betacoronavirus that emerged in Guangdong, southern China, in November 2002 and resulted in more than 8000 human infections and 774 deaths in 37 countries in 2002-03. Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) was first detected in Saudi Arabia in 2012 and was responsible for 2494 laboratory-confirmed cases of infection and 858 deaths from 2012-20. In December, 2019, a cluster of pneumonia cases caused by a newly identified β-coronavirus were found to be epidemiologically-associated with the Huanan seafood market in Wuhan, China, where a number of non-aquatic animals, such as birds and rabbits were on sale before the outbreak. This coronavirus was named January 2020 by the World Health Organization (WHO) as the 2019-novel coronavirus (2019-nCov or COVID-19), and February 2020 by the International Committee as SARS-CoV-2. SARS-CoV-2 was declared a pandemic due to its rapid, uncontrolled and vast worldwide spread.

[0004] Coronavirus virions are spherical with diameters of approximately 125 nanometers, as demonstrated in studies by cryo-electron tomography and cryo-electron microscopy. A prominent feature of coronaviruses is the club-shape spike projections emanating from the surface of the virion, giving the virion the appearance of a solar corona and resulting in the name, coronaviruses. Within the envelope of the coronavirus virion is the helically-symmetrical nucleocapsid, which binds to and creates a shell around the coronavirus RNA genome. The

spike (S) and nucleocapsid (N) proteins are the main immunogens of the coronavirus. The other two main structural proteins of the coronavirus particles are the membrane (M) and envelope (E) proteins. All four proteins are encoded within the 3′ end of the viral genome.

[0005] The S protein (~150 kDa) is heavily N-linked glycosylated and utilizes an N-terminal signal sequence to gain access to the endoplasmic reticulum (ER). Homotrimers of the virus-encoding S protein make up the distinctive spike structure on the surface of the virus. In many, but not all, coronaviruses, the S protein is cleaved by a host cell furin-like protease into two separate polypeptides known as S1 and S2. S1 makes up the large receptor-binding domain of the S protein while S2 forms the stalk of the spike molecule. The trimeric S glycoprotein mediates attachment of the coronavirus virion to the host cell by interactions between the S protein and its receptor. In humans, angiotensin-converting enzyme 2 (ACE2) is the receptor for SARS-CoV. The sites of receptor binding domains (RBD) within the S1 region of a coronavirus S protein vary depending on the virus, with some having the RBD at the N-terminus of S1 (e.g., murine hepatitis virus) while others (e.g., SARS-CoV) have the RBD at the C-terminus of S1. The S-protein/receptor interaction is the primary determinant for the coronavirus to infect a host species and also governs the tissue tropism of the virus.

[0006] The M protein is the most abundant structural protein in the virion. It is a small (~25–30 kDa) protein with 3 transmembrane domains and is believed to give the virion its shape. It has a small N-terminal glycosylated ectodomain and a much larger C-terminal endodomain that extends 6–8 nm into the viral particle.

[0007] The E protein (~8–12 kDa) is found in small quantities within the virion. E proteins in coronaviruses are highly divergent but have a common architecture. Data suggests that the E protein is a transmembrane protein with an N-terminal ectodomain and a C-terminal endodomain that has ion channel activity. Recombinant viruses lacking the E protein are not always lethal - although this is virus-type dependent. The E protein facilitates assembly and release of the virus, but also has other functions (e.g., ion channel activity in SARS-CoV E protein is not required for viral replication but is required for pathogenesis).

[0008] The N protein is the only protein present in the nucleocapsid. It is composed of two separate domains, an N-terminal domain (NTD) and a C-terminal domain (CTD), both capable of binding RNA *in vitro* using different mechanisms, which may suggest that optimal RNA binding requires contributions from both domains. The N protein is heavily phosphorylated, and

phosphorylation has been suggested to trigger a structural change enhancing the affinity for viral versus non-viral RNA. The N protein binds the viral genome in a beads-on-a-string type conformation. Two specific RNA substrates have been identified for N protein; the transcriptional regulatory sequences and the genomic packaging signal. The genomic packaging signal has been found to bind specifically to the second, or C-terminal RNA binding domain. The N protein also binds nsp3, a key component of the replicase complex, and the M protein. These protein interactions likely help tether the viral genome to the replicase-transcriptase complex, and subsequently package the encapsidated genome into viral particles.

[0009] In February 2020, Lu et al. reported obtaining complete and partial SARS-CoV-2 genome sequences using next-generation sequencing of bronchoalveolar lavage fluid samples and cultured isolates from nine patients from Wuhan diagnosed with viral pneumonia but negative for common respiratory pathogens. Lu et al., *The Lancet*, 395: 565-574 (February 22, 2020). Based on their analysis, Lu et al. further reported that SARS-CoV-2 was closely related (with 88% identity) to two bat-derived severe acute respiratory syndrome (SARS)-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21, collected in eastern China in 2018, but was more distant from SARS-CoV (about 79%) and MERS-CoV (about 50%). Additionally, Zhou et al. confirmed that SARS-CoV-2 uses the same cellular entry receptor, ACE2, as SARS-CoV. Zhou et al., *Nature*, 579:270-273 (March 2020).

[0010] SARS-CoV-2 primarily spreads through the respiratory tract, by droplets, respiratory secretions, and direct contact. Additionally, SARS-CoV-2 has been found in fecal swabs and blood, indicating the possibility of multiple routs of transmission. Zhang et al., *Microbes* 9(1):386-9 (2020). SARS-CoV-2 is highly transmissible in humans, especially in the elderly and people with underlying diseases. Symptoms can appear 2 to 14 days after exposure. Patients present with symptoms such as fever, malaise, cough, and/or shortness of breath. Most adults or children with SARS-CoV-2 infection present with mild flu-like symptoms, however, critical patients rapidly develop acute respiratory distress syndrome, respiratory failure, multiple organ failure and even death.

[0011] Because of the health risks imposed by SARS-CoV-2 transmission, there is a need for methods and kits to assess coronavirus transmission in humans, in combination with methods to assess an anti-β-coronavirus antibody (such as an IgG and/or IgM antibody) in one or more samples obtained from a subject.

SUMMARY

[0012] In one aspect, the present disclosure relates to a method for detecting a presence or determining an amount, level or concentration of (e.g., quantitating or semi-quantitating) at least one type of anti-β-coronavirus antibody in a subject. The method can comprise the steps of:

[0013] a) contacting at least one biological sample from the subject, either simultaneously or sequentially, in any order, with

[0014] at least one type of first specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof, selected from a C-terminal domain of a nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain of a nucleocapsid protein and a a receptor binding domain of a spike protein, wherein the polypeptide specifically binds to at least one type of anti-β-coronavirus antibody in the sample, and

[0015] at least one type of second specific binding partner comprising a detectable label,

[0016] thereby producing one or more types of first complexes comprising the first specific binding partner-anti-β-coronavirus antibody-second specific binding partner; and

[0017] b) assessing a signal from the one or more types of first complexes, wherein the amount of detectable signal from the detectable label indicates the presence or amount of anti-β-coronavirus antibody in the sample.

[0018] In some aspects, the above methods are (a) semi-quantitative; (b) quantitative; or (c) qualitative. In some aspects, when the method described herein is semi-quantitative, e.g., the method is not standardized against an internationally recognized standard (such as a WHO international standard (e.g., such as in BAU/mL)). In other aspects, when the method described herein is quantitative, the method e.g., can be standardized against an internationally recognized standard, such as, for example, a WHO international standard (e.g., such as in BAU/mL). In yet further aspects, when the method described herein is qualitative, a single-to-calibrator ratio (S/CO) is obtained or determined. An understanding of whether a method is semi-quantitative, quantitative or qualitative, and adaption of a method to either semi-quantitative, quantitative or qualitative is well known and done using routine techniques known in the art.

[0019] In some aspects, the above method quantifies up to (a) about 91% or (b) about 99% of anti-SARS-CoV-2 IgG antibodies.

[0020] In some aspects of the above method, the at least one type of anti-β-coronavirus antibody is an anti-SARS-CoV antibody or an anti-SARS-CoV-2 antibody. In yet other aspects

of the above method, the at least one type of anti-β-coronavirus antibody is an anti-SARS-CoV-2 antibody.

[0021] In some aspects of the above method, when the type of anti-β-coronavirus antibody is an anti-SARS-CoV-2 antibody, the method further comprises detecting at least one anti-SARS-CoV-2 neutralizing IgG antibody. Specifically, in this aspect, the method demonstrates high qualitative agreement with a plaque reduction neutralization assay. Additionally, increasing amounts of anti-SARS-CoV-2 IgG antibodies as detected by the method are associated with increasing amounts of anti-SARS-CoV-2 IgG neutralizing antibodies. Moreover, in this aspect, the probability profile for the method corresponds to high titer levels in the plaque reduction neutralization assay such that there is a high probability of the levels of anti-SARS-CoV-2 IgG antibodies determined by the method being at or above the levels of anti-SARS-CoV-2 IgG neutralizing antibodies determined in the plaque reduction neutralization assay. Finally, the method further demonstrates high qualitative agreement with an ACE2 binding inhibition assay.

[0022] In still further aspects of the above method, a level of anti-SARS-CoV-2 IgG antibodies of at least about 4160 AU/mL (about 590 BAU/mL) used as a cutoff or threshold corresponds to about 0.95 probability of obtaining a plaque reduction neutralization assay ID50 at 1:250 dilution.

[0023] In still further aspects of the above method, when the at least one type of anti-β-coronavirus antibody is an anti-SARS-CoV-2 antibody, the method comprises obtaining the at least one biological sample from the subject at a critical time of from about fourteen (14) days to about thirty-five (35) days after onset of symptoms of SARS-CoV-2. Specifically, in these aspects, the method further comprises determining that the subject: (i) more likely than not will develop or experience at least one of a cytokine storm, acute respiratory distress syndrome (ARDS), or a combination of a cytokine storm and ARDS if anti-SARS-CoV-2 IgM antibody, anti-SARS-CoV-2 IgG antibody, or anti-SARS-CoV-2 IgM antibody and anti-SARS-CoV-2 IgG antibody are not detected in the biological sample within the critical time; or (ii) more likely than not will not develop or experience at least one of a cytokine storm, ARDS, or a combination of a cytokine storm and ARDS if anti-SARS-CoV-2 IgM antibody, anti-SARS-CoV-2 IgG antibody, or anti-SARS-CoV-2 IgM antibody and anti-SARS-CoV-2 IgG antibody are not detected in the biological sample within the critical time.

[0024] In some aspects of the above method, the at least one type of anti-β-coronavirus antibody is an anti-β-coronavirus IgG antibody, an anti-β-coronavirus IgM antibody, or an anti-β-coronavirus IgG antibody and an anti-β-coronavirus IgM antibody.

[0025] In other aspects of the above method, the biological sample is whole blood, serum, plasma, saliva, a nasal mucus specimen, an oropharyngeal specimen, or a nasopharyngeal specimen. In some aspects, the biological sample is whole blood. In other aspects, the biological sample is serum. In yet other aspects, the biological sample is plasma. In yet other aspects, the biological sample is saliva. In still other aspects, the biological sample is an oropharyngeal specimen. In still yet other aspects, the biological sample is a nasopharyngeal specimen. In still yet other aspects, the biological sample is a nasal mucus specimen. In still yet further aspects, the biological sample is an anal swab specimen.

[0026] In some additional aspects, the isolated polypeptide used in the above method is a fusion polypeptide.

[0027] In still further additional aspects, the isolated polypeptide used in the above method has a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349,

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[0028] In other aspects, the isolated polypeptide used in the above method is a C-terminal domain of a nucleocapsid protein, said polypeptide having a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404 or 405 amino acids.

[0029] In yet still further aspects, the isolated polypeptide used in the above method is a C-terminal domain of a nucleocapsid protein, said polypeptide having a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34,

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[0030] In yet still further aspects the isolated polypeptide used in the above method is a receptor binding domain (RBD) of a spike protein, said polypeptide having a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373,

374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962,

963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000, 1001, 1002, 1003, 1004, 1005, 1006, 1007, 1008, 1009, 1010, 1011, 1012, 1013, 1014, 1015, 1016, 1017, 1018, 1019, 1020, 1021, 1022, 1023, 1024, 1025, 1026, 1027, 1028, 1029, 1030, 1031, 1032, 1033, 1034, 1035, 1036, 1037, 1038, 1039, 1040, 1041, 1042, 1043, 1044, 1045, 1046, 1047, 1048, 1049, 1050, 1051, 1052, 1053, 1054, 1055, 1056, 1057, 1058, 1059, 1060, 1061, 1062, 1063, 1064, 1065, 1066, 1067, 1068, 1069, 1070, 1071, 1072, 1073, 1074, 1075, 1076, 1077, 1078, 1079, 1080, 1081, 1082, 1083, 1084, 1085, 1086, 1087, 1088, 1089, 1090, 1091, 1092, 1093, 1094, 1095, 1096, 1097, 1098, 1099, 1100, 1101, 1102, 1103, 1104, 1105, 1106, 1107, 1108, 1109, 1110, 1111, 1112, 1113, 1114, 1115, 1116, 1117, 1118, 1119, 1120, 1121, 1122, 1123, 1124, 1125, 1126, 1127, 1128, 1129, 1130, 1131, 1132, 1133, 1134, 1135, 1136, 1137, 1138, 1139, 1140, 1141, 1142, 1143, 1144, 1145, 1146, 1147, 1148, 1149, 1150, 1151, 1152, 1153, 1154, 1155, 1156, 1157, 1158, 1159, 1160, 1161, 1162, 1163, 1164, 1165, 1166, 1167, 1168, 1169, 1170, 1171, 1172, 1173, 1174, 1175, 1176, 1177, 1178, 1179, 1180, 1181, 1182, 1183, 1184, 1185, 1186, 1187, 1188, 1189, 1190, 1191, 1192, 1193, 1194, 1195, 1196, 1197, 1198, 1199, 1200, 1201, 1202, 1203, 1204, 1205, 1206, 1207, 1208, 1209, 1210, 1211, 1212, 1213, 1214, 1215, 1216, 1217, 1218, 1219, 1220, 1221, 1222, 1223, 1224, 1225, 1226, 1227, 1228, 1229, 1230, 1231, 1232, 1233, 1234, 1235, 1236, 1237, 1238, 1239, 1240, 1241, 1242, 1243, 1244, 1245, 1246, 1247, 1248, 1249, 1250, 1251, 1252, 1253, 1254, 1255, 1256, 1257, 1258, 1259, 1260, 1261, 1262, 1263, 1264, 1265, 1266, 1267, 1268, 1269, 1270, 1272, 1272, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1286, 1287, 1288, 1289, 1290, 1291, 1292, 1293, 1294, 1295, 1296, 1297, 1298, 1299, 1300, 1301, 1302, 1303, 1304, 1305, 1306, 1307, 1308, 1309, 1310, 1311, 1312, 1313, 1314, 1315, 1316, 1317, 1318, 1319, 1320, 1321, 1322, 1323, 1324, 1325, 1326, 1327, 1328, 1329, 1330, 1331, 1332, 1333, 1334, 1335, 1336, 1337, 1338, 1339, 1340, 1341, 1342, 1343, 1344, 1345, 1346, 1347, 1348, 1349, 1350, 1351, 1352, 1353, 1354, 1355, 1356, 1357, 1358, 1359, 1360, 1361, 1362, 1363, 1364, 1365, 1366, 1367, 1368, 1369, 1370, 1371, 1372, 1373, 1374, 1375, 1376, 1377, 1378, 1379, 1380, 1381, 1382, 1383, 1384, 1385, 1386, 1387, 1388, 1389, 1390, 1391, 1392, 1393, 1394, 1395, 1396, 1397, 1398, 1399, 1400, 1401, 1402, 1403, 1404, 1405, 1406, 1407, 1408, 1409, 1410, 1411, 1412, 1413, 1414, 1415, 1416, 1417, 1418, 1419, 1420, 1421, 1422, 1423, 1424, 1425, 1426, 1427, 1428, 1429, 1430, 1431, 1432, 1433, 1434, 1435,

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[0031] In some aspects, the at least one type of first specific binding partner comprises at least one isolated polypeptide from a C-terminal domain of a nucleocapsid protein from a β-coronavirus, wherein the method is carried out so that said specific binding partner specifically binds to (a) an anti-β-coronavirus IgG antibody; (b) an anti-β-coronavirus IgM antibody; or (b) both an anti-β-coronavirus IgG and an anti-β-coronavirus IgM antibody.

[0032] In other aspects, wherein the at least one type of first specific binding partner comprises at least one isolated polypeptide from a receptor binding domain (RBD) of a spike protein from a β-coronavirus, wherein the method is carried out so that said specific binding partner specifically binds to (a) an anti-β-coronavirus IgG antibody; (b) an anti-β-coronavirus IgM antibody; or (b) both an anti-β-coronavirus IgG antibody and anti-β-coronavirus IgM antibody.

[0033] In still yet further aspects, the above method is carried out so that the at least one type of first specific binding partner binds to (a) an anti-SARS-CoV-2 IgG antibody; (b) an anti-SARS CoV-2 IgM antibody; or (c) both an anti-β-coronavirus IgG antibody and anti-β-coronavirus IgM antibody.

[0034] In still yet other aspects, the at least one type of first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof from that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2. In some aspects, the variant of the nucleocapsid protein comprises: (i) an amino acid substitution replacing serine with phenylalanine at position 235 (S235F) within amino acids 210 to 419 of SEQ ID NO:2; or (ii) SEQ ID NO:24. In these aspects, the at least one type of first specific binding partner binds

to an (a) anti-β-coronavirus IgG antibody; (b) anti-β-coronavirus IgM antibody; or (c) a combination of (a) and (b). In other aspects, the at least one type of first specific binding partner is a receptor binding domain of a spike protein or variant thereof from SARS-CoV-2 (e.g., amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17). In some aspects, the variant of the RBD of a spike protein comprises one or more amino substitutions within amino acids 319 to 542 of SEQ ID NOS:15 or 25 replacing lysine with asparagine at position 417, replacing glutamic acid with lysine at position 484, and/or replacing asparagine with tyrosine at position 501.

[0035] In still further aspect of the above method, the at least one type of first specific binding partner comprises at least one isolated polypeptide from a C-terminal domain of a nucleocapsid protein from a β-coronavirus, wherein said specific binding partner specifically binds to (a) an anti-β-coronavirus IgG antibody; (b) an anti-β-coronavirus IgM antibody; or (b) both an anti-β-coronavirus IgG and anti-β-coronavirus IgM antibody. In some aspects, the at least one first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.) In some aspects, the variant of the nucleocapsid protein comprises: (i) an amino acid substitution replacing serine with phenylalanine at position 235 (S235F) within amino acids 210 to 419 of SEQ ID NO:2; or (ii) SEQ ID NO:24. In these aspects, the at least one first specific binding partner binds to an (a) anti-β-coronavirus IgG antibody; (b) anti-β-coronavirus IgM antibody; and (c) a combination of (a) and (b).

[0036] In still further aspects of the above method, the at least one type of first specific binding partner comprises at least one isolated polypeptide from a receptor binding domain of a spike protein from a β-coronavirus, wherein said specific binding partner specifically binds to (a) an anti-β-coronavirus IgG antibody; and (b) an anti-β-coronavirus IgM antibody; or (b) both an anti-β-coronavirus IgG and anti-β-coronavirus IgM antibody. In some aspects, the at least one type of first specific binding partner is a receptor binding domain of a spike protein or variant thereof from SARS-CoV-2 (e.g., amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID

NO:17). In some aspects, the variant of the RBD of a spike protein comprises one or more amino substitutions within amino acids 319 to 542 of SEQ ID NOS:15 or 25 replacing lysine with asparagine at position 417, replacing glutamic acid with lysine at position 484, and/or replacing asparagine with tyrosine at position 501.

[0037] In some aspects of the above method, the at least one type of second specific binding partner is an antibody. Specifically, in some aspects, at least one type of second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM (e.g., anti-human-IgM IgG) antibody, or an anti-species IgG (e.g., anti-human-IgG Ig) antibody, and an anti-species IgM (e.g., anti-human-IgM IgG) antibody.

[0038] In still yet further aspects, the method involves detecting at least one anti-SARS-CoV-2 IgG antibody and/or at least one anti-SARS-CoV-2 IgM antibody in at least one (e.g., single or multiple) biological sample(s) from a subject. For example, in some aspects, the method involves detecting:

[0039] (a) at least one anti-SARS-CoV-2 IgG antibody in at least one biological sample obtained from a subject wherein the at least one type of first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2 and specifically binds to at least one anti-SARS-CoV-2 IgG antibody, and/or

[0040] (b) at least one anti-SARS-CoV-2 IgM antibody in at least one biological sample obtained in a subject wherein the at least one type of first specific binding partner wherein the at least one type of first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17 and specifically binds to at least one anti-SARS-CoV-2 IgM antibody; and

[0041] wherein the at least one anti-SARS-CoV-2 IgG antibody and/or at least one anti-SARS-CoV-2 IgM antibody are detected in a single biological sample obtained from the subject or in multiple biological samples obtained from the subject; and

[0042] further wherein, when at least one anti-SARS-CoV-2 IgG antibody and at least one anti-SARS-CoV-2 IgM antibody are detected simultaneously or sequentially, in any order, in a single biological sample obtained from the subject or in multiple biological samples obtained from the subject.

[0043] In some aspects, the above method employs a small volume of biological sample. More specifically, in some aspects, the small volume of biological sample used in the method is from about 0.30 μL to about 0.40 μL. In some aspects, the small volume of biological sample used in the method is at least about 0.30 μL, at least about 0.31 μL, at least about 0.32 μL, at least about 0.33 μL, at least about 0.34 μL, at least about 0.35 μL, at least about 0.36 μL, at least about 0.37 μL, at least about 0.38 μL, at least about 0.39 μL, or at least about 0.40 μL.

[0044] In yet other aspects, the above method comprises diluting the biological sample prior to or during the method. Specifically, in some aspects, the diluting comprises mixing about 10 μL of the biological sample with about 290 μL of at least one buffer to form a biological sampling mixture and using about 10 μL of the biological sampling mixture in the method. More specifically, in some aspects, the above method, when determining the amount of at least one anti-SARS-CoV-2 IgG antibody in at least one biological sample, comprises diluting the biological sample prior to or during the method. Specifically, in some aspects, the diluting comprises mixing about 10 μL of the biological sample with about 290 μL of at least one buffer to form a biological sampling mixture and using about 10 μL of the biological sampling mixture in the method.

[0045] In yet still further aspects, the method further comprises detecting SARS-CoV-2 from at least one (e.g., a single or multiple) biological sample(s) obtained from the subject. In some aspects, the SARS-CoV-2 is detected by its viral RNA using polymerase chain reaction, or by its viral antigen. In other aspects, when multiple biological samples are obtained, these can be at the same or at different times. When the method comprises detecting (i) at least one anti-SARS-CoV-2 IgG antibody; (ii) at least one anti-SARS-CoV-2 IgM antibody; and (iii) viral RNA or viral antigen in a single or multiple biological samples obtained from a subject, the method further comprises detecting whether the subject is in:

[0046] (a) an initial period of infection without any antibodies being produced, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen, negative for anti-SARS-CoV-2 IgM antibodies, and negative for anti-SARS-CoV-2 IgG antibodies; and/or

[0047] (b) an early acute phase of infection, and is developing an immune response to the virus and producing antibodies, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen, positive for an anti-SARS-CoV-2 IgM antibodies, and negative for anti-SARS-CoV-2 IgG antibodies; and/or

[0048] (c) an early acute phase of infection or had a false negative viral RNA result or viral antigen result, or a false positive anti-SARS-CoV-2 IgM antibody result, when the at least one biological sample obtained from the subject is negative for viral RNA, positive for anti-SARS-CoV-2 IgM antibodies, and negative for anti-SARS-CoV-2 IgG antibodies; and/or

[0049] (d) an acute phase of infection, and is progressing in an immune response, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen, positive for anti-β-coronavirus IgM antibodies, and positive for anti-SARS-CoV-2 IgG antibodies; and/or

[0050] (e) a late acute phase of infection, or has developed a recurrent infection with SARS-CoV-2, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen, negative for anti-SARS-CoV-2 IgM antibodies, and positive for anti-SARS-CoV-2 IgG antibodies; and/or

[0051] (f) a late acute phase of infection or recovery phase, or had a false negative viral RNA result, when the at least one biological sample obtained from the subject is negative for viral RNA or viral antigen, positive for anti-β-coronavirus IgM antibodies, and positive for anti-SARS-CoV-2 IgG antibodies; and/or

[0052] (g) recovery phase when the at least one biological sample obtained from the subject is negative for viral RNA or viral antigen, negative for anti-SARS-CoV-2 IgM antibodies and positive for anti-SARS-CoV-2 IgG antibodies; and/or

[0053] (h) recovery phase when the at least one biological sample obtained from the subject is negative for viral RNA or viral antigen and negative for anti-SARS-CoV-2 IgM antibodies and anti-SARS-CoV-2 IgG antibodies.

[0054] Still further aspects of the above method involve (i) at least one type of first specific binding partner comprising at least one isolated polypeptide from a C-terminal domain of a nucleocapsid protein from a β-coronavirus wherein said first specific binding partner specifically binds to (a) an anti-β-coronavirus IgG antibody; (b) an anti-β-coronavirus IgM antibody; or (b) both an anti-β-coronavirus IgG antibody and anti-β-coronavirus IgM antibody; (ii) at least one type of second specific binding partner comprising isolated polypeptide from a receptor binding domain of a spike protein from a β-coronavirus, wherein said second specific binding partner specifically binds to (a) an anti-β-coronavirus IgG antibody; and (b) an anti-β-coronavirus IgM antibody; or (b) both an anti-β-coronavirus IgG antibody and anti-β-coronavirus IgM antibody; and (iii) at least one third specific binding partner comprising a detectable label. For example, in one aspect, the method involves (i) at least one first type of specific binding partner comprising at least one isolated polypeptide from a C-terminal domain of a nucleocapsid protein that (1) has an amino acid sequence of SEQ ID NO:1 (e.g., SARS-CoV-2); (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2, wherein said at least one type of first specific binding partner specifically binds to an anti-β-coronavirus IgG antibody; (ii) at least one type of second specific binding partner comprising an isolated polypeptide from a receptor binding domain (RBD) of a spike protein from SARS-CoV-2 (e.g., amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17), wherein said at least one type of second specific binding partner specifically binds to an anti-β-coronavirus IgM antibody; aspects (iii) at least one type of third specific binding partner comprising a detectable label. In some further aspects, the method comprises at least one type of fourth specific binding partner comprising a detectable label. The detectable label used in the at least one type of fourth specific binding partner may be the same or different than the label used in the at least one type of third specific binding partner.

[0055] In yet another aspect, the above method involves determining an amount or quantity of at least one type of anti-β-coronavirus antibody, such as an anti-SARS-CoV-2 IgG antibody, in a subject based on the amount of detectable signal assessed. In some aspects, the at least one type

of first specific binding partner used in the method is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17 and specifically binds to at least one anti-SARS-CoV-2 IgG antibody. In yet other aspects, the at least one type of second specific binding partner used in the method is anti-human-IgG IgG antibody.

[0056] In yet other aspects of the above method, the method further comprises a pre-treatment step done at the same time as, or prior to contacting the at least one type of first specific binding partner, the at least one type of second specific binding partner, or the at least one first specific binding partner and the at least one type of second specific binding partner, with the biological sample, and wherein the pretreatment set optionally comprises treatment with anti-human IgG (e.g., anti-human-IgG IgG), anti-human IgM (e.g., anti-human-IgM IgG), or anti-human IgG and anti-human IgM (e.g., anti-human-IgG IgG and anti-human-IgM IgG).

[0057] In yet still further aspects of the above method, the at least one type of first specific binding partner is immobilized on a solid support. In other aspects, at least one type of second specific binding partner is immobilized on a solid support. In other aspects, at least one type of first specific binding partner and at least one type of second specific binding partner are immobilized on different solid supports. In yet further aspects, at least one type of first specific binding partner and at least one type of second specific binding partner are immobilized on the same solid support.

[0058] In yet further aspects of the above method, the method is performed in less than about 20 minutes. In some aspect, the above method is performed in less than about 5 minutes. In some aspects, the above method is performed in less than about 10 minutes. In yet other aspects, the above method is performed in less than about 20 minutes. In still further aspects, the above method is performed in about 1 minute to about 20 minutes. In some aspects, the method is performed in about 5 minutes to about 20 minutes. In some aspects, the method is performed in about 15 minutes.

[0059] In still further aspects, the method is selected from the group consisting of an immunoassay or a clinical chemistry assay. In other aspects, the method is performed using single molecule detection, a lateral flow assay, or a point-of-care assay. . In some aspects, the method is performed using an immunoassay. In other aspects, the method is performed using a clinical chemistry assay. In yet other aspects, the method is performed using single molecule

detection. In still other aspects, the method is performed using a lateral flow assay. In still other aspects, the method is performed using a point-of-care assay.

[0060] In still further aspects, the above method further comprises use with at least one calibrator reagent, at least one control reagent, or at least one calibrator reagent and at least one control reagent.Some aspects of the above method further comprise identifying a subject having one or more anti-β-coronavirus IgG and/or anti-β-coronavirus IgM antibodies as a candidate subject to provide a biological sample for use in convalescent therapy against a β-coronavirus. In this aspect, a subject is identified as a candidate to provide a biological sample for use in convalescent therapy if the level of one or more anti-β-coronavirus IgG and/or anti-β-coronavirus IgM antibodies is from at least about 100 BAU/mL to about 490 BAU/mL; at least about 110 BAU/mL to about 490 BAU/mL; at least about 200 BAU/mL to about 490 BAU/mL; at least from about 300 BAU mL to about 490 BAU/mL; at least about 400 BAU mL to about 490 BAU/mL; at least about 100 BAU/mL; at least about 110 BAU/mL; at least about 120 BAU/mL; at least about 130 BAU/mL; at least about 140 BAU/mL; at least about 150 BAU/mL; at least about 160 BAU/mL; at least about 170 BAU/mL; at least about 180 BAU/mL; at least about 190 BAU/mL; at least about 200 BAU/mL; at least about 210 BAU/mL; at least about 220 BAU/mL; at least about 230 BAU/mL; at least about 240 BAU/mL; at least about 250 BAU/mL; at least about 260 BAU/mL; at least about 270 BAU/mL; at least about 280 BAU/mL; at least about 290 BAU/mL; at least about 300 BAU/mL; at least about 300 BAU/mL; at least about 310 BAU/mL; at least about 320 BAU/mL; at least about 330 BAU/mL; at least about 340 BAU/mL; at least about 350 BAU/mL; at least about 360 BAU/mL; at least about 370 BAU/mL; at least about 380 BAU/mL; at least about 390 BAU/mL; at least about 400 BAU/mL; at least about 400 BAU/mL; at least about 410 BAU/mL; at least about 420 BAU/mL; at least about 430 BAU/mL; at least about 440 BAU/mL; at least about 450 BAU/mL; at least about 460 BAU/mL; at least about 470 BAU/mL; at least about 480 BAU/mL; or at least about 490 BAU/mL.

[0061] Other aspects of the above method further comprise identifying a subject having one or more anti-β-coronavirus IgG and/or anti-β-coronavirus IgM antibodies as a candidate subject to provide a biological sample for use in convalescent therapy against a β-coronavirus. In this aspect, a subject is identified as a candidate to provide a biological sample for use in convalescent therapy if the level of one or more anti-β-coronavirus IgG and/or anti-β-coronavirus IgM antibodies is from at least about 500 BAU/mL to about 650 BAU/mL; at least

about 550 BAU/mL to about 650 BAU/mL; at least about 500 BAU/mL; from at least about 510 BAU/mL; at least about 520 BAU/mL; at least about 530 BAU/mL; at least about 540 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL; at least about 560 BAU/mL; at least about 570 BAU/mL; at least about 580 BAU/mL; at least about 590 BAU/mL; at least about 600 BAU/mL; at least about 610 BAU/mL; at least about 620 BAU/mL; at least about 630 BAU/mL; at least about 639 BAU/mL; at least about 640 BAU/mL; or at least about 650 BAU/mL.

[0062] In still further aspects, in the above method, the subject: (1) is naïve and was not previously vaccinated against SARS-CoV-2; (2) is naïve (a naïve subject is a subject that has not been exposed to the SARS-CoV-2 antigen previously) and was previously vaccinated against SARS-CoV-2; (3) is currently infected with SARS-CoV-2 and was not previously vaccinated against SARS-CoV-2; (4) is currently infected with SARS-CoV-2 and was previously vaccinated against SARS-CoV-2; (5) was previously infected with SARS-CoV-2, recovered, and was not previously vaccinated against SARS-CoV-2; or (6) was previously infected with SARS-CoV-2, recovered, and then was vaccinated against SARS-CoV-2.

[0063] In still further aspects, the above method is performed to: (a) determine whether the subject can be administered a current vaccine for SARS-CoV-2; or (b) monitor the subject following the current vaccine or previous vaccination, based on detecting the presence of at least one type of anti-SARS-CoV-2 antibody in the sample (e.g., for example, the subject can be monitored for one or more post-vaccine symptoms or side-effects, such as, for example, one or more of fatigue or malaise, headache, dizziness, or lightheadedness, fever or chills, muscle, bone, joint or nerve symptoms, nausea, vomiting, diarrhea, or other digestive symptoms, sleep changes, swollen lymph node, skin/nail or face changes, eye, ear, mouth or throat changes, cought, chest or breathing symptoms and/or memory or mood changes). Still further, in this aspect, the method is

performed regardless of variation in timing and/or severity of prior infection with SARS-CoV-2.

[0064] In still further aspects, the above method comprises determining that the a current vaccine for SARS-CoV-2:

[0065] (a) can be administered to the subject when no anti-SARS-CoV-2 antibodies, no anti-SARS-CoV-2 neutralizing IgG antibodies, or an amount of anti-SARS-CoV-2 IgG antibody or

anti-SARS-CoV-2 neutralizing IgG antibody that is insufficient to impart immunity, is detected in the biological sample; and/or

[0066] (b) should not be administered to the subject if an amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 neutralizing IgG antibody that is sufficient to impart immunity is detected in the biological sample.

[0067] In still yet further aspects, the above method comprises obtaining the biological sample within a time frame after the subject has received either the current vaccine or previous vaccination for SARS-CoV-2 of at least one day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, at least 15 days, at least 16 days, at least 17 days, at least 18 days, at least 19 days, at least 20 days, at least 21 days, at least 22 days, at least 23 days, at least 24 days, at least 25 days, at least 26 days, at least 27 days, at least 28 days, at least 29 days, at least 30 days, at least 31 days, at least 32 days, at least 33 days, at least 34 days, at least 35 days, at least 36 days, at least 37 days, at least 38 days, at least 39 days, at least 40 days, at least 41 days, at least 42 days, at least 43 days, at least 44 days, at least 45 days, at least 46 days, at least 47 days, at least 48 days, at least 49 days, or at least 50 days.

[0068] In still yet other aspects, the biological sample is obtained within about 7 to about 21 days after a subject has received either the current vaccine or a previous vaccination.

[0069] In still further aspects, the above method comprises determining that at least one further vaccine for SARS-CoV-2:

[0070] (a) can be administered to the subject after the current vaccine if no anti-SARS-CoV-2 antibodies, no anti-SARS-CoV-2 neutralizing IgG antibodies, and/or an amount of anti-SARS-CoV-2 IgG antibody or anti-SARS-CoV-2 neutralizing IgG antibody that is insufficient to impart immunity, is detected in the biological sample obtained from the subject; or

[0071] (b) should not be administered to the subject after the current vaccine if an amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 IgG neutralizing antibody that is sufficient to impart immunity is detected in the biological sample obtained from the subject.

[0072] As used in the above methods, the amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 neutralizing IgG antibody sufficient to impart immunity is from at least about 100 BAU/mL to about 490 BAU/mL; at least about 110 BAU/mL to about 490 BAU/mL; at least about 200 BAU/mL to about 490 BAU/mL; at least from about 300 BAU mL to about 490

BAU/mL; at least about 400 BAU mL to about 490 BAU/mL; at least about 100 BAU/mL; at least about 110 BAU/mL; at least about 120 BAU/mL; at least about 130 BAU/mL; at least about 140 BAU/mL; at least about 150 BAU/mL; at least about 160 BAU/mL; at least about 170 BAU/mL; at least about 180 BAU/mL; at least about 190 BAU/mL; at least about 200 BAU/mL; at least about 210 BAU/mL; at least about 220 BAU/mL; at least about 230 BAU/mL; at least about 240 BAU/mL; at least about 250 BAU/mL; at least about 260 BAU/mL; at least about 270 BAU/mL; at least about 280 BAU/mL; at least about 290 BAU/mL; at least about 300 BAU/mL; at least about 300 BAU/mL; at least about 310 BAU/mL; at least about 320 BAU/mL; at least about 330 BAU/mL; at least about 340 BAU/mL; at least about 350 BAU/mL; at least about 360 BAU/mL; at least about 370 BAU/mL; at least about 380 BAU/mL; at least about 390 BAU/mL; at least about 400 BAU/mL; at least about 400 BAU/mL; at least about 410 BAU/mL; at least about 420 BAU/mL; at least about 430 BAU/mL; at least about 440 BAU/mL; at least about 450 BAU/mL; at least about 460 BAU/mL; at least about 470 BAU/mL; at least about 480 BAU/mL; or at least about 490 BAU/mL.

[0073] In other aspects, in the above methods, the amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 IgG neutralizing antibody sufficient to impart immunity is from at least about 500 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 500 BAU/mL; from at least about 510 BAU/mL; at least about 520 BAU/mL; at least about 530 BAU/mL; at least about 540 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL; at least about 560 BAU/mL; at least about 570 BAU/mL; at least about 580 BAU/mL; at least about 590 BAU/mL; at least about 600 BAU/mL; at least about 610 BAU/mL; at least about 620 BAU/mL; at least about 630 BAU/mL; at least about 639 BAU/mL; at least about 640 BAU/mL; or at least about 650 BAU/mL.

[0074] As used in the above methods, the phrase "at least one further vaccine" encompasses a scenario where a first or current vaccine is administered to a subject followed (at some later period in time) by an additional or further at least one vaccine (e.g., N + 1 (where N is a first or current vaccine plus an additional or further vaccine), N+2 (where N is a first or current vaccine plus an additional or further two vaccines), N+3, N+4, N+5, N+6, N+7, N+8, N+9, N+10 to N + N' (where N' is an integer from 1 to 1000, from 1 to 500, from 1 to 100)).

[0075] In still further aspects, the anti-SARS-CoV-2 neutralizing antibody is an anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof.

[0076] In still further aspects, the above method comprises (a) monitoring the subject for anti-β-coronavirus, optionally, SARS-CoV-2, IgG and/or IgM antibodies; (b) treating the subject for a β-coronavirus, optionally, SARS-CoV-2; (c) monitoring the subject for anti-β-coronavirus, optionally, SARS-CoV-2, IgG and/or IgM antibodies and treating the subject for a β-coronavirus, optionally, SARS-CoV-2; or (d) treating the subject for a β-coronavirus, optionally, SARS-CoV-2 and monitoring the subject for anti-β-coronavirus, optionally, anti-SARS-CoV-2, IgG and/or IgM antibodies.

[0077] In yet other aspects, the above method can be used in an automated system or a semi-automated system.

[0078] In yet another aspect, the present disclosure relates to a method of predicting outcome in a subject that is or was infected with SARS-CoV-2. This method comprises the steps of:

[0079] a) detecting an anti-SARS-CoV-2 IgG antibody in at least one biological sample obtained from the subject within a first ten days after onset of symptoms of SARS-CoV-2 infection;

[0080] b) detecting an anti-SARS-CoV-2 IgM antibody in the at least one biological sample obtained from the subject within the first ten days after onset of symptoms of SARS-CoV-2 infection;

[0081] c) determining which of the anti-SARS-CoV-2 IgG or anti-SARS-CoV-2 IgM antibody detected in a) and b) first appears in the subject;

[0082] d) predicting that the subject is more likely to have an unfavorable outcome if anti-SARS-CoV-2 IgG antibody first appears in the subject prior to the appearance of anti-SARS-CoV-2 IgM antibody; and

[0083] e) predicting that the subject is more likely to have an favorable outcome if anti-SARS-CoV-2 IgG antibody first appears in the subject at the same time or after the appearance of anti-SARS-CoV-2 IgM antibody.

[0084] In some aspects in the above method, the subject is hospitalized for symptoms of SARS-CoV-2.

[0085] In some aspects in the above method, (i) the anti-SARS-CoV-2 IgG antibody and the one anti-SARS-CoV-2 IgM antibody are detected in the same biological sample; or (ii) the anti-SARS-CoV-2 IgG antibody and the anti-SARS-CoV-2 IgM antibody are detected in different biological samples. In yet other aspects of the above method, the anti-SARS-2 IgG antibody and the anti-SARS-CoV-2 IgM antibody are (i) detected on the same day within the first ten days after onset of symptoms; (ii) detected on different days within the first ten days after the onset of symptoms.

[0086] In still yet other aspects of the above method, the anti-SARS-CoV-2 IgG antibody is detected in at least one biological sample obtained from the subject within the first day after the onset of symptoms, within the second day after the onset of symptoms, within the third day after the onset of symptoms, within the fourth day after the onset of symptoms, within the fifth day after the onset of symptoms, within the sixth day after the onset of symptoms, within the seventh date after the onset of symptoms, within the eighth day after the onset of symptoms or within the ninth day after the onset of symptoms.

[0087] In still yet other aspects of the above method, the anti-SARS-CoV-2 IgM antibody is detected in at least one biological sample obtained from the subject within the first day after the onset of symptoms, within the second day after the onset of symptoms, within the third day after the onset of symptoms, within the fourth day of infection, within the fifth day after the onset of symptoms, within the sixth day after the onset of symptoms, within the seventh date after the onset of symptoms, within the eighth day after the onset of symptoms or within the ninth day after the onset of symptoms.

[0088] In still further aspects, the unfavorable outcome is death. In yet further aspect, the favorable outcome is survival, particularly, for subjects that are hospitalized. In still further aspects, the method is selected from the group consisting of an immunoassay or a clinical chemistry assay. In other aspects, the method is performed using single molecule detection, a lateral flow assay, or a point-of-care assay. In some aspects, the method is performed using an immunoassay. In other aspects, the method is performed using a clinical chemistry assay. In yet other aspects, the method is performed using single molecule detection. In still other aspects, the method is performed using a lateral flow assay. In still other aspects, the method is performed using a point-of-care assay.

[0089] In yet another aspects, the present disclosure relates to a method of determining SARS-CoV-2 immune status of a subject. The method involves the steps of:

[0090] a) determining an amount of at least one anti-SARS-CoV-2 IgG antibody and/or anti-SARS-CoV-2 IgG neutralizing antibody in at least one biological sample obtained from a subject; and

[0091] b) determining the subject's SARS-CoV-2 immune status, wherein the subject is determined to have immunity to SARS-CoV-2 when the amount of anti-SARS-CoV-2 IgG antibody and/or anti-SARS-CoV-2 IgG neutralizing antibody is (a) from at least about 550 BAU/mL to about 650 BAU/mL; or (b) from at least about 100 BAU/mL to about 490 BAU/mL, where the method is performed irrespective of (a) the subject's prior infection and/or vaccination history with SARS-CoV-2, or (b) whether there is any knowledge of the subject's prior infection and/or vaccination history with SARS-CoV-2.

[0092] In some aspects of the above method, the subject has not previously been infected with SARS-CoV-2. In other aspects of the above method, the subject has previously been infected with SARS-CoV-2. In still yet other aspects of the above method, the subject has not previously been vaccinated for SARS-CoV-2. In yet other aspects of the above method, the subject has previously been vaccinated for SARS-CoV-2.

[0093] In some aspects of the above method, the immune status of the subject is that the subject has immunity to SARS-CoV-2. In yet other aspects of the above method, the immune status of the subject is that subject does not have immunity to SARS-CoV-2. In still further aspects, the method is selected from the group consisting of an immunoassay or a clinical chemistry assay. In other aspects, the method is performed using single molecule detection, a lateral flow assay, or a point-of-care assay. In some aspects, the method is performed using an immunoassay. In other aspects, the method is performed using a clinical chemistry assay. In yet other aspects, the method is performed using single molecule detection. In still other aspects, the method is performed using a lateral flow assay. In still other aspects, the method is performed using a point-of-care assay.

[0094] In yet other aspects, the above method is for use in an automated system or a semi-automated system.

[0095] In yet another aspect, the present disclosure relates to a method of predicting outcome in a subject that is or was infected with SARS-CoV-2. The method comprises the steps of:

[0096] a) obtaining a signal-to-calibrator ratio (S/CO) from an assay of at least one anti-SARS-CoV-2 IgM antibody in at least one biological sample obtained from the subject at least ten days after onset of symptoms of SARS-CoV-2; and

[0097] b) predicting that the subject is more likely than not to have an unfavorable outcome if the S/CO ratio of anti-SARS-CoV-2 IgM antibody determined in the biological sample is equal to or greater than about 10 S/CO.

[0098] In the above method, the subject is hospitalized for symptoms of SARS-CoV-2.

[0099] In still further aspects, the method is selected from the group consisting of an immunoassay or a clinical chemistry assay. In other aspects, the method is performed using single molecule detection, a lateral flow assay, or a point-of-care assay. In some aspects, the method is performed using an immunoassay. In other aspects, the method is performed using a clinical chemistry assay. In yet other aspects, the method is performed using single molecule detection. In still other aspects, the method is performed using a lateral flow assay. In still other aspects, the method is performed using a point-of-care assay.In yet other aspects, the above method is for use in an automated system or a semi-automated system.

[0100] Additionally, the present disclosure relates to a kit for performing the above method. Such a kit can comprise:

[0101] a. at least one type of first specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof selected from a C-terminal domain nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain nucleocapsid protein and a receptor binding domain of a spike protein from a β-coronavirus; and

[0102] b. at least one type of second specific binding partner comprising at least one detectable label.

[0103] In the above kit, the at least one type of first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino

acid sequence of amino acids 210 to 419 of SEQ ID NO:2. In some aspects, the variant of the nucleocapsid protein comprises: (i) an amino acid substitution replacing serine with phenylalanine at position 235 (S235F) within amino acids 210 to 419 of SEQ ID NO:2; or (ii) SEQ ID NO:24.

[0104] Alternatively, in the above kit, the at least one type of first specific binding partner is a receptor binding domain (RBD) of a spike protein that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17. In some aspects, the variant of the RBD of a spike protein comprises one or more amino substitutions within amino acids 319 to 542 of SEQ ID NOS:15 or 25 replacing lysine with asparagine at position 417, replacing glutamic acid with lysine at position 484, and/or replacing asparagine with tyrosine at position 501.

[0105] In yet other aspects, the kit can comprise:

[0106] a. at least one type of first specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof selected from a C-terminal domain nucleocapsid protein;

[0107] b. at least one type of second specific binding partner comprising a receptor binding (RBD) domain of a spike protein; and

[0108] c. at least one type of third specific binding partner comprising at least one detectable label. Optionally, in some aspects, the kit can contain at least one type of fourth specific binding partner that comprises a detectable label.

[0109] In the above kit, the at least one type of first specific binding partner comprises a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2 and the at least one second specific binding partner comprises a receptor binding domain of a spike protein or a variant thereof from SARS-CoV-2 (e.g., amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17). In some aspects, the variant of the nucleocapsid protein comprises: (i) an amino acid substitution replacing serine with phenylalanine at position 235 (S235F) within amino acids 210 to 419 of

SEQ ID NO:2; or (ii) SEQ ID NO:24. In some aspects, the variant of the RBD of a spike protein comprises one or more amino substitutions within amino acids 319 to 542 of SEQ ID NOS:15 or 25 replacing lysine with asparagine at position 417, replacing glutamic acid with lysine at position 484, and/or replacing asparagine with tyrosine at position 501.

[0110] Additionally, in some aspects, the kit can further comprise or be configured to be used with, at least one calibrator reagent, at least one control reagent, or at least one calibrator reagent and at least one control reagent.

[0111] In some further aspects, the kit can further comprise at least one solid support. In some further aspects, the kit can further comprise at least two solid supports.

[0112] In still yet further aspect, the kit comprises or can be configured to be used with, at least one pretreatment reagent.

[0113] In some aspects, in the above kit, the isolated polypeptide is a fusion polypeptide.

[0114] In still further aspects, the isolated polypeptide in the kit has a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374,

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[0115] In still further aspects, the isolated polypeptide in the kit comprises a C-terminal domain nucleocapsid protein, said polypeptide having a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404 or 405 amino acids.

[0116] In yet still further aspects, the isolated polypeptide in the kit is a C-terminal domain of a nucleocapsid protein, said polypeptide having a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66,

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[0117] In still further aspects, the isolated polypeptide in the kit is a receptor binding domain (RBD) of a spike protein, said polypeptide having a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397,

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1454, 1455, 1456, 1457, 1458, 1459, 1460, 1461, 1462, 1463, 1464, 1465, 1466, 1467, 1468, 1469, 1470, 1471, 1472, 1473, 1474, 1475, 1476, 1477, 1478, 1479, 1480, 1481, 1482, 1483, 1484, 1485, 1486, 1487, 1488, 1489, 1490, 1491, 1492, 1493, 1494, 1495, 1496, 1497, 1498, 1499 or 1500 amino acids.

[0118] In yet other aspects, the kit contains at least one second, third, fourth, or fifth type of specific binding partner which is an anti-human-IgG IgG antibody, an anti-human-IgM IgG antibody, or an anti-human-IgG IgG antibody and an anti-human-IgM IgG antibody. For example, in some aspects, the kit contains at least one type of second specific binding partner which is an anti-human-IgG IgG antibody, an anti-human-IgM IgG antibody, or an anti-human-IgG IgG antibody and an anti-human-IgM IgG antibody. For example, in yet other aspects, the kit contains at least one type of third specific binding partner which is an anti-human-IgG IgG antibody and at least one type of fourth specific binding partner which is an anti-human-IgM IgG antibody.

[0119] In still further aspects, the kit can be adapted for use with an automated or semi-automated system.

[0120] The present disclosure also relates to a system for detecting an anti-β-coronavirus antibody in a biological sample obtained from a subject. Such a system can comprise:

[0121] at least one type of first specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof selected from a C-terminal domain nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain nucleocapsid protein and a receptor binding domain of a spike protein from a β-coronavirus that specifically binds to at least one anti-β-coronavirus antibody and at least one type of second specific binding partner comprising at least one detectable label; and

[0122] at least one device for detecting the at least one label from the complex, wherein the amount of signal from the label indicates the presence or amount of anti-β-coronavirus antibody in the sample.

[0123] In some aspects, the system for comprises determining the amount, level and/or concentration of at least one type of anti-β-coronavirus (such as anti-SARS-CoV or anti-SARS-CoV-2) antibody in a biological sample based on the amount of at least one label detected from the complex. In yet further aspects, the amount, level and/or concentration of an anti-SARS-

CoV-2 antibody is determined in the system. For example, in yet other aspects, the amount, level and/or concentration of a anti-SARS-CoV-2 IgG antibody is determined in the system.

[0124] In some aspects, the at least one type of first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2. In some aspects, the variant of the nucleocapsid protein comprises: (i) an amino acid substitution replacing serine with phenylalanine at position 235 (S235F) within amino acids 210 to 419 of SEQ ID NO:2; or (ii) SEQ ID NO:24. In these aspects, the at least one type of first specific binding partner binds to an (a) anti-β-coronavirus IgG antibody or (b) anti-β-coronavirus IgM antibody. In other aspects, the at least one type of first specific binding partner is a receptor binding domain of a spike protein or variant thereof from SARS-CoV-2 (e.g., amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17). In some aspects, the variant of the RBD of a spike protein comprises one or more amino substitutions within amino acids 319 to 542 of SEQ ID NOS:15 or 25 replacing lysine with asparagine at position 417, replacing glutamic acid with lysine at position 484, and/or replacing asparagine with tyrosine at position 501.

[0125] In yet other aspects, the system for detecting at least one type of anti-β-coronavirus antibody in a biological sample obtained from a subject can comprise:

[0126] at least one type of first specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof from a C-terminal domain nucleocapsid protein, at least one type of second specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof from a receptor binding domain of a spike protein and at least one type of third specific binding partner comprising at least one detectable label; and

[0127] at least one type of device for detecting the at least one label from the complex. Optionally, in some aspects, the system can comprise at least one type of fourth specific binding partner comprising at least one detectable label.

[0128] In some aspects, the at least one type of first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2 and the at least one type of second specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof from SARS-CoV-2 (e.g., amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17). In some aspects, the variant of the nucleocapsid protein comprises: (i) an amino acid substitution replacing serine with phenylalanine at position 235 (S235F) within amino acids 210 to 419 of SEQ ID NO:2; or (ii) SEQ ID NO:24. In some aspects, the variant of the RBD of a spike protein comprises one or more amino substitutions within amino acids 319 to 542 of SEQ ID NOS:15 or 25 replacing lysine with asparagine at position 417, replacing glutamic acid with lysine at position 484, and/or replacing asparagine with tyrosine at position 501.

[0129] In some aspects, the device for detecting the label from the complex in the above system is automated or semi-automated.

[0130] In yet other aspects, the at least one type of anti-β-coronavirus antibody detected in the above system is an anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody. Specifically, in other aspects, the at least one type of anti-β-coronavirus antibody is an anti-SARS-CoV IgG antibody, an anti-SARS-CoV IgM antibody, an anti-SARS-CoV-2 IgG antibody, an anti-SARS-CoV-2 IgM antibody, or any combination thereof.

[0131] In still a further aspect, the at least one type of second specific binding partner in the system is an anti-human-IgG IgG antibody, an anti-human-IgM IgG antibody, or an anti-human-IgG IgG and anti-human-IgM IgG antibody. In still a further aspect, the system comprises at least one type of third specific binding partner which is an anti-human-IgG IgG antibody and at least one type of fourth specific binding partner which is an anti-human-IgM IgG antibody.

[0132] In yet other aspects, the above system further comprises determining an amount of at least one type of anti-β-coronavirus antibody, such as an anti-SARS-CoV-2 IgG antibody, based on the amount of label detected from the at least one complex. In yet other aspects, the at least

one type of first specific binding partner used in the system is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17 and specifically binds to at least one anti-SARS-CoV-2 IgG antibody. In still further aspects, the at least one type of second specific binding partner used in the system is an anti-human-IgG IgG antibody.

[0133] In still further aspects, in any of the above methods, the anti-SARS-CoV-2 antibody detected or determined specifically binds to a variant:

[0134] a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

[0135] b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

[0136] c) from a SARS-CoV-2 virus comprising any combination of a) and b).

[0137] In any of the above mentioned kits, the at least one type of anti-β-coronavirus antibody detected or determined is an anti-SARS-CoV-2 antibody that specifically binds to a variant:

[0138] a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position

373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

[0139] b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

[0140] c) from a SARS-CoV-2 virus comprising any combination of a) and b).

[0141] In any of the above systems, the at least one type of anti-β-coronavirus antibody detected is an anti-SARS-CoV-2 antibody that specifically binds to a variant:

[0142] a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T), or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

[0143] b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or

combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

[0144] c) from a SARS-CoV-2 virus comprising any combination of a) and b).

[0145] The present disclosure also relates to an improvement of a method of detecting an at least one type of anti-β-coronavirus antibody in a biological sample, wherein the method comprises detecting a complex comprising at least one type of first specific binding partner, said sample anti-β-coronavirus antibody, and at least one type of second specific binding partner comprising at least one detectable label, wherein the improvement comprises using at least one type of first specific binding partner comprising at least one isolated polypeptide from a C-terminal domain of a β-coronavirus nucleocapsid protein or a variant thereof.

[0146] The present disclosure also relates to an improvement of a method of detecting at least one type of anti-β-coronavirus antibody in a biological sample, wherein the method comprises detecting a complex comprising at least one first specific binding partner, said sample anti-β-coronavirus antibody, and at least one type of second specific binding partner comprising at least one detectable label, wherein the improvement comprises using at least one type of first specific binding partner comprising at least one isolated polypeptide from a receptor binding domain (RBD) of a β-coronavirus spike protein or a variant thereof.

[0147] The present disclosure also relates to an improvement of a method or system for determining an amount of an anti-SARS-CoV-2 IgG antibody in a subject based on the amount of detectable signal assessed, wherein the improvement comprises: (a) a linear assay range of up to 50,000 AU/mL; (b) determining an amount in arbitrary units (e.g., AU/mL) rather than an index; and/or (c) improved detection of positive samples as compared to other assays.

[0148] In any of the above improvements, the at least one type of anti-β-coronavirus antibody detected is an anti-SARS-CoV-2 antibody that specifically binds to a variant:

[0149] a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of

(1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

[0150] b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

[0151] c) from a SARS-CoV-2 virus comprising any combination of a) and b).

[0152] In yet another aspect, the present disclosure relates to a system for assessing whether a subject is likely to have immunity from infection from SARS-CoV-2 in a biological sample obtained from a subject. In this aspect, the system comprises:

[0153] at least one type of first specific binding partner comprising at least one type of β-coronavirus isolated polypeptide or variant thereof selected from a C-terminal domain nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain nucleocapsid protein and a receptor binding domain (RBD) of a spike protein from at least one type β-coronavirus that specifically binds to at least one type of anti-SARS-CoV-2 antibody and at least one type of second specific binding partner comprising at least one detectable label;

[0154] at least one device for detecting the at least one label from the complex, wherein the amount of signal from the label indicates the presence or amount of anti-SARS-CoV-2 antibody in the sample; and

[0155] a means for assigning a differentiative rating indicating whether the subject is likely to have immunity from infection from SARS-CoV-2 based on the presence or amount of at least one type of anti-SARS-CoV-2 antibody detected in the sample.

[0156] In some aspects of the system, the differentiative rating is a color and/or number rating. In other aspects of the system, the color and/or number rating is displayed on a mobile device through a mobile application.

[0157] In still further aspects of this system, the variant:

[0158] a) is of the C-terminal domain of a nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

[0159] b) is the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

[0160] c) comprises any combination of a) and b).

[0161] In some aspects of the system, the device for detecting the label from the complex is automated or semi-automated. In further aspects in the system, the device for detecting the label from the complex is semi-automated and comprises uploading the differentiative rating using a bar code.

[0162] In still further aspects of the system, the at least one type of anti-SARS-CoV-2 antibody is an anti-SARS-CoV-2 IgG antibody, an anti-SARS-CoV-2 IgM antibody, or any combination thereof. In yet still further aspects of the system, the at least one type of second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM (e.g., anti-human-IgM IgG) antibody, or an anti-species IgG (e.g., anti-human-IgG IgG) antibody, and anti-species IgM (e.g., anti-human-IgM IgG) antibody. In still further aspects of the system, the at least one type of first specific binding partner is a receptor binding domain

(RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17. In yet further aspects of the system, the at least one type of first specific binding partner binds to (a) an anti-SARS-CoV-2 IgG antibody, (b) an anti-SARS-CoV-2 IgM antibody, or (c) combinations of (a) and (b).

Brief Description Of The Drawings

[0163] FIG. 1 provides the amino acid sequence of a nucleocapsid protein from a strain of SARS-CoV-2 (SEQ ID NO:2) and SARS-CoV (SEQ ID NO:14).

[0164] FIG. 2 provides an alignment between amino acids 1-422 of CoV (SARS-CoV; SEQ ID NO:14) and amino acids 1-419 of Cov2 (SARS-CoV-2; SEQ ID NO:2).

[0165] FIG. 3 provides a diagram illustrating the structure of a fusion protein comprising at least 5 amino acids of the N-terminal domain (NTD) of a β-coronavirus operably linked or grafted on to at least 5 amino acids of the C-terminal domain (CTD) of the same or different β-coronavirus. In some aspects, a fusion protein can comprise a methionine initiator residue operably linked or grafted on to amino acids 5 to 25 of SEQ ID NO:14 or amino acids 5 to 24 of SEQ ID NO:2 operably linked or grafted (such as by one or more polypeptide linkers and/or HIS tags) on to amino acids 211 to 419 of SEQ ID NO:2 (or the analogous region from SEQ ID NO:14). Other variations are possible. For example, in some aspects, the fusion protein can comprise an epitope for the monoclonal antibody CR3018 (described in van den Brink et al., *J. Virol.* 79(3):1635-1644 (February 2005) and U.S. Patent No. 7,696,330) fused at the N-terminal end of the CTD ("epitope-grafted CTD" fusion protein or peptide). More specifically, in this aspect, a fusion protein comprising methionine as the starting amino acid operably linked or grafted on to an epitope comprising at least amino acids RNAPRITFG (SEQ ID NO:6) or RSAPRITFG (SEQ ID NO:7), which in turn, are operably linked or grafted on to the N-terminal end of the CTD, e.g., amino acids 211 to 419 of SEQ ID NO:2 (or the analogous region from SEQ ID NO:14). In some aspects, there may be one or more additional sequences operably linked or grafted on to the starting amino acid methionine, such as, for example, GPQNQ (SEQ ID NO:21) or GPQSNQ (SEQ ID NO:22), which in turn, can be operably linked or grafted on to an epitope comprising at least amino acids RNAPRITFG (SEQ ID NO:6) or RSAPRITFG (SEQ ID NO:7), which furthermore, are operably linked or grafted on to the N-terminal end of the CTD, e.g., amino acids 211 to 419 of SEQ ID NO:2 (or the analogous region from SEQ ID

NO:14). In another aspect, the fusion protein comprises the initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2. In still yet another aspect, the fusion protein comprises the initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2.

[0166] FIG. 4 provides a sequence listing comprising SEQ ID NOS. 1 – 25. For SEQ ID NO:2, the CTD of the nucleocapsid protein of SARS-CoV-2 (amino acids 210-419) is indicated in underlining and italics. Additionally, at amino acid position 235 shown in underlining, italics and bold in SEQ ID NO:2 a serine can be replaced with a phenylalanine at this position (known as S235F).

[0167] FIG. 5 provides the kinetics of viral and host biomarkers in SARS-CoV-2 infection. The incubation phase or initial period of infection is an asymptomatic period when a subject does not exhibit any symptoms of a SARS-CoV-2 infection. The incubation phase or initial period can last for a period of about 0 to about 14 days after infection. During the incubation phase or initial period, viral RNA and/or viral antigen become detectable. After incubation phase or initial period of infection, the subject progresses to an active phase of infection. Initally, the subject enters an early acute phase of infection. The early acute phase is the phase when the subject starts to experience the onset of one or more symptoms of a SARS-CoV-2 infection and begins to develop an immune response to the virus and begins to produce antibodies. The early acute phase may last for a period of about 0 to about 14 days after the onset of symptoms. During the early acute phase, viral RNA and/or viral antigen are detectable and anti-SARS-CoV-2 IgM antibodies become detectable. After the early acute phase, the subject progresses to the acute or active phase of infection (not shown). During the acute phase of infection, the symptoms of SARS-CoV-2 infection worsen (e.g., the subject may have to be hospitalized, may need oxygen support, etc.), the immune response of the subject continues to progress, viral RNA and/or viral antigen are detectable, anti-SARS-CoV-2 IgM antibodies are detectable and anti-SARS-CoV-2 IgG antibodies become detectable. The acute phase may last for for a period of about 0 to about 14 days after the onset of worsening symptoms of the virus. After the acute phase, the subject progresses into the late acute phase. The late acute phase is the stage when the subject starts to recover from the SARS-CoV-2 infection and one or more symptoms of infection begin to

improve, lessen and/or disappear. The late acute phase may last for a period of about 1 day to about 28 days. During the late acute phase, viral RNA and viral antigen levels start to decline and eventually are not detectable, anti-SARS-CoV-2 IgM antibodies are detectable and anti-SARS-CoV-2 IgG antibodies are detectable. The convalescence or recovery phase of infection begins after the late acute phase. During this period, the subject no longer exhibits any symptoms of SARS-CoV-2 infection and viral RNA and viral antigen are no longer detectable, anti-SARS-CoV-2 IgM antibodies begin to decline and eventually become non-detectable, and anti-SARS-CoV-2 IgG antibodies remain in the blood and provide long-term immunity for the subject.

[0168] FIG. 6, as described in Example 8, shows that subjects who had an unfavorable outcome (i.e., died) had a significantly higher anti-SARS-CoV-2 IgM signal (13.8 S/CO, 11.2-12.5) later post-symptom onset (e.g., at least 10 days post-symptom onset, or, e.g., day eleven or later).

[0169] FIG. 7 shows the probability profile using a plaque reduction neutralization test (PRNT) at ID_{50} at a 1:250 dilution as a representative high titer as described in Example 9.

[0170] FIG. 8 shows that the assay of Example 7 can be used without additional dilution on an Abbott ARCHITECT instrument when monitoring vaccine response in subjects receiving a SARS-CoV-2 vaccine. Dose 1 was measured at about 3 weeks post-immunization and dose 2 was measured about 2 weeks after subjects received a booster (e.g., second vaccination with a SARS-CoV-2 vaccine). Specifically, the assay of Example 7 shows quantification of 98.8% of anti-SARS-CoV-2 IgG antibodies within the extended measuring interval (EMI) with automated dilution and 90.6% within the upper limit measuring interval (ULMI) with no dilution.

[0171] FIG. 9 shows the SARS-CoV-2 anti-spike (S) protein IgG responses of persons to vaccination with BNT162b2 (Pfizer-BiNTech) mRNA vaccine as described in Example 10. The box plots display the median values with the interquartile range (lower and upper hinge) and ±1.5 fold the interquartile range from the first and third quartile (lower and upper whiskers). Two-sided Wilcoxon tests were used, without adjustment for multiple testing, to conduct the following between-group comparisons: (1) infection naïve (n=903) and those with prior infection (n=78), both at baseline ($P<0.001$); (2) infection naïve (n=490) and those with prior infection (n=35) both following dose 1 ($P<0.001$); (3) infection naïve (n=228) and those with prior infection (n=11), both following dose 2 ($P<0.001$); (4) infection naïve (n=490) following

dose 1 and those with prior infection (n=78) at baseline (P<0.001); and (5) infection naïve (n=228) following dose 2 and prior infection (n=35) following dose 1 (P=0.92).

[0172] FIG. 10 shows the SARS-CoV-2 anti-nucleocapsid (N) protein IgG responses to mRNA SARS-CoV-2 vaccination with BNT162b2 (Pfizer-BiNTech) in persons with and without prior SARS-CoV-2 infection as described in Example 10. The box plots display the median values with the interquartile range (lower and upper hinge) and ±1.5 fold the interquartile range from the first and third quartile (lower and upper whiskers). Two-sided Wilcoxon tests were used, without adjustment for multiple testing, to conduct the following between-group comparisons: (1) infection naïve (n=903) and those with prior infection (n=78), both at baseline (P<0.001); (2) infection naïve (n=490) and those with prior infection (n=35) both following dose 1 (P<0.001); (3) infection naïve (n=228) and those with prior infection (n=11), both following dose 2 (P<0.001); (4) infection naïve (n=490) following dose 1 and those with prior infection (n=78) at baseline (P<0.001); and (5) infection naïve (n=228) following dose 2 and prior infection (n=35) following dose 1 (P<0.001).

[0173] FIG. 11 shows ACE2 antibody binding capacity in persons with and without prior SARS-CoV-2 infection as described in Example 10. The box plots display the median values with the interquartile range (lower and upper hinge) and ±1.5 fold the interquartile range from the first and third quartile (lower and upper whiskers). Two-sided Wilcoxon tests without adjustments were used for multiple testing, to conduct the following between-group comparisons: (1) infection naïve (n=490) and those with prior infection (n=35) both following dose 1 (P<0.001); (2) infection naïve (n=228) and those with prior infection (n=11), both following dose 2 (P<0.001); and (3) infection naïve (n=228) following dose 2 and those with prior infection (n=35) following dose 1 (P=0.52).

[0174] FIG. 12 shows the difference in post-vaccination symptoms in persons with and without prior SARS-CoV-2 infection who received the BNT162b2 (Pfizer-BiNTech) mRNA vaccine as described in Example 10. Specifically, the bar graphs display the frequency and severity of symptoms reported following each dose of vaccine administered, by prior infection status.

[0175] FIG. 13 shows various aspects of the ULMI and EMI for the assay described in Example 7. For example, in one aspect, the ULMI for the assay described in Example 7 can be from about 15,500 to about 40,000 AU/mL and/or the EMI can be from about 40,000 to about

80,000 AU/mL, or in other aspects, the ULMI can be from about 15,500 to about 25,000 AU/mL and/or the EMI can be from about 25,000 to about 50,000 AU/mL.

DETAILED DESCRIPTION

[0176] The present disclosure relates to methods, kits, systems and algorithms to detect the presence of or determine the amount, concentration and/or level of at least one type of anti-β-coronavirus (such as SARS-CoV or SARS-CoV-2) antibody (e.g., IgG and/or IgM) in a sample. In some aspects, the methods, kits and systems described herein are used as an aid in the determination of a subject's immune status to SARS-CoV-2. Specifically, in one aspect, the methods of the present disclosure involve contacting at least one sample obtained from a subject (either simultaneously or sequentially, in any order), with at least one type of first specific binding partner comprising at least one type of β-coronavirus (such as SARS-CoV or SARS-CoV-2) isolated polypeptide or variant thereof, (e.g., a β-coronavirus (such as SARS-CoV or SARS-CoV-2) recombinant antigen comprising an isolated polypeptide or variant thereof) which is (a) a C-terminal domain nucleocapsid protein or variant thereof from a β-coronavirus (such as, for example, SARS-CoV or SARS-CoV-2); (b) a receptor binding domain (RBD) of a spike protein or variant thereof from a β-coronavirus (such as, for example, SARS-CoV or SARS-CoV-2); or (c) a C-terminal domain nucleocapsid protein or variant thereof and a receptor binding domain of a spike protein or variant thereof from a β-coronavirus (such as, for example, SARS-CoV or SARS-CoV-2), wherein said polypeptide specifically binds to at least one anti-β-coronavirus antibody (e.g., IgG and/or IgM) in the sample and at least one type of second specific binding partner comprising a detectable label to produce one or more types of first complexes comprising the first specific binding partner-β-coronavirus antibody-second specific binding partner. In some aspects, the least one type of second specific binding partner is a human IgG antibody, a human IgM antibody or a human IgG and/or IgM antibody. In other aspects, the at least one type of specific binding partner is a human IgG anti-β-coronavirus antibody, human IgM anti-β-coronavirus antibody, or a human IgG anti-β-coronavirus antibody and human IgM anti-β-coronavirus antibody. A signal from the one or more types of first complexes are assessed (e.g., detected). Specifically, the amount of the detectable signal from the detectable label indicates the presence or amount of at least one type of anti-β-coronavirus antibody in the sample.

[0177] The biological sample used in the methods of the present disclosure may be obtained from an asymptomatic subject or from a subject exhibiting one or more symptoms of infection with at least one type of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). The methods of the present disclosure also include treating a subject identified as having at least one type of β-coronavirus with one or more β-coronavirus treatments and optionally, monitoring such subjects, such as before, during and/or after receiving such treatments.

[0178] In another aspect, the present disclosure relates to kits for performing such methods.

[0179] In still yet another aspect, the present disclosure relates to systems for detecting a β-coronavirus in a biological sample.

[0180] Section headings as used in this section and the entire disclosure herein are merely for organizational purposes and are not intended to be limiting.

1. Definitions

[0181] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present disclosure. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

[0182] The terms "comprise(s)," "include(s)," "having," "has," "can," "contain(s)," and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms "a," "an" and "the" include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments "comprising," "consisting of" and "consisting essentially of," the embodiments or elements presented herein, whether explicitly set forth or not.

[0183] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

[0184] The clause, "at least" followed directly or indirectly by a number refers to type and/or quantity, unless it is evident from the context in which the clause is applied that it refers to only type, or only quantity, and not both type and quantity.

[0185] "Affinity matured antibody" is used herein to refer to an antibody with one or more alterations in one or more CDRs, which result in an improvement in the affinity (i.e., K_D, k_d or k_a) of the antibody for a target antigen compared to a parent antibody, which does not possess the alteration(s). Exemplary affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. A variety of procedures for producing affinity matured antibodies are known in the art, including the screening of a combinatory antibody library that has been prepared using bio-display. For example, Marks et al., *BioTechnology*, 10: 779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by Barbas et al., *Proc. Nat. Acad. Sci. USA*, 91: 3809-3813 (1994); Schier *et al.*, *Gene*, 169: 147-155 (1995); Yelton et al., *J. Immunol.*, 155: 1994-2004 (1995); Jackson et al., *J. Immunol.*, 154(7): 3310-3319 (1995); and Hawkins et al., J. Mol. Biol., 226: 889-896 (1992). Selective mutation at selective mutagenesis positions and at contact or hypermutation positions with an activity-enhancing amino acid residue is described in U.S. Patent No. 6,914,128 B1.

[0186] "Antibody" and "antibodies" as used herein refers to monoclonal antibodies, monospecific antibodies (e.g., which can either be monoclonal, or may also be produced by other means than producing them from a common germ cell), multispecific antibodies, human antibodies, humanized antibodies (fully or partially humanized), animal antibodies such as, but not limited to, a bird (for example, a duck or a goose), a shark, a whale, and a mammal, including a non-primate (for example, a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, etc.) or a non-human primate (for example, a monkey, a chimpanzee, etc.), recombinant antibodies, chimeric antibodies, single-chain Fvs ("scFv"), single chain antibodies, single domain antibodies, Fab fragments, F(ab') fragments, $F(ab')_2$ fragments, disulfide-linked Fvs ("sdFv"), and anti-idiotypic ("anti-Id") antibodies, dual-domain antibodies, dual variable domain (DVD) or triple variable domain (TVD) antibodies (dual-variable domain immunoglobulins and methods for making them are described in Wu, C., et al., *Nature Biotechnology*, 25(11):1290-1297 (2007) and PCT International Application WO 2001/058956, the contents of each of which are herein

incorporated by reference), or domain antibodies (dAbs) (e.g., such as described in Holt et al., *Trends in Biotechnology* 21:484-490 (2014)), and including single domain antibodies sdAbs that are naturally occurring, e.g., as in cartilaginous fishes and camelid, or which are synthetic, e.g., nanobodies, VHH, or other domain structure), and functionally active epitope-binding fragments of any of the above. In particular, antibodies include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, namely, molecules that contain an analyte-binding site. Immunoglobulin molecules can be of any type (for example, IgG, IgE, IgM, IgD, IgA, and IgY), class (for example, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2), or subclass. For simplicity sake, an antibody against an analyte is frequently referred to herein as being either an "anti-analyte antibody" or merely an "analyte antibody".

[0187] "Antibody fragment" as used herein refers to a portion of an intact antibody comprising the antigen-binding site or variable region. The portion does not include the constant heavy chain domains (i.e., CH2, CH3, or CH4, depending on the antibody isotype) of the Fc region of the intact antibody. Examples of antibody fragments include, but are not limited to, Fab fragments, Fab' fragments, Fab'-SH fragments, $F(ab')_2$ fragments, Fd fragments, Fv fragments, diabodies, single-chain Fv (scFv) molecules, single-chain polypeptides containing only one light chain variable domain, single-chain polypeptides containing the three CDRs of the light-chain variable domain, single-chain polypeptides containing only one heavy chain variable region, and single-chain polypeptides containing the three CDRs of the heavy chain variable region.

[0188] "Anti-species antibodies" as used herein refers to an antibody, such as an IgG and/or IgM antibody, that recognize antibodies of another species of interest. For example, anti-human antibodies, e.g., anti-human IgG or IgM antibodies, recognize, respectively, other human IgG or IgM antibodies.

[0189] The "area under curve" or "AUC" refers to area under a ROC curve. AUC under a ROC curve is a measure of accuracy. An AUC of 1 represents a perfect test, whereas an AUC of 0.5 represents an insignificant test. A preferred AUC may be at least approximately 0.700, at least approximately 0.750, at least approximately 0.800, at least approximately 0.850, at least approximately 0.900, at least approximately 0.910, at least approximately 0.920, at least approximately 0.930, at least approximately 0.940, at least approximately 0.950, at least

approximately 0.960, at least approximately 0.970, at least approximately 0.980, at least approximately 0.990, or at least approximately 0.995.

[0190] "AU/mL" as used herein refers to antibody units per milliliter.

[0191] "BAU/mL" as used herein refers to the WHO International Standard of binding antibody units per milliliter. For the SARS-CoV-2 IgG antibody quantitative assay described and claimed herein, the measure AU/mL can be converted to BAU/mL using the following formula: BAU/mL=0.142 x AU/mL (e.g., about 4500 AU/mL is about 639 BAU/mL (4500 AU/mL x 0.142 = 639 BAU/mL)). Those skilled in the art are readily aware of how to harmonize or standardize results between different assays (e.g., by comparing each against a known standard) thus converting to common units. Those skilled in the art also are readily aware of how to harmonize or standardize results between different assays using different (e.g., alternate), or multiple know assay standards. Typically for assays there are multiple standards created, including iterations of standards, e.g., from WHO.

[0192] "Bead" and "particle" are used herein interchangeably and refer to a substantially spherical solid support. One example of a bead or particle is a microparticle. Microparticles that can be used herein can be any type known in the art. For example, the bead or particle can be a magnetic bead or magnetic particle. Magnetic beads/particles may be ferromagnetic, ferrimagnetic, paramagnetic, superparamagnetic or ferrofluidic. Exemplary ferromagnetic materials include Fe, Co, Ni, Gd, Dy, CrO_2, MnAs, MnBi, EuO, and NiO/Fe. Examples of ferrimagnetic materials include $NiFe_2O_4$, $CoFe_2O_4$, Fe_3O_4 (or $FeO{\cdot}Fe_2O_3$). Beads can have a solid core portion that is magnetic and is surrounded by one or more non-magnetic layers. Alternately, the magnetic portion can be a layer around a non-magnetic core. The microparticles can be of any size that would work in the methods described herein, e.g., from about 0.75 to about 5 nm, or from about 1 to about 5 nm, or from about 1 to about 3 nm.

[0193] "Binding protein" is used herein to refer to a monomeric or multimeric protein that binds to and forms a complex with a binding partner, such as, for example, a polypeptide, an antigen, a chemical compound or other molecule, or a substrate of any kind. A binding protein specifically binds a binding partner. Binding proteins include antibodies, as well as antigen-binding fragments thereof and other various forms and derivatives thereof as are known in the art and described herein below, and other molecules comprising one or more antigen-binding domains that bind to an antigen molecule or a particular site (epitope) on the antigen molecule.

Accordingly, a binding protein includes, but is not limited to, an antibody a tetrameric immunoglobulin, an IgG molecule, an IgG1 molecule, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, an affinity matured antibody, and fragments of any such antibodies that retain the ability to bind to an antigen.

[0194] "Bispecific antibody" is used herein to refer to a full-length antibody that is generated by quadroma technology (see Milstein et al., *Nature*, 305(5934): 537-540 (1983)), by chemical conjugation of two different monoclonal antibodies (see, Staerz et al., *Nature*, 314(6012): 628-631 (1985)), or by knob-into-hole or similar approaches, which introduce mutations in the Fc region (see Holliger et al., *Proc. Natl. Acad. Sci. USA*, 90(14): 6444-6448 (1993)), resulting in multiple different immunoglobulin species of which only one is the functional bispecific antibody. A bispecific antibody binds one antigen (or epitope) on one of its two binding arms (one pair of HC/LC), and binds a different antigen (or epitope) on its second arm (a different pair of HC/LC). By this definition, a bispecific antibody has two distinct antigen-binding arms (in both specificity and CDR sequences), and is monovalent for each antigen to which it binds to.

[0195] As used herein, the term "coronavirus" refers to viruses that belonging to the family *Coronaviridae* that have a positive-sense, RNA genome ranging from 26 to 32 kilobases in length. Coronaviruses having four main structural proteins: the spike glycoprotein (S protein), the membrane protein (M protein), the envelope protein (E protein) and the nucleocapsid protein (N protein). Coronavirus can be further subdivided into four groups, alpha, beta, gamma and delta coronaviruses. Examples of alpha coronaviruses include HCoV-229E and HCoV-NL63. Examples of beta coronaviruses are HCoV-OC43, HCoV-HKU1, Middle East Respiratory Syndrome (MERS-CoV), severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) and SARS-CoV-2 (also known as 2019-nCov, COVID-19, coronavirus disease, and Coronavirus Disease 2019).

[0196] In one aspect, the present disclosure relates to β-coronaviruses. In another aspect, the β-coronaviruses are MERS-CoV, SARS-CoV and SARS-CoV-2. In still yet another aspect, the β-coronaviruses are SARS-CoV and SARC-CoV-2. In still yet another aspect, the β-coronavirus is SARS-CoV-2. The sequence of SARS-CoV-2 has been described in a variety of publications, such as, for example, Lu et al., *Lancet*, 395:565-574 (February 2020) and https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/, the contents of each are herein incorporated by reference.

[0197] "CDR" is used herein to refer to the "complementarity determining region" within an antibody variable sequence. There are three CDRs in each of the variable regions of the heavy chain and the light chain. Proceeding from the N-terminus of a heavy or light chain, these regions are denoted "CDR1", "CDR2", and "CDR3", for each of the variable regions. The term "CDR set" as used herein refers to a group of three CDRs that occur in a single variable region that binds the antigen. An antigen-binding site, therefore, may include six CDRs, comprising the CDR set from each of a heavy and a light chain variable region. A polypeptide comprising a single CDR, (e.g., a CDR1, CDR2, or CDR3) may be referred to as a "molecular recognition unit." Crystallographic analyses of antigen-antibody complexes have demonstrated that the amino acid residues of CDRs form extensive contact with bound antigen, wherein the most extensive antigen contact is with the heavy chain CDR3. Thus, the molecular recognition units may be primarily responsible for the specificity of an antigen-binding site. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

[0198] The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as "Kabat CDRs". Chothia and coworkers (Chothia and Lesk, *J. Mol. Biol.*, 196: 901-917 (1987); and Chothia et al., *Nature*, 342: 877-883 (1989)) found that certain sub-portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as "L1", "L2", and "L3", or "H1", "H2", and "H3", where the "L" and the "H" designate the light chain and the heavy chain regions, respectively. These regions may be referred to as "Chothia CDRs", which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan, *FASEB J.*, 9: 133-139 (1995), and MacCallum, *J. Mol. Biol.*, 262(5): 732-745 (1996). Still other CDR boundary definitions may not strictly follow one of the herein systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs

defined according to any of these systems, although certain embodiments use Kabat- or Chothia-defined CDRs.

[0199] "Component," "components," or "at least one component," refer generally to a capture antibody, a detection or conjugate a calibrator, a control, a sensitivity panel, a container, a buffer, a diluent, a salt, an enzyme, a co-factor for an enzyme, a detection reagent, a pretreatment reagent/solution, a substrate (e.g., as a solution), a stop solution, and the like that can be included in a kit for assay of a test sample, such as a patient saliva, a nasal mucus specimen, oropharyngeal specimens, nasopharyngeal specimens, an anal swab specimen, urine, whole blood, serum or plasma sample (e.g., as per "Sample" below), in accordance with the methods described herein and other methods known in the art. Some components can be in solution or lyophilized for reconstitution for use in an assay

[0200] "Controls" as used herein generally refers to a reagent whose purpose is to evaluate the performance of a measurement system in order to assure that it continues to produce results within permissible boundaries (e.g., boundaries ranging from measures appropriate for a research use assay on one end to analytic boundaries established by quality specifications for a commercial assay on the other end). To accomplish this, a control should be indicative of patient results and optionally should somehow assess the impact of error on the measurement (e.g., error due to reagent stability, calibrator variability, instrument variability, and the like). As used herein, a "control subject" relates to a subject or subjects that has not been infected with a coronavirus, such as, a β-coronavirus or been exposed to any subject that has had a coronavirus, such as a β-coronavirus.

[0201] As used herein, the term "control zone" or "control line" is a region of a test strip in which a label can be observed to shift location, appear, change color, or disappear to indicate that an assay performed correctly. Detection or observation of the control zone (e.g., of a control line) may be done by any convenient means, depending upon the particular choice of label, especially, for example but not limited to, visually, fluorescently, by reflectance, radiographically, and the like. As will be described, the label may or may not be applied directly to the control zone, depending upon the design of the control being used.

[0202] "Cytokine storm" refers to a complex network of severe molecular events that are unified by a clinical phenotype of systemic inflammation, multi-organ failure and hyper-ferritinemia. A cytokine storm is induced by activation of large number of white blood cells

(including B cells, T cells, NK cells, macrophages, dendritic cells, neutrophils, monocytes) and resident tissue cells (such as epithelial and endothelial cells) which release high amounts of pro-inflammatory cytokines. A number of molecules increase in serum in a cytokine storm including complements, IL-10, IFN-γ, IL-1β, IL-6, IL-12, IL-17 and tumor-necrosis factor-α (TNF-α). In a study of 41 hospitalized SARS-CoV-2 patients, high-levels of proinflammatory cytokines were observed in severe cases, including IL-2, IL-7, IL-10, granulocyte-colony stimulating factor, IP-10, MCP-1, macrophase infallamtory protein 1 alpha and TNF-α (See, Akdis et al., "Immune Response to SARS-CoV-2 and Mechanisms of Immunopathological Changes in COVID-19", *Allergy*, 75(7):1564-1581 (July 2020)). Subjects that experience a cytokine storm are more likely to experience a severe and fatal disease outcome.

[0203] "Derivative" of an antibody as used herein may refer to an antibody having one or more modifications to its amino acid sequence when compared to a genuine or parent antibody and exhibit a modified domain structure. The derivative may still be able to adopt the typical domain configuration found in native antibodies, as well as an amino acid sequence, which is able to bind to targets (antigens) with specificity. Typical examples of antibody derivatives are antibodies coupled to other polypeptides, rearranged antibody domains, or fragments of antibodies. The derivative may also comprise at least one further compound, e.g., a protein domain, said protein domain being linked by covalent or non-covalent bonds. The linkage can be based on genetic fusion according to the methods known in the art. The additional domain present in the fusion protein comprising the antibody may preferably be linked by a flexible linker, advantageously a peptide linker, wherein said peptide linker comprises plural, hydrophilic, peptide-bonded amino acids of a length sufficient to span the distance between the C-terminal end of the further protein domain and the N-terminal end of the antibody or vice versa. The antibody may be linked to an effector molecule having a conformation suitable for biological activity or selective binding to a solid support, a biologically active substance (*e.g.*, a cytokine or growth hormone), a chemical agent, a peptide, a protein, or a drug, for example.

[0204] "Determined by an assay" is used herein to refer to the determination of a reference level by any appropriate assay. The determination of a reference level may, in some embodiments, be achieved by an assay of the same type as the assay that is to be applied to the sample from the subject (for example, by an immunoassay, clinical chemistry assay, a single molecule detection assay, protein immunoprecipitation, immunoelectrophoresis, a point-of-care

assay, chemical analysis, SDS-PAGE and Western blot analysis, or protein immunostaining, electrophoresis analysis, a protein assay, a competitive binding assay or a functional protein assay. The determination of a reference level may, in some embodiments, be achieved by an assay of the same type and under the same assay conditions as the assay that is to be applied to the sample from the subject. As noted herein, this disclosure provides exemplary reference levels (e.g., calculated by comparing reference levels at different time points). It is well within the ordinary skill of one in the art to adapt the disclosure herein for other assays to obtain assay-specific reference levels for those other assays based on the description provided by this disclosure. For example, a set of training samples comprising samples obtained from subjects known to have been infected by a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2), and samples obtained from human subjects known not to have been infected with a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2), or been exposed to a subject that has been infected with a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2), may be used to obtain assay-specific reference levels. It will be understood that a reference level "determined by an assay" and having a recited level of "sensitivity" and/or "specificity" is used herein to refer to a reference level which has been determined to provide a method of the recited sensitivity and/or specificity when said reference level is adopted in the methods of the disclosure. It is well within the ordinary skill of one in the art to determine the sensitivity and specificity associated with a given reference level in the methods of the disclosure, for example by repeated statistical analysis of assay data using a plurality of different possible reference levels.

[0205] Practically, when discriminating between a subject as having been infected by a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2), or not having been infected by a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2), the skilled person will balance the effect of raising a cutoff on sensitivity and specificity. Raising or lowering a cutoff will have a well-defined and predictable impact on sensitivity and specificity, and other standard statistical measures. It is well known that raising a cutoff will improve specificity but is likely to worsen sensitivity (proportion of those with disease who test positive). In contrast, lowering a cutoff will improve sensitivity but will worsen specificity (proportion of those without disease who test negative). The ramifications for detecting or measuring a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2), will be readily

apparent to those skilled in the art. In discriminating whether a subject has or has not been infected by a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2), the higher the cutoff, specificity improves as more true negatives (i.e., subjects not having been infected by a coronavirus, such as β-coronavirus (such as SARS-CoV or SARS-CoV-2)) are distinguished from those having been infected by a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2). But at the same time, raising the cutoff decreases the number of cases identified as positive overall, as well as the number of true positives, so the sensitivity must decrease. Conversely, the lower the cutoff, sensitivity improves as more true positives (i.e., subjects having been infected with a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2)) are distinguished from those who have not been infected (e.g., do not have) with a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2). But at the same time, lowering the cutoff increases the number of cases identified as positive overall, as well as the number of false positives, so the specificity must decrease.

[0206] Generally, a high sensitivity value helps one of skill rule out disease or condition (such as infection with a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2)), and a high specificity value helps one of skill rule in disease or condition. Whether one of skill desires to rule out or rule in disease depends on what the consequences are for the patient for each type of error. Accordingly, one cannot know or predict the precise balancing employed to derive a test cutoff without full disclosure of the underlying information on how the value was selected. The balancing of sensitivity against specificity and other factors will differ on a case-by-case basis. This is why it is sometimes preferable to provide alternate cutoff (e.g., reference) values so a physican or practitioner can choose.

[0207] "Dual-specific antibody" is used herein to refer to a full-length antibody that can bind two different antigens (or epitopes) in each of its two binding arms (a pair of HC/LC) (see PCT International Application WO 02/02773). Accordingly, a dual-specific binding protein has two identical antigen binding arms, with identical specificity and identical CDR sequences, and is bivalent for each antigen to which it binds.

[0208] "Dual variable domain" is used herein to refer to two or more antigen binding sites on a binding protein, which may be divalent (two antigen binding sites), tetravalent (four antigen binding sites), or multivalent binding proteins. DVDs may be monospecific, i.e., capable of binding one antigen (or one specific epitope), or multispecific, i.e., capable of binding two or

more antigens (i.e., two or more epitopes of the same target antigen molecule or two or more epitopes of different target antigens). A preferred DVD binding protein comprises two heavy chain DVD polypeptides and two light chain DVD polypeptides and is referred to as a "DVD immunoglobulin" or "DVD-Ig." Such a DVD-Ig binding protein is thus tetrameric and reminiscent of an IgG molecule, but provides more antigen binding sites than an IgG molecule. Thus, each half of a tetrameric DVD-Ig molecule is reminiscent of one half of an IgG molecule and comprises a heavy chain DVD polypeptide and a light chain DVD polypeptide, but unlike a pair of heavy and light chains of an IgG molecule that provides a single antigen binding domain, a pair of heavy and light chains of a DVD-Ig provide two or more antigen binding sites.

[0209] Each antigen binding site of a DVD-Ig binding protein may be derived from a donor ("parental") monoclonal antibody and thus comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) with a total of six CDRs involved in antigen binding per antigen binding site. Accordingly, a DVD-Ig binding protein that binds two different epitopes (*i.e.*, two different epitopes of two different antigen molecules or two different epitopes of the same antigen molecule) comprises an antigen binding site derived from a first parental monoclonal antibody and an antigen binding site of a second parental monoclonal antibody.

[0210] A description of the design, expression, and characterization of DVD-Ig binding molecules is provided in PCT International Application WO 2007/024715, U.S. Patent No. 7,612,181, and Wu et al., *Nature Biotech.*, 25: 1290-1297 (2007). An example of such DVD-Ig molecules comprises a heavy chain that comprises the structural formula VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, X2 is an Fc region, and n is 0 or 1, but preferably 1; and a light chain that comprises the structural formula VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region; and n is 0 or 1, but preferably 1. Such a DVD-Ig may comprise two such heavy chains and two such light chains, wherein each chain comprises variable domains linked in tandem without an intervening constant region between variable regions, wherein a heavy chain and a light chain associate to form tandem functional antigen binding sites, and a pair of heavy and light chains may associate with another pair of heavy and light chains to form a tetrameric binding protein with four functional

antigen binding sites. In another example, a DVD-Ig molecule may comprise heavy and light chains that each comprise three variable domains (VD1, VD2, VD3) linked in tandem without an intervening constant region between variable domains, wherein a pair of heavy and light chains may associate to form three antigen binding sites, and wherein a pair of heavy and light chains may associate with another pair of heavy and light chains to form a tetrameric binding protein with six antigen binding sites.

[0211] In another embodiment, a DVD-Ig binding protein not only binds the same target molecules bound by its parental monoclonal antibodies, but also possesses one or more desirable properties of one or more of its parental monoclonal antibodies. Such an additional property is an antibody parameter of one or more of the parental monoclonal antibodies. Antibody parameters that may be contributed to a DVD-Ig binding protein from one or more of its parental monoclonal antibodies include, but are not limited to, antigen specificity, antigen affinity, potency, biological function, epitope recognition, protein stability, protein solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.

[0212] A DVD-Ig binding protein binds at least one epitope of nucleocapsid protein, spike protein or nucleocapsid protein and spike protein from a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2). Non-limiting examples of a DVD-Ig binding protein include a DVD-Ig binding protein that binds one or more epitopes of a nucleocapsid protein, spike protein, or nucleocapsid protein and spike protein of a β-coronavirus (such as SARS-CoV or SARS-CoV-2), a DVD-Ig binding protein that binds an epitope of a human nucleocapsid protein, spike protein, or nucleocapsid protein and spike protein of a β-coronavirus (such as SARS-CoV or SARS-CoV-2) and an epitope of a nucleocapsid protein, spike protein, or nucleocapside protein and spike protein of a β-coronavirus (such as SARS-CoV or SARS-CoV-2) of another species (for example, mouse, rat, bat, etc.), and a DVD-Ig binding protein that binds an epitope of a human β-coronavirus (such as SARS-CoV or SARS-CoV-2) and an epitope of another target molecule.

[0213] "Dynamic range" as used herein refers to range over which an assay readout is proportional to the amount of target molecule or analyte in the sample being analyzed.

[0214] "Epitope," or "epitopes," or "epitopes of interest" refer to a site(s) on any molecule that is recognized and can bind to a complementary site(s) on its specific binding partner. The

molecule and specific binding partner are part of a specific binding pair. For example, an epitope can be on a polypeptide, a protein, a hapten, a carbohydrate antigen (such as, but not limited to, glycolipids, glycoproteins or lipopolysaccharides), or a polysaccharide. Its specific binding partner can be, but is not limited to, an antibody.

[0215] "Extended measuring interval" (EMI) as used herein refers to the interval in which concentrations are measured with appropriate accuracy by diluting the specimen before taking a measurement with a developed measurement process. A "Measuring Interval" refers to where a set of values of the same kind can be measured by a given measuring instrument or measuring system with specified instrumental uncertainty, under defined conditions. A Measuring Interval is bounded by an upper limit.

[0216] "Fragment antigen-binding fragment" or "Fab fragment" as used herein refers to a fragment of an antibody that binds to antigens and that contains one antigen-binding site, one complete light chain, and part of one heavy chain. Fab is a monovalent fragment consisting of the VL, VH, CL and CH1 domains. Fab is composed of one constant and one variable domain of each of the heavy and the light chain. The variable domain contains the paratope (the antigen-binding site), comprising a set of complementarity determining regions, at the amino terminal end of the monomer. Each arm of the Y thus binds an epitope on the antigen. Fab fragments can be generated such as has been described in the art, e.g., using the enzyme papain, which can be used to cleave an immunoglobulin monomer into two Fab fragments and an Fc fragment, or can be produced by recombinant means.

[0217] "$F(ab')_2$ fragment" as used herein refers to antibodies generated by pepsin digestion of whole IgG antibodies to remove most of the Fc region while leaving intact some of the hinge region. $F(ab')_2$ fragments have two antigen-binding F(ab) portions linked together by disulfide bonds, and therefore are divalent with a molecular weight of about 110 kDa. Divalent antibody fragments ($F(ab')_2$ fragments) are smaller than whole IgG molecules and enable a better penetration into tissue thus facilitating better antigen recognition in immunohistochemistry. The use of $F(ab')_2$ fragments also avoids unspecific binding to Fc receptor on live cells or to Protein A/G. $F(ab')_2$ fragments can both bind and precipitate antigens.

[0218] "Framework" (FR) or "Framework sequence" as used herein may mean the remaining sequences of a variable region minus the CDRs. Because the exact definition of a CDR sequence can be determined by different systems (for example, see above), the meaning of a

framework sequence is subject to correspondingly different interpretations. The six CDRs (CDR-L1, -L2, and -L3 of light chain and CDR-H1, -H2, and -H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3, and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3, or FR4, a framework region, as referred by others, represents the combined FRs within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, and FRs represents two or more of the four sub-regions constituting a framework region.

[0219] Human heavy chain and light chain FR sequences are known in the art that can be used as heavy chain and light chain "acceptor" framework sequences (or simply, "acceptor" sequences) to humanize a non-human antibody using techniques known in the art. In one embodiment, human heavy chain and light chain acceptor sequences are selected from the framework sequences listed in publicly available databases such as V-base (hypertext transfer protocol://vbase.mrc-cpe.cam.ac.uk/) or in the international ImMunoGeneTics® (IMGT®) information system (hypertext transfer protocol://imgt.cines.fr/texts/IMGTrepertoire/LocusGenes/).

[0220] "Functional antigen binding site" as used herein may mean a site on a binding protein (e.g., an antibody) that is capable of binding a target antigen. The antigen binding affinity of the antigen binding site may not be as strong as the parent binding protein, *e.g.*, parent antibody, from which the antigen binding site is derived, but the ability to bind antigen must be measurable using any one of a variety of methods known for evaluating protein, *e.g.*, antibody, binding to an antigen. Moreover, the antigen binding affinity of each of the antigen binding sites of a multivalent protein, e.g., multivalent antibody, herein need not be quantitatively the same.

[0221] The term "fusion protein" as used herein relates to a protein or polypeptide comprising at least one first protein or polyeptide joined or linked to at least one second protein or polypeptide. In some aspects, the at least one protein or polypeptide is joined or linked to at least one second protein or polypeptide through one or more linking peptide sequences. An example of a fusion protein is a chimeric protein. A fusion protein can be created using routine techniques known in the art such as recombinant DNA technology, through joining or linking of two or more genes that originally coded for separate proteins. Thus, a fusion protein may

comprise a multimer of different or identical binding proteins which are expressed as a single, linear polypeptide.

[0222] "Humanized antibody" is used herein to describe an antibody that comprises heavy and light chain variable region sequences from a non-human species (e.g., a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like," i.e., more similar to human germline variable sequences. A "humanized antibody" is an antibody or a variant, derivative, analog, or fragment thereof, which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (*i.e.*, donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. In an embodiment, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In some embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain.

[0223] A humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA, and IgE, and any isotype, including without limitation IgG1, IgG2, IgG3, and IgG4. A humanized antibody may comprise sequences from more than one class or isotype, and particular constant domains may be selected to optimize desired effector functions using techniques well-known in the art.

[0224] The framework regions and CDRs of a humanized antibody need not correspond precisely to the parental sequences, e.g., the donor antibody CDR or the consensus framework

may be mutagenized by substitution, insertion, and/or deletion of at least one amino acid residue so that the CDR or framework residue at that site does not correspond to either the donor antibody or the consensus framework. In a preferred embodiment, such mutations, however, will not be extensive. Usually, at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences. As used herein, the term "consensus framework" refers to the framework region in the consensus immunoglobulin sequence. As used herein, the term "consensus immunoglobulin sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related immunoglobulin sequences (see, *e.g.*, Winnaker, *From Genes to Clones* (Verlagsgesellschaft, Weinheim, 1987)). A "consensus immunoglobulin sequence" may thus comprise a "consensus framework region(s)" and/or a "consensus CDR(s)". In a family of immunoglobulins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence.

[0225] "Identical" or "identity," as used herein in the context of two or more polypeptide or polynucleotide sequences, can mean that the sequences have a specified percentage of residues that are the same over a specified region. The percentage can be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of the single sequence are included in the denominator but not the numerator of the calculation.

[0226] "Isolated polynucleotide" as used herein may mean a polynucleotide (*e.g.*, of genomic, cDNA, or synthetic origin, or a combination thereof) that, by virtue of its origin, the isolated polynucleotide is not associated with all or a portion of a polynucleotide with which the "isolated polynucleotide" is found in nature; is operably linked to a polynucleotide that it is not linked to in nature; or does not occur in nature as part of a larger sequence. As used herein, "isolated polypeptide" refers to a polypeptide (e.g., of recombinant, synthetic or chemical original or a

combination thereof), that, by virtue of its origin, the isolated polypeptide is not associated with all or a portion of a polypeptide and/or other protein(s) with which the "isolated polypeptide" is found in nature; is operably linked to a polypeptide and/or protein that it is not linked to in nature; or does not occur in nature as part of a larger sequence. When associated with a particular species, virus or strain (e.g., "β-coronavirus isolated polypeptide"), the isolated polypeptide optionally can be made by recombinant means rather than by isolation from in vivo.

[0227] "Label" and "detectable label" as used herein refer to a moiety attached to an antibody or an analyte to render the reaction between the antibody and the analyte detectable, and the antibody or analyte so labeled is referred to as "detectably labeled." A label can produce a signal that is detectable by visual or instrumental means. Various labels include signal-producing substances, such as chromagens, fluorescent compounds, chemiluminescent compounds, radioactive compounds, and the like. Representative examples of labels include moieties that produce light, e.g., acridinium compounds, and moieties that produce fluorescence, e.g., fluorescein. Other labels are described herein. In this regard, the moiety, itself, may not be detectable but may become detectable upon reaction with yet another moiety. Use of the term "detectably labeled" is intended to encompass such labeling.

[0228] Any suitable detectable label as is known in the art can be used. For example, the detectable label can be a radioactive label (such as 3H, 14C, 32P, 33P, 35S, 90Y, 99Tc, 111In, 125I, 131I, 177Lu, 166Ho, and 153Sm), an enzymatic label (such as horseradish peroxidase, alkaline peroxidase, glucose 6-phosphate dehydrogenase, and the like), a chemiluminescent label (such as acridinium esters, thioesters, or sulfonamides; luminol, isoluminol, phenanthridinium esters, and the like), a fluorescent label (such as fluorescein (e.g., 5-fluorescein, 6-carboxyfluorescein, 3'6-carboxyfluorescein, 5(6)-carboxyfluorescein, 6-hexachloro-fluorescein, 6-tetrachlorofluorescein, fluorescein isothiocyanate, and the like)), rhodamine, phycobiliproteins, R-phycoerythrin, quantum dots (e.g., zinc sulfide-capped cadmium selenide), a thermometric label, or an immuno-polymerase chain reaction label. An introduction to labels, labeling procedures and detection of labels is found in Polak and Van Noorden, *Introduction to Immunocytochemistry*, 2nd ed., Springer Verlag, N.Y. (1997), and in Haugland, *Handbook of Fluorescent Probes and Research Chemicals* (1996), which is a combined handbook and catalogue published by Molecular Probes, Inc., Eugene, Oregon. A fluorescent label can be used in FPIA (see, e.g., U.S. Patent Nos. 5,593,896, 5,573,904, 5,496,925, 5,359,093, and 5,352,803,

which are hereby incorporated by reference in their entireties). An acridinium compound can be used as a detectable label in a homogeneous chemiluminescent assay (see, e.g., Adamczyk *et al.*, Bioorg. *Med. Chem. Lett.* 16: 1324-1328 (2006); Adamczyk et al., *Bioorg. Med. Chem. Lett.* 4: 2313-2317 (2004); Adamczyk et al., *Biorg. Med. Chem. Lett.* 14: 3917-3921 (2004); and Adamczyk et al., *Org. Lett.* 5: 3779-3782 (2003)).

[0229] In one aspect, the acridinium compound is an acridinium-9-carboxamide. Methods for preparing acridinium 9-carboxamides are described in Mattingly, *J. Biolumin. Chemilumin.* 6: 107-114 (1991); Adamczyk et al., *J. Org. Chem.* 63: 5636-5639 (1998); Adamczyk et al., *Tetrahedron* 55: 10899-10914 (1999); Adamczyk et al., *Org. Lett.* 1: 779-781 (1999); Adamczyk et al., *Bioconjugate Chem.* 11: 714-724 (2000); Mattingly et al., *In Luminescence Biotechnology: Instruments and Applications*; Dyke, K. V. Ed.; CRC Press: Boca Raton, pp. 77–105 (2002); Adamczyk et al., *Org. Lett.* 5: 3779-3782 (2003); and U.S. Patent Nos. 5,468,646, 5,543,524 and 5,783,699 (each of which is incorporated herein by reference in its entirety for its teachings regarding same).

[0230] Another example of an acridinium compound is an acridinium-9-carboxylate aryl ester. An example of an acridinium-9-carboxylate aryl ester of formula II is 10-methyl-9-(phenoxycarbonyl)acridinium fluorosulfonate (available from Cayman Chemical, Ann Arbor, MI). Methods for preparing acridinium 9-carboxylate aryl esters are described in McCapra et al., *Photochem. Photobiol.* 4: 1111-21 (1965); Razavi et al., *Luminescence* 15: 245-249 (2000); Razavi et al., *Luminescence* 15: 239-244 (2000); and U.S. Patent No. 5,241,070 (each of which is incorporated herein by reference in its entirety for its teachings regarding same). Such acridinium-9-carboxylate aryl esters are efficient chemiluminescent indicators for hydrogen peroxide produced in the oxidation of an analyte by at least one oxidase in terms of the intensity of the signal and/or the rapidity of the signal. The course of the chemiluminescent emission for the acridinium-9-carboxylate aryl ester is completed rapidly, i.e., in under 1 second, while the acridinium-9-carboxamide chemiluminescent emission extends over 2 seconds. Acridinium-9-carboxylate aryl ester, however, loses its chemiluminescent properties in the presence of protein. Therefore, its use requires the absence of protein during signal generation and detection. Methods for separating or removing proteins in the sample are well-known to those skilled in the art and include, but are not limited to, ultrafiltration, extraction, precipitation, dialysis, chromatography, and/or digestion (see, e.g., Wells, *High Throughput Bioanalytical Sample*

Preparation. Methods and Automation Strategies, Elsevier (2003)). The amount of protein removed or separated from the test sample can be about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%. Further details regarding acridinium-9-carboxylate aryl ester and its use are set forth in U.S. Patent No. 7,906,293. Acridinium-9-carboxylate aryl esters can be dissolved in any suitable solvent, such as degassed anhydrous N,N-dimethylformamide (DMF) or aqueous sodium cholate.

[0231] "Linking sequence" or "linking peptide sequence" refers to a natural or artificial polypeptide sequence that is connected to one or more polypeptide sequences of interest (*e.g.*, full-length, fragments, etc.). The term "connected" refers to the joining of the linking sequence to the polypeptide sequence of interest. Such polypeptide sequences are preferably joined by one or more peptide bonds. Linking sequences can have a length of from about 4 to about 50 amino acids. Preferably, the length of the linking sequence is from about 6 to about 30 amino acids. Natural linking sequences can be modified by amino acid substitutions, additions, or deletions to create artificial linking sequences. Linking sequences can be used for many purposes, including in recombinant Fabs. Exemplary linking sequences include, but are not limited to: (i) Histidine (His) tags, such as a 6X His tag, which has an amino acid sequence of HHHHHH (SEQ ID NO: 3), are useful as linking sequences to facilitate the isolation and purification of polypeptides and antibodies of interest; or (ii) Enterokinase cleavage sites, like His tags, are used in the isolation and purification of proteins and antibodies of interest. Often, enterokinase cleavage sites are used together with His tags in the isolation and purification of proteins and antibodies of interest. Various enterokinase cleavage sites are known in the art. Examples of enterokinase cleavage sites include, but are not limited to, the amino acid sequence of DDDDK (SEQ ID NO: 4) and derivatives thereof (e.g., ADDDDK (SEQ ID NO: 5), etc.). Additionally, miscellaneous sequences can be used to link or connect the light and/or heavy chain variable regions of single chain variable region fragments. Examples of other linking sequences can be found in Bird et al., *Science* 242: 423-426 (1988); Huston et al., *PNAS USA* 85: 5879-5883 (1988); and McCafferty et al., *Nature* 348: 552-554 (1990). Linking sequences also can be modified for additional functions, such as attachment of drugs or attachment to solid supports. In the context of the present disclosure, the monoclonal antibody, for example, can contain a linking sequence, such as a His tag, an enterokinase cleavage site, or both.

[0232] "Monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological.

[0233] "Multivalent binding protein" is used herein to refer to a binding protein comprising two or more antigen binding sites (also referred to herein as "antigen binding domains"). A multivalent binding protein is preferably engineered to have three or more antigen binding sites, and is generally not a naturally occurring antibody. The term "multispecific binding protein" refers to a binding protein that can bind two or more related or unrelated targets, including a binding protein capable of binding two or more different epitopes of the same target molecule.

[0234] "Negative predictive value" or "NPV" as used interchangeably herein refers to the probability that a subject has a negative outcome (i.e., the proposed result is absent) given that they have a negative test result (i.e., the subject that tested negative for the proposed result does not have the proposed result).

[0235] "Nucleocapsid protein" or "N" protein as used interchangeably herein, refers to one of four main structural proteins of a coronavirus. The N protein is the only protein present in the nucleocapsid. It is composed of two separate domains, an N-terminal domain (NTD) and a C-terminal domain (CTD), both capable of binding RNA *in vitro* using different mechanisms, which may suggest that optimal RNA binding requires contributions from both domains. For example, in SARS-CoV-2, the NTD can be found at amino acids 1 to 209 of SEQ ID NO.2. In SARS-CoV, the NTD can be found at amino acids 1 to 210 of SEQ ID NO:14. For example, in

SARS-CoV-2, the CTD can be found at amino acids 210 to 419 of SEQ ID NO.2. In SARS-CoV, the CTD can be found at amino acids 211 to 422 of SEQ ID NO:14.

[0236] In some aspects described herein, a nucleocapsid protein is at least a portion (e.g., at least 5 amino acids or more) or the entirety of a nucleocapsid protein from a SARS-CoV-2 strain of β-coronavirus comprising the sequence of SEQ ID NO:1, and is referred to here as a "CTD peptide", "Nc-CTD peptide", or "Nc-Cbt peptide".

[0237] In some aspects described herein, at least a portion (e.g., at least 5 amino acids or more) of a NTD or CTD of a nucleocapsid protein from one strain of β-coronavirus (such as SARS-CoV or SARS-CoV-2) can be operably linked, fused or grafted directly or indirectly (such as through one or more linking peptide sequences and/or HIS tags) to all or at least a portion (at least 5 amino acids or more) of a NTD or CTD from the same or different strain of β-coronavirus (such as SARS-CoV or SARS-CoV-2) resulting in a fusion protein, which is an example of a type of epitope-grafted fusion protein or peptide. For example, in some aspects described herein, at least a portion (e.g., at least 5 amino acids or more) of a NTD of a nucleocapsid protein from one strain of β-coronavirus (such as SARS-CoV or SARS-CoV-2) can be operably linked, fused or grafted directly or indirectly (such as through one or more linking peptide sequences and/or HIS tags) to all or at least a portion (at least 5 amino acids or more) of a NTD from the same or different strain of β-coronavirus (such as SARS-CoV or SARS-CoV-2) resulting in a fusion protein which is referred to herein as a "Nc-NTD/NTD" fusion protein or peptide (e.g., "Nc" referring to nucleocapsid). In some aspects described herein, at least a portion (e.g., at least 5 amino acids or more) of a CTD of a nucleocapsid protein from one strain of β-coronavirus (such as SARS-CoV or SARS-CoV-2) can be operably linked, fused or grafted directly or indirectly (such as through one or more linking peptide sequences and/or HIS tags) to all or at least a portion (at least 5 amino acids or more) of a CTD from the same or different strain of β-coronavirus (such as SARS-CoV or SARS-CoV-2) resulting in a fusion protein which is referred to herein as a "Nc-CTD/CTD" fusion protein or peptide. In still other aspects described herein, at least a portion (e.g., at least 5 amino acids or more) of a NTD of a nucleocapsid protein from one strain of β-coronavirus (such as SARS-CoV or SARS-CoV-2) can be operably linked, fused or grafted directly or indirectly (such as through one or more linking peptide sequences and/or HIS tags) to all or at least a portion (at least 5 amino acids or more) of a CTD from the same or different strain of β-coronavirus (such as SARS-CoV or SARS-CoV-2) resulting in a fusion

protein which is referred to herein as a "eNc-CTD" fusion protein or peptide, "eNc-Cbt" fusion protein or peptide," or "epitope-grafted CTD" fusion protein or peptide, as used interchangeably herewith.

[0238] "Point-of-care device" refers to a device used to provide medical diagnostic testing at or near the point-of-care (namely, outside of a laboratory), at the time and place of patient care (such as in a hospital, physician's office, urgent or other medical care facility, a patient's home, a nursing home and/or a long-term care and/or hospice facility). Examples of point-of-care devices include those produced by Abbott Laboratories (Abbott Park, IL) (*e.g.*, i-STAT and i-STAT Alinity, Universal Biosensors (Rowville, Australia) (see US 2006/0134713), Axis-Shield PoC AS (Oslo, Norway) and Clinical Lab Products (Los Angeles, USA).

[0239] "Positive predictive value" or "PPV" as used interchangeably herein refers to the probability that a subject has a positive outcome (i.e., the proposed result is present) given that they have a positive test result (i.e., the subject that tested positive for the proposed result has the proposed result).

[0240] "Quality control reagents" in the context of immunoassays and kits described herein, include, but are not limited to, calibrators, controls, and sensitivity panels. A "calibrator" or "standard" typically is used (e.g., one or more, such as a plurality) in order to establish calibration (standard) curves for interpolation of the concentration of an analyte, such as an antibody or an analyte. Alternatively, a single calibrator, which is near a reference level or control level (e.g., "low", "medium", or "high" levels), can be used. Multiple calibrators (i.e., more than one calibrator or a varying amount of calibrator(s)) can be used in conjunction to comprise a "sensitivity panel."

[0241] A "receiver operating characteristic" curve or "ROC" curve refers to a graphical plot that illustrates the performance of a binary classifier system as its discrimination threshold is varied. For example, a ROC curve can be a plot of the true positive rate against the false positive rate for the different possible cutoff points of a diagnostic test. It is created by plotting the fraction of true positives out of the positives (TPR = true positive rate) versus. the fraction of false positives out of the negatives (FPR = false positive rate), at various threshold settings. TPR is also known as sensitivity, and FPR is one minus the specificity or true negative rate. The ROC curve demonstrates the tradeoff between sensitivity and specificity (any increase in sensitivity will be accompanied by a decrease in specificity); the closer the curve follows the left-hand

border and then the top border of the ROC space, the more accurate the test; the closer the curve comes to the 45-degree diagonal of the ROC space, the less accurate the test; the slope of the tangent line at a cutoff point gives the likelihood ratio (LR) for that value of the test; and the area under the curve is a measure of test accuracy.

[0242] "Recombinant antibody" and "recombinant antibodies" refer to antibodies prepared by one or more steps, including cloning nucleic acid sequences encoding all or a part of one or more monoclonal antibodies into an appropriate expression vector by recombinant techniques and subsequently expressing the antibody in an appropriate host cell. The terms include, but are not limited to, recombinantly produced monoclonal antibodies, chimeric antibodies, humanized antibodies (fully or partially humanized), multi-specific or multi-valent structures formed from antibody fragments, bifunctional antibodies, heteroconjugate Abs, DVD-Ig®s, and other antibodies as described herein (Dual-variable domain immunoglobulins and methods for making them are described in Wu, C., *et al.*, *Nature Biotechnology*, 25:1290-1297 (2007)). The term "bifunctional antibody," as used herein, refers to an antibody that comprises a first arm having a specificity for one antigenic site and a second arm having a specificity for a different antigenic site, i.e., the bifunctional antibodies have a dual specificity.

[0243] "Reference level" as used herein refers to an assay cutoff value (or level) that is used to assess diagnostic, prognostic, or therapeutic efficacy and that has been linked or is associated herein with various clinical parameters (e.g., presence of disease, stage of disease, severity of disease, progression, non-progression, or improvement of disease, etc.). As used herein, the term "cutoff" refers to a limit (e.g., such as a number) above which there is a certain or specific clinical outcome and below which there is a different certain or specific clinical outcome.

[0244] This disclosure provides exemplary reference levels. However, it is well-known that reference levels may vary depending on the nature of the immunoassay (e.g., capture and detection reagents employed, reaction conditions, sample purity, etc.) and that assays can be compared and standardized. It further is well within the ordinary skill of one in the art to adapt the disclosure herein for other immunoassays to obtain immunoassay-specific reference levels for those other immunoassays based on the description provided by this disclosure. Whereas the precise value of the reference level may vary between assays, the findings as described herein should be generally applicable and capable of being extrapolated to other assays.

[0245] "Sample," "test sample," "specimen," "sample from a subject," "biological sample," and "patient sample" as used interchangeably herein may be a sample of blood, such as whole blood (including for example, capillary blood, venous blood, dried blood spot, etc.), tissue, urine, serum, plasma, amniotic fluid, an anal sample (such as an anal swab specimen), lower respiratory specimens such as, but not limited to, sputum, endotracheal aspirate or bronchoalveolar lavage, nasal mucus, cerebrospinal fluid, placental cells or tissue, endothelial cells, leukocytes, or monocytes. The sample can be used directly as obtained from a patient or can be pre-treated, such as by filtration, distillation, extraction, concentration, centrifugation, inactivation of interfering components, addition of reagents, and the like, to modify the character of the sample in some manner as discussed herein or otherwise as is known in the art. Additionally, the sample can be a nasopharyngeal or oropharyngeal sample obtained using one or more swabs that, once obtained, is placed in a sterile tube containing a virus transport media (VTM) or universal transport media (UTM), for testing.

[0246] A variety of cell types, tissue, or bodily fluid may be utilized to obtain a sample. Such cell types, tissues, and fluid may include sections of tissues such as biopsy and autopsy samples, oropharyngeal specimens, nasopharyngeal specimens, nasal mucus specimens, frozen sections taken for histologic purposes, blood (such as whole blood, dried blood spots, etc.), plasma, serum, red blood cells, platelets, an anal sample (such as an anal swab specimen), interstitial fluid, cerebralspinal fluid, etc. Cell types and tissues may also include lymph fluid, cerebrospinal fluid, or any fluid collected by aspiration. A tissue or cell type may be provided by removing a sample of cells from a human and a non-human animal, but can also be accomplished by using previously isolated cells (e.g., isolated by another person, at another time, and/or for another purpose). Archival tissues, such as those having treatment or outcome history, may also be used. Protein or nucleotide isolation and/or purification may not be necessary. In some embodiments, the sample is a whole blood sample. In some embodiments, the sample is a capillary blood sample. In some embodiments, the sample is a dried blood spot. In some embodiments, the sample is a serum sample. In yet other embodiments, the sample is a plasma sample. In some embodiments, the sample is an oropharyngeal specimen. In other embodiments, the sample is a nasopharyngeal specimen. In other embodiments, the sample is sputum. In other embodiments, the sample is endotracheal aspirate. In still yet other

embodiments, the sample is bronchoalveolar lavage. In still yet other aspects, the sample is nasal mucus. In still yet further aspects, the sample is an anal swab specimen.

[0247] "Sensitivity" of an assay as used herein refers to the proportion of subjects for whom the outcome is positive that are correctly identified as positive (e.g., correctly identifing those subjects with a disease or medical condition for which they are being tested). For example, this might include correctly identifying subjects as having been infected with a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2), from those who do not have not been infected with a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2).

[0248] "Signal-to-calibrator" or "S/C" ratio as used herein, refers to the ratio of detectable signal (e.g., such as in reference light units) to a signal for a calibrator for at least one biological sample obtained for at least one subject pursuant to the methods of the present disclosure. When the signal-to-calibrator value is greater than a reference level or cutoff, then the subject is reactive (e.g., positive) for the analyte (e.g., anti-β-coronavirus antibody (e.g., SARS-CoV or SARS-CoV-2)) detected. For example, in the methods described herein, if the reference level or cutoff for the signal-to-calibrator ratio is 1.0, any subject tested having a signal-to-calibrator ratio greater than or equal to 1.0 will be considered reactive, namely, positive for at least one anti-β-coronavirus antibody (e.g., SARS-CoV or SARS-CoV-2). However, if the subject tested has a signal-to-calibrator ratio less than the reference level or cutoff of 1.0, then the subject will be considered not to be reactive, namely, negative for at least anti-β-coronavirus antibody (e.g., SARS-CoV or SARS-CoV-2).

[0249] "Specimen-to-calibrator" or "S/CO" ratio as used herein, refers to the ratio of detectable signal (e.g., such as in reference light units (RLU)) of a specimen/sample to detectable signal for a calibrator in at least one biological sample obtained from at least one subject pursuant to the methods of the present disclosure. A S/CO ratio is determined when a calibrator is not directly associated with a cutoff or reference level, and thus requires the use of an equation. Example equations that can be used to calculate the S/CO are: 1. Detectable signal of the specimen/sample (e.g., RLU's)/(detectable signal of the calibrator (e.g., RLUs)/X), where X is a correction factor (e.g., multiplier) of the calibrator to a cutoff or reference level; or 2. Detectable signal of the specimen/sample (e.g., RLU's) multiplied by (X/detectable signal of the calibrator (e.g., RLUs)). For example, if the correction factor is 4 times a cutoff due to the precision of assay being higher at 4 times at the cutoff, X would be 4. If the calibrator RLU's are

24,000 RLU's and the specimen/sample RLU's are 12,000, the S/CO would be calculated: (1) Equation 1: 12,000 RLU's/(24,000 RLU's/4)=2.0; or (2) Equation 2: 12,000 RLU's x (4/24,000 RLU's) = 2.0. Thus, in this example, the S/CO ratio would be 2.0.

[0250] "Specificity" of an assay as used herein refers to the proportion of subjects for whom the outcome is negative that are correctly identified as negative (e.g., correctly identifying those subjects who do not have a disease or medical condition for which they are being tested). For example, this might include correctly identifying subjects having being infected with a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2), from those who have not been infected with a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2).

[0251] "Series of calibrating compositions" refers to a plurality of compositions comprising a known concentration of the analytes, such as one or more antibodies (such as anti-β-coronavirus antibodies (such as anti-SARS-CoV (IgG or IgM) antibodies or anti-SARS-CoV-2 (IgG or IgM) antibodies)), or polypeptides (such as one or more peptides derived from a β-coronavirus, such as SARS-CoV or SARS-CoV-2) wherein each of the compositions differs from the other compositions in the series by the concentration of the analytes, such as anti-β-coronavirus antibodies (such as anti-SARS-CoV (IgG or IgM) antibodies or anti-SARS-CoV-2 (IgG or IgM) antibodies).

[0252] As used herein the term "single molecule detection" refers to the detection and/or measurement of a single molecule of an analyte in a test sample at very low levels of concentration (such as pg/mL or femtogram/mL levels). A number of different single molecule analyzers or devices are known in the art and include nanopore and nanowell devices. Examples of nanopore devices are described in PCT International Application WO 2016/161402, which is hereby incorporated by reference in its entirety. Examples of nanowell device are described in PCT International Application WO 2016/161400, which is hereby incorporated by reference in its entirety.

[0253] "Solid phase" or "solid support" as used interchangeably herein, refers to any material that can be used to attach and/or attract and immobilize (1) one or more capture agents or capture specific binding partners, or (2) one or more detection agents or detection specific binding partners. The solid phase can be chosen for its intrinsic ability to attract and immobilize a capture agent. Alternatively, the solid phase can have affixed thereto a linking agent that has the

ability to attract and immobilize the (1) capture agent or capture specific binding partner, or (2) detection agent or detection specific binding partner. For example, the linking agent can include a charged substance that is oppositely charged with respect to the capture agent (e.g., capture specific binding partner) or detection agent (e.g., detection specific binding partner) itself or to a charged substance conjugated to the (1) capture agent or capture specific binding partner, or (2) detection agent or detection specific binding partner. In general, the linking agent can be any binding partner (preferably specific) that is immobilized on (attached to) the solid phase and that has the ability to immobilize the (1) capture agent or capture specific binding partner, or (2) detection agent or detection specific binding partner through a binding reaction. The linking agent enables the indirect binding of the capture agent to a solid phase material before the performance of the assay or during the performance of the assay. For examples, the solid phase can be plastic, derivatized plastic, magnetic, or non-magnetic metal, glass or silicon, including, for example, a test tube, microtiter well, sheet, bead, microparticle, chip, and other configurations known to those of ordinary skill in the art.

[0254] "Specific binding" or "specifically binding" as used herein may refer to the interaction of an antibody, a protein, or a peptide with a second chemical species, wherein the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

[0255] "Specific binding partner" or "Specific binding member", as used interchangeable herein, is a member of a specific binding pair. A specific binding pair comprises two different molecules, which specifically bind to each other through chemical or physical means. Therefore, in addition to antigen and antibody specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin (or streptavidin), carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzymes and enzyme inhibitors, and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, and

antibodies, including monoclonal and polyclonal antibodies as well as complexes and fragments thereof, whether isolated or recombinantly produced.

[0256] "Spike protein" or "S" protein as used interchangeably herein refers to one of four main structural proteins of a coronavirus. The spike protein is heavily N-linked glycosylated and utilizes an N-terminal signal sequence to gain access to the endoplasmic reticulum (ER). Homotrimers of the virus-encoding S protein make up the distinctive spike structure on the surface of the virus. In many coronaviruses, the S protein is cleaved by a host cell furin-like protease into two separate polypeptides noted S1 and S2. S1 makes up the large receptor-binding domain (RBD) of the S protein while S2 forms the stalk of the spike molecule. In SARS-CoV-2, the RBD can be found at amino acids 319 to 542 of SEQ ID NO:15. For example, in SARS-CoV, the RBD can be found at amino acids 306 to 528 of SEQ ID NO:16 (Yuan et al., *Science*, published on-line on April 3, 2020 (10.1126/science.abb7269) refers to residue "529", however, based on the true counting of the last residues of the sequence this appears to be an error). The trimeric S glycoprotein mediates attachment of the coronavirus virion to the host cell by interactions between the S protein and its receptor. In humans, angiotensin-converting enzyme 2 (ACE2) is the receptor for SARS-CoV and SARS-CoV-2.

[0257] In some aspects described herein, at least portion (e.g., at least 5 amino acids or more) of (a) a S1 polypeptide, a S2 polypeptide, and/or a RBD of a spike protein from one strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2); or (b) an epitope from monoclonal antibody CR3022 (described in U.S. Patent No. 8,106,170, ter Meulen, et al., *PLOS Medicine*, 3(7):1071-1079 (July 2006) and Yuan et a., *Science*, published on-line on April 3, 2020 (10.1126/science.abb7269) the contents of which are herein incorporated by reference) is operably linked, fused or grafted directly or indirectly (such as through one or more linking peptide sequences and/or HIS tags) to all or at least a portion (at least 5 amino acids or more) of a S1 polypeptide, a S2 polypeptide, and/or a RBD from the same or different strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) resulting in a fusion protein which is referred to herein as an "epitope-grafted spike" fusion protein or peptide. In some aspects, at least portion (e.g., at least 5 amino acids or more) of an epitope from monoclonal antibody CR3022 (described in U.S. Patent No. 8,106,170, ter Meulen, et al., *PLOS Medicine*, 3(7):1071-1079 (July 2006) and Yuan et al., *Science*, published on-line on April 3, 2020 (10.1126/science.abb7269) the contents of which are herein incorporated by reference) is operably linked, fused or grafted directly or

indirectly (such as through one or more linking peptide sequences and/or HIS tags) to all or at least a portion (at least 5 amino acids or more) of a RBD of a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) resulting in a fusion protein which is referred to herein as an "epitope-grafted RBD" fusion protein or peptide.

[0258] "Statistically significant" as used herein refers to the likelihood that a relationship between two or more variables is caused by something other than random chance. Statistical hypothesis testing is used to determine whether the result of a data set is statistically significant. In statistical hypothesis testing, a statistical significant result is attained whenever the observed *p*-value of a test statistic is less than the significance level defined of the study. The *p*-value is the probability of obtaining results at least as extreme as those observed, given that the null hypothesis is true. Examples of statistical hypothesis analysis include Wilcoxon signed-rank test, t-test, Chi-Square or Fisher's exact test. "Significant" as used herein refers to a change that has not been determined to be statistically significant (e.g., it may not have been subject to statistical hypothesis testing).

[0259] "Subject" and "patient" as used herein interchangeably refers to any vertebrate, including, but not limited to, a mammal (e.g., a bear, cow, cattle, pig, camel, llama, horse, goat, rabbit, sheep, hamster, guinea pig, cat, tiger, lion, cheetah, jaguar, bobcat, mountain lion, dog, wolf, coyote, rat, mouse, and a non-human primate (for example, a monkey, such as a cynomolgous or rhesus monkey, chimpanzee, etc.) and a human). In some embodiments, the subject may be a human, a non-human primate or a cat. In some embodiments, the subject is a human. The subject or patient may be undergoing other forms of treatment. In some embodiments, the subject is a human that may be undergoing other forms of treatment. In some embodiments, the subject is suspected to have, have had or has been exposed to a subject that has had or tested positive for infection with a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2). In other embodiments, the subject is completely asymptomatic and does not exhibit any symptoms of a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2), and may or may not have been exposed to a subject that has or has been exposed or infected with a coronavirus, such as a β-coronavirus (such as SARS-CoV or SARS-CoV-2).

[0260] As used herein, a "system" refers to a plurality of real and/or abstract elements operating together for a common purpose. In some embodiments, a "system" is an integrated assemblage of hardware and/or software elements. In some embodiments, each component of the

system interacts with one or more other elements and/or is related to one or more other elements. In some embodiments, a system refers to a combination of elements and software for controlling and directing methods.

[0261] As used herein, the term "test strip" can include one or more bibulous or non-bibulous materials. If a test strip comprises more than one material, the one or more materials are preferably in fluid communication. One material of a test strip may be overlaid on another material of the test strip, such as for example, filter paper overlaid on nitrocellulose. Alternatively or in addition, a test strip may include a region comprising one or more materials followed by a region comprising one or more different materials. In this case, the regions are in fluid communication and may or may not partially overlap one another. Suitable materials for test strips include, but are not limited to, materials derived from cellulose, such as filter paper, chromatographic paper, nitrocellulose, and cellulose acetate, as well as materials made of glass fibers, nylon, dacron, PVC, polyacrylamide, cross-linked dextran, agarose, polyacrylate, ceramic materials, and the like. The material or materials of the test strip may optionally be treated to modify their capillary flow characteristics or the characteristics of the applied sample. For example, the sample application region of the test strip may be treated with buffers to correct the pH, salt concentration, or specific gravity of an applied sample to optimize test conditions.

[0262] The material or materials can be a single structure such as a sheet cut into strips or it can be several strips or particulate material bound to a support or solid surface such as found, for example, in thin-layer chromatography and may have an absorbent pad either as an integral part or in liquid contact. The material can also be a sheet having lanes thereon, capable of spotting to induce lane formation, wherein a separate assay can be conducted in each lane. The material can have a rectangular, circular, oval, triangular, or other shape provided that there is at least one direction of traversal of a test solution by capillary migration. Other directions of traversal may occur such as in an oval or circular piece contacted in the center with the test solution. However, the main consideration is that there be at least one direction of flow to a predetermined site.

[0263] The support for the test strip, where a support is desired or necessary, will normally be water insoluble, frequently non-porous and rigid but may be elastic, usually hydrophobic, and porous and usually will be of the same length and width as the strip but may be larger or smaller. The support material can be transparent, and, when a test device of the present technology is assembled, a transparent support material can be on the side of the test strip that can be viewed

by the user, such that the transparent support material forms a protective layer over the test strip where it may be exposed to the external environment, such as by an aperture in the front of a test device. A wide variety of non-mobilizable and non-mobilizable materials, both natural and synthetic, and combinations thereof, may be employed provided only that the support does not interfere with the capillary action of the material or materials, or non-specifically bind assay components, or interfere with the signal producing system. Illustrative polymers include polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate), nylon, poly(vinyl butyrate), glass, ceramics, metals, and the like. Elastic supports may be made of polyurethane, neoprene, latex, silicone rubber and the like.

[0264] "Treat," "treating" or "treatment" are each used interchangeably herein to describe reversing, alleviating, or inhibiting the progress of a disease and/or injury, or one or more symptoms of such disease, to which such term applies. Depending on the condition of the subject, the term also refers to preventing a disease, and includes preventing the onset of a disease, or preventing the symptoms associated with a disease. A treatment may be either performed in an acute or chronic way. The term also refers to reducing the severity of a disease or symptoms associated with such disease prior to affliction with the disease. Such prevention or reduction of the severity of a disease prior to affliction refers to administration of a pharmaceutical composition to a subject that is not at the time of administration afflicted with the disease. "Preventing" also refers to preventing the recurrence of a disease or of one or more symptoms associated with such disease. "Treatment" and "therapeutically," refer to the act of treating, as "treating" is defined above.

[0265] "Variant" is used herein to describe a peptide or polypeptide that differs from a reference peptide or polypeptide in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retains at least one biological activity. Representative examples of "biological activity" include the ability to be bound by a specific antigen or antibody, or to promote an immune response. Variant is also used herein to describe a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree, and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by

considering the hydropathic index of amino acids, as understood in the art. Kyte et al., *J. Mol. Biol.* 157:105-132 (1982). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ±2 are substituted. The hydrophilicity of amino acids can also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity. U.S. Patent No. 4,554,101, incorporated fully herein by reference. Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions may be performed with amino acids having hydrophilicity values within ±2 of each other. Both the hydrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties. "Variant" also can be used to refer to an antigenically-reactive fragment of an anti-analyte antibody that differs from the corresponding fragment of anti-analyte antibody in amino acid sequence but is still antigenically reactive and can compete with the corresponding fragment of anti-analyte antibody for binding with the analyte. "Variant" also can be used to describe a polypeptide or a fragment thereof that has been differentially processed, such as by proteolysis, phosphorylation, or other post-translational modification, yet retains its antigen reactivity.

[0266] "Vector" is used herein to describe a nucleic acid molecule that can transport another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double-stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors can replicate autonomously in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated

along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. "Plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions, can be used. In this regard, RNA versions of vectors (including RNA viral vectors) may also find use in the context of the present disclosure.

[0267] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. For example, any nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those that are well known and commonly used in the art. The meaning and scope of the terms should be clear; in the event, however of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

2. Methods for detecting the presence of or determining the quantity, amount, level and/or concentration of an antibody against at least one type of β-coronavirus (such as SARS-CoV or SARS-CoV-2) in a subject

[0268] The present disclosure relates to methods for (a) detecting the presence of at least one type of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) (sometimes referred to as a qualitative method or assay); or (b) determining or measuring the quantity, amount, level or concentration (e.g., quantitating (which includes semi-quantitating)) of at least one type of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2), in one or more biological samples obtained from one or more subjects. In some aspects, the methods described herein may be (a) semi-quantitative; (b) quantitative; or (c) qualitative. In some aspects, the method described herein is semi-quantitative, e.g., the method is not standardized against an internationally recognized standard (such as a WHO international standard (e.g., such as in BAU/mL)). In other aspects, the method described herein is quantitative, e.g., the method can be standardized against an internationally recognized standard, such as, for example, a WHO international standard (e.g., such as in

BAU/mL). In yet further aspects, the method described herein is qualitative, e.g., a single-to-calibrator ratio (S/CO) is obtained or determined. An understanding of whether a method is semi-quantitative, quantitative or qualitative, and adaptation of the method to either semi-quantitative, quantitative or qualitative is well known and done using routine techniques known in the art. In still yet other aspects, the methods described herein can be used as an aid in the diagnosis of a SARS-CoV-2 infection. For example, the methods described herein can be used in conjunction with clinical presentation and other laboratory tests to aid in the diagnosis of SARS-CoV-2 infection in a subject (e.g., who may or may not exhibit signs and/or symptoms of infection and suspected of having SARS-CoV-2).

[0269] In some aspects, the detection in samples of antibodies directed against at least one type of β-coronavirus (such as SARS-CoV or SARS-CoV-2) signals a reaction to β-coronavirus (such as SARS-CoV or SARS-CoV-2), and thus the past or current presence in the subject of at least one type of β-coronavirus (such as SARS-CoV or SARS-CoV-2). In one aspect, the methods relate to (a) detecting the presence of (e.g., which can be qualitative as well as semi-quantitative or quantitative) at least one type of anti-β-coronavirus antibody (e.g., an anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody); or (b) determining or measuring the quantity, amount, level or concentration (e.g., quantitating (which includes semi-quantitating)) of at least one type of anti-β-coronavirus antibody (e.g., an anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody), in one or more biological samples obtained from one or more subjects (e.g., who may or may not exhibit signs and/or symptoms of infection and is suspected of having at least one type of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2)). In some aspects, when the methods measure the quantity, amount, level or concentration (e.g., quantitating (which includes semi-quantitating)) of at least one type anti-β-coronavirus antibody (such as at least one anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody), the method can be performed without dilution of the biological sample. In yet other aspects, when the methods measure the quantity, amount, level or concentration (e.g., quantitating (which includes semi-quantitating)) of at least one type of anti-β-coronavirus antibody (such as at least one anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody), the method can be performed without dilution of the biological sample when monitoring vaccine response in subjects receiving or administered at least one type of β-coronavirus (e.g, SARS-CoV or SARS-CoV-2) vaccine (e.g., a first or initial vaccine and/or one or more subsequent (e.g., booster) vaccines).In yet another aspect, the methods relate to detecting

the presence of (which can be qualitative as well as semi-quantitative or quantitative) at least one type of anti-β-coronavirus (IgG and/or IgM) antibody, such as at least one anti-SARS-CoV (IgG and/or IgM) antibody or anti-SARS-CoV-2 (IgG and/or IgM) antibody, in one or more biological samples obtained from a subject (e.g., such as a human, a non-human primate, a cat, a dog, etc.). In another aspect, the methods relate to determining or measuring the quantity, amount, level or concentration of at least one type of anti-β-coronavirus (IgG and/or IgM) antibody, such as at least one anti-SARS-CoV (IgG and/or IgM) antibody or anti-SARS-CoV-2 (IgG and/or IgM) antibody, in one or more biological samples obtained from a subject (e.g., such as a human, a non-human primate, a cat, a dog, etc.). In yet a further aspect, the methods relate to detecting the presence of at least one type of anti-SARS-CoV (IgG and/or IgM) antibody or anti-SARS-CoV-2 (IgG and/or IgM) antibody in one or more biological samples obtained from one or more subjects (e.g., who may or may not exhibit signs and/or symptoms of infection and suspected of having SARS-CoV or SARS-CoV-2).

In still yet a further aspect, the methods relate to determining or measuring the quantity, amount, level or concentration of at least one type of anti-SARS-CoV (IgG and/or IgM) antibody or anti-SARS-CoV-2 (IgG and/or IgM) antibody in one or more biological samples obtained from one or more subjects (e.g., who may or may not exhibit signs and/or symptoms of infection and are suspected of having SARS-CoV or SARS-CoV-2). In this aspect, the method the method can further comprise the step of assigning the subject differentiative rating (e.g., color and/or number rating) (such as that described in Section 14), indicating whether the subject is likely to have immunity (e.g., or has likely developed protective immunity) from infection from at least one type of SARS-CoV-2 based on the quantity, amount, level or concentration of at least one type of anti-SARS-CoV-2 antibody detected or determined in the biological sample. Such a differentiative rating (e.g., color and/or number rating) can be displayed on a mobile device, such as through a mobile application. In yet a further aspect, the methods relate to detecting the presence of at least one type of human anti-SARS-CoV (IgG and/or IgM) antibody or human anti-SARS-CoV-2 (IgG and/or IgM) antibody in one or more biological samples obtained from one or more human subjects (e.g., who may or may not exhibit signs and/or symptoms of infection and are suspected of having SARS-CoV or SARS-CoV-2). In this aspect, the method further comprises the step of assigning the subject differentiative rating (e.g., color and/or number rating) (such as that described in Section 14), indicating whether the subject is likely to

have immunity (e.g., or has likely developed protective immunity) from infection from at least one type of SARS-CoV-2 based on the presence of at least one type of anti-SARS-CoV-2 antibody detected or determined in the biological sample. Such a differentiative rating (e.g., color and/or number rating) can be displayed on a mobile device, such as through a mobile application. In still yet another aspect, the methods relate to detecting the presence of at least one human anti-SARS-CoV IgG antibody and at least one human anti-SARS-CoV IgM antibody or of at least one human anti-SARS-CoV-2 IgG antibody and at least one human anti-SARS-CoV-2 IgM antibody. In still yet a further aspect, the methods relate to determing or measuring the quantity, amount, level or concentration of at least one type of human anti-SARS-CoV (IgG and/or IgM) antibody or human anti-SARS-CoV-2 (IgG and/or IgM) antibody in one or more biological samples obtained from one or more human subjects (e.g., who may or may not exhibit signs and/or symptoms of infection and suspected of having SARS-CoV or SARS-CoV-2). In yet still a further aspect, the methods relate to determining or measuring the quantity, amount, level or concentration of at least one human anti-SARS-CoV IgG antibody and at least one human anti-SARS-CoV IgM antibody or of at least one human anti-SARS-CoV-2 IgG antibody and at least one human anti-SARS-CoV-2 IgM antibody. It should be understood that a "negative" result obtained using the methods described herein (e.g., where the presence of at least one type of anti-β-coronavirus (IgG and/or IgM) antibody is not detected and/or the quantity, amount, level or concentration of at least one type of anti-β-coronavirus (IgG and/or IgM) antibody cannot be determined or is below a predetermined level or cutoff) does not rule out prior or concurrent infection with at least one type of β-coronavirus (e.g., such as SARS-CoV or SARS-CoV-2), particularly in those subjects who have been in contact with the virus (e.g., health care workers). Typically such subjects might receive follow-up or further testing with a molecular diagnostic to further rule out infection in said individuals.

[0270] In some embodiments, detecting the presence of or measuring the quantity, amount, level or concentration of at least one type of anti-β-coronavirus antibody (such as at least one anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) includes contacting the sample, either simultaneously or sequentially, in any order, with: (1) at least one type of first specific binding partner which specifically binds to at least one type of anti-β-coronavirus antibody to form at least one type of first specific binding partner-anti-β-coronavirus antibody complex; and (2) at least one type of second specific binding partner comprising at least one detectable label (e.g.,

detection reagent) that specifically binds to the at least one type of β-coronavirus (such as SARS-CoV or SARS-CoV-2) antibody at a different location than the at least one type of first specific binding partner such that an at least one type of first specific binding partner-anti-β-coronavirus antibody-second specific binding partner complex is formed, and detecting the presence or determining or measuring the amount or concentration of at least one type of anti-β-coronavirus antibody (such as an anti-SARS-CoV (e.g., IgG and/or IgM) antibody or anti-SARS-CoV-2 (e.g., IgG and/or IgM) antibody) in the sample based on the signal generated by the detectable label in the at least one type of first specific binding partner-anti-β-coronavirus antibody-second specific binding partner complex. In some aspects, when measuring the quantity, amount, level or concentration of at least one type of anti-β-coronavirus antibody (such as at least one anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody), the method can be performed without dilution of the biological sample. In yet other aspects, when the methods measure the quantity, amount, level or concentration of at least one type of anti-β-coronavirus antibody (such as at least one anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody), the method can be performed without dilution of the biological sample when monitoring vaccine response in subjects receiving or administered at least one type of β-coronavirus (e.g, SARS-CoV or SARS-CoV-2) vaccine (e.g., a first or initial vaccine and/or one or more subsequent (e.g., booster) vaccines). See, for example, as shown in Fig. 8.

[0271] In some embodiments, the at least one first specific binding partner (e.g., capture reagent) comprises at least one recombinant antigen. The at least one recombinant antigen comprises at least one β-coronavirus (such as SARS-CoV or SARS-CoV-2) isolated polypeptide or variant thereof from (a) a nucleocapsid protein or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) a spike protein or a variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); or (c) a nucleocapsid protein and spike protein or a variant of a nucleocapsid protein and/or a spike protein from a β-coronavirus (such as SARS-CoV or SARS-CoV-2). Any nucleocapsid protein or spike protein from a β-coronavirus, such as SARS-CoV or SARS-CoV-2, known in the art can be used in the at least one first specific binding partner. For example, nucleocapsid and/or spike proteins from SARS-CoV, such as from strains FM1 (GenBank No. Accession No. AY291315), GZ02 (GenBank Accession No. AY390556), Sin3408 (GenBank Accession No. AY559083), Shanghai LY (GenBank Accession No. AY322207), GZ-C (GenBank Accession No. AY394979), Sino1-11 (GenBank Accession

No. AY485277), BJ302 cl.2 (GenBank Accession No. AY429073) GD03T0013 (GenBank Accession No. AY525636) , and/or GB01 (GenBank Accession No. AY278489) described in van den Brink et al., *J. Virol.* 79(3):1635-1644 (February 2005) the contents of which are herein incorporated by reference, can be used. Alternatively, nucleocapsid and/or spike proteins from SARS-CoV-2 such as those described, for example, in Lu et al., *Lancet*, 395:565-574 (February 2020) and deposited in the China National Microbiological Data Center (Accession number NMDC10013002 and Genome accession numbers NMDC60013002-01 to NMDC60013002-10), Wuhan-Hu-1 (GenBank Accession No. NC_045512.2), Wuhan-Hu-1 (GenBank Accession No. MN908947.3) and https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/, the contents of which are herein incorporated by reference, can also be used.

At least one First Specific Binding Partner

[0272] In some aspects, the at least one first specific binding partner (e.g., capture reagent) comprises at least one recombinant antigen. The recombinant antigen comprises at least one β-coronavirus (such as SARS-CoV or SARS-CoV-2) isolated polypeptide or variant thereof from a nucleocapsid protein or variant thereof. The nucleocapsid protein of β-coronaviruses (such as SARS-CoV or SARS-CoV-2) comprise two separate domains (a) a N-terminal domain (NTD) or N-terminal binding domain (NBD) and (b) a C-terminal domain (CTD) or C-terminal binding domain (CBD). For example, FIG. 1 and SEQ ID NO:2 provide a nucleocapsid protein from a strain of SARS-CoV-2. The NTD can be found in amino acids 1-209 of FIG. 1 and SEQ ID NO:2. The CTD can be found in amino acids 210-419 of FIG. 1 and SEQ ID NO:2 (shown in underlining and italics in SEQ ID NO:2 in FIG. 4). In some aspects, an isolated SARS-CoV-2 variant polypeptide can comprise one or more substitutions in one or more of the following amino acid positions within SEQ ID NO:2 as shown below in Table B.

[0273] Table B

		Strain							
	Country	Ohio	Ohio	Canada	Brazil	Brazil	USCF	UK	SA
Protein	Mutation	COH.26G/ 677H	COH.20G/ 501Y	L00311131	P.1	B.1.1.28	UC301-3	B.1.1.7	501Y.V2
Nucleocapsid	D3L								
Nucleocapsid	S235F						X	X	
Nucleocapsid	M234I			X		X			
Nucleocapsid	K373N						X		
Nucleocapsid	D377Y	X							
Nucleocapsid	A376T			X					

[0274] In other aspects, the isolated SARS-CoV-2 variant polypeptide can comprise one or more of substitutions and/or deletions in one or more of the following amino acid positions within SEQ ID NO:2: (1) replacing asparagine with leucine at amino acid position 3 (D3L); (2) replacing serine with phenylalanine at amino acid position 235 (S235F) (shown in underlining, italics and bold in SEQ ID NO:2 in FIG. 4); (3) replacing methionine with isoleucine at amino acid position 234 (M234I); (4) replacing lysine with asparagine at amino acid position 373 (K373N); (5) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (6)

replacing alanine with threonine at amino acid position 376 (A376T); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in SEQ ID NO:2 other than those recited in (1)-(6).

[0275] In other aspects, the isolated SARS-CoV-2 variant polypeptide can comprise one or more substitutions and/or deletions in one or more positions of amino acids 210 to 419 of SEQ ID NO:2: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210 to 419 of SEQ ID NO:2 other than those recited in (1)-(5).

[0276] The nucleocapsid protein or variant thereof of the at least one first specific binding partner can have a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384,

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974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000, 1001, 1002, 1003, 1004, 1005, 1006, 1007, 1008, 1009, 1010, 1011, 1012, 1013, 1014, 1015, 1016, 1017, 1018, 1019, 1020, 1021, 1022, 1023, 1024, 1025, 1026, 1027, 1028, 1029, 1030, 1031, 1032, 1033, 1034, 1035, 1036, 1037, 1038, 1039, 1040, 1041, 1042, 1043, 1044, 1045, 1046, 1047, 1048, 1049, 1050, 1051, 1052, 1053, 1054, 1055, 1056, 1057, 1058, 1059, 1060, 1061, 1062, 1063, 1064, 1065, 1066, 1067, 1068, 1069, 1070, 1071, 1072, 1073, 1074, 1075, 1076, 1077, 1078, 1079, 1080, 1081, 1082, 1083, 1084, 1085, 1086, 1087, 1088, 1089, 1090, 1091, 1092, 1093, 1094, 1095, 1096, 1097, 1098, 1099, 1100, 1101, 1102, 1103, 1104, 1105, 1106, 1107, 1108, 1109, 1110, 1111, 1112, 1113, 1114, 1115, 1116, 1117, 1118, 1119, 1120, 1121, 1122, 1123, 1124, 1125, 1126, 1127, 1128, 1129, 1130, 1131, 1132, 1133, 1134, 1135, 1136, 1137, 1138, 1139, 1140, 1141, 1142, 1143, 1144, 1145, 1146, 1147, 1148, 1149, 1150, 1151, 1152, 1153, 1154, 1155, 1156, 1157, 1158, 1159, 1160, 1161, 1162, 1163, 1164, 1165, 1166, 1167, 1168, 1169, 1170, 1171, 1172, 1173, 1174, 1175, 1176, 1177, 1178, 1179, 1180, 1181, 1182, 1183, 1184, 1185, 1186, 1187, 1188, 1189, 1190, 1191, 1192, 1193, 1194, 1195, 1196, 1197, 1198, 1199, 1200, 1201, 1202, 1203, 1204, 1205, 1206, 1207, 1208, 1209, 1210, 1211, 1212, 1213, 1214, 1215, 1216, 1217, 1218, 1219, 1220, 1221, 1222, 1223, 1224, 1225, 1226, 1227, 1228, 1229, 1230, 1231, 1232, 1233, 1234, 1235, 1236, 1237, 1238, 1239, 1240, 1241, 1242, 1243, 1244, 1245, 1246, 1247, 1248, 1249, 1250, 1251, 1252, 1253, 1254, 1255, 1256, 1257, 1258, 1259, 1260, 1261, 1262, 1263, 1264, 1265, 1266, 1267, 1268, 1269, 1270, 1272, 1272, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1286, 1287, 1288, 1289, 1290, 1291, 1292, 1293, 1294, 1295, 1296, 1297, 1298, 1299, 1300, 1301, 1302, 1303, 1304, 1305, 1306, 1307, 1308, 1309, 1310, 1311, 1312, 1313, 1314, 1315, 1316, 1317, 1318, 1319, 1320, 1321, 1322, 1323, 1324, 1325, 1326, 1327, 1328, 1329, 1330, 1331, 1332, 1333, 1334, 1335, 1336, 1337, 1338, 1339, 1340, 1341, 1342, 1343, 1344, 1345, 1346, 1347, 1348, 1349, 1350, 1351, 1352, 1353, 1354, 1355, 1356, 1357, 1358, 1359, 1360, 1361, 1362, 1363, 1364, 1365, 1366, 1367, 1368, 1369, 1370, 1371, 1372, 1373, 1374, 1375, 1376, 1377, 1378, 1379, 1380, 1381, 1382, 1383, 1384, 1385, 1386, 1387, 1388, 1389, 1390, 1391, 1392, 1393, 1394, 1395, 1396, 1397, 1398, 1399, 1400, 1401, 1402, 1403, 1404, 1405, 1406, 1407, 1408, 1409, 1410, 1411, 1412, 1413, 1414, 1415, 1416, 1417, 1418, 1419, 1420, 1421, 1422, 1423, 1424, 1425, 1426, 1427, 1428, 1429, 1430, 1431, 1432, 1433, 1434, 1435, 1436, 1437, 1438, 1439, 1440, 1441, 1442, 1443, 1444,

1445, 1446, 1447, 1448, 1449, 1450, 1451, 1452, 1453, 1454, 1455, 1456, 1457, 1458, 1459, 1460, 1461, 1462, 1463, 1464, 1465, 1466, 1467, 1468, 1469, 1470, 1471, 1472, 1473, 1474, 1475, 1476, 1477, 1478, 1479, 1480, 1481, 1482, 1483, 1484, 1485, 1486, 1487, 1488, 1489, 1490, 1491, 1492, 1493, 1494, 1495, 1496, 1497, 1498, 1499 or 1500 amino acids.

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193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781,

782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, or 800 amino acids.

[0279] In some aspects, the nucleocapsid protein or variant thereof of the at least one first specific binding partner can have a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553,

554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, or 700 amino acids.

[0280] In some aspects, the nucleocapsid protein or variant thereof of the at least one first specific binding partner can have a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439,

440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, or 600 amino acids.

[0281] In some aspects, the nucleocapsid protein or variant thereof in the at least one first recombinant antigen of the at least one first specific binding partner can have a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411,

412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, or 500 amino acids.

[0282] In some aspects, the nucleocapsid protein or variant thereof of the at least one first specific binding partner can have a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404 or 405 amino acids.

[0283] In some aspects, the nucleocapsid protein or variant thereof of the at least first specific binding partner can have a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72,

73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249 or 250 amino acids.

[0284] In another aspect, the nucleocapsid protein or variant thereof of the first specific binding partner can have a length of about 5 amino acids to about 1500 amino acids, about 10 amino acids to about 1500 amino acids, about 15 amino acids to about 1500 amino acids, about 20 amino acids to about 1500 amino acids, about 25 amino acids to about 1500 amino acids, about 30 amino acids to about 1500 amino acids, about 40 amino acids to about 1500 amino acids, about 50 amino acids to about 1500 amino acids, about 60 amino acids to about 1500 amino acids, about 70 amino acids to about 1500 amino acids, about 75 amino acids to about 1500 amino acids, about 80 amino acids to about 1500 amino acids, about 90 amino acids to about 1500 amino acids, about 100 amino acids to about 1500 amino acids, about 5 amino acids to about 1400 amino acids, about 10 amino acids to about 1400 amino acids, about 15 amino acids to about 1400 amino acids, about 20 amino acids to about 1400 amino acids, about 25 amino acids to about 1400 amino acids, about 30 amino acids to about 1400 amino acids, about 40 amino acids to about 1400 amino acids, about 50 amino acids to about 1400 amino acids, about 60 amino acids to about 1400 amino acids, about 70 amino acids to about 1400 amino acids, about 75 amino acids to about 1400 amino acids, about 80 amino acids to about 1400 amino acids, about 90 amino acids to about 1400 amino acids, about 100 amino acids to about 1400 amino acids, about 5 amino acids to about 1300 amino acids, about 10 amino acids to about 1300 amino acids, about 15 amino acids to about 1300 amino acids, about 20 amino acids to about 1300 amino acids, about 25 amino acids to about 1300 amino acids, about 30 amino acids to about 1300 amino acids, about 40 amino acids to about 1300 amino acids, about 50 amino acids to about 1300 amino acids, about 60 amino acids to about 1300 amino acids, about 70

amino acids to about 1300 amino acids, about 75 amino acids to about 1300 amino acids, about 80 amino acids to about 1300 amino acids, about 90 amino acids to about 1300 amino acids, about 100 amino acids to about 1300 amino acids, about 5 amino acids to about 1200 amino acids, about 10 amino acids to about 1200 amino acids, about 15 amino acids to about 1200 amino acids, about 20 amino acids to about 1200 amino acids, about 25 amino acids to about 1200 amino acids, about 30 amino acids to about 1200 amino acids, about 40 amino acids to about 1200 amino acids, about 50 amino acids to about 1200 amino acids, about 60 amino acids to about 1200 amino acids, about 70 amino acids to about 1200 amino acids, about 75 amino acids to about 1020 amino acids, about 80 amino acids to about 1200 amino acids, about 90 amino acids to about 1200 amino acids, about 100 amino acids to about 1200 amino acids, about 5 amino acids to about 1100 amino acids, about 10 amino acids to about 1100 amino acids, about 15 amino acids to about 1100 amino acids, about 20 amino acids to about 1100 amino acids, about 25 amino acids to about 1100 amino acids, about 30 amino acids to about 1100 amino acids, about 40 amino acids to about 1100 amino acids, about 50 amino acids to about 1100 amino acids, about 60 amino acids to about 1100 amino acids, about 70 amino acids to about 1100 amino acids, about 75 amino acids to about 1100 amino acids, about 80 amino acids to about 1100 amino acids, about 90 amino acids to about 1100 amino acids, about 100 amino acids to about 1100 amino acids, about 5 amino acids to about 1000 amino acids, about 10 amino acids to about 1000 amino acids, about 15 amino acids to about 1000 amino acids, about 20 amino acids to about 1000 amino acids, about 25 amino acids to about 1000 amino acids, about 30 amino acids to about 1000 amino acids, about 40 amino acids to about 1000 amino acids, about 50 amino acids to about 1000 amino acids, about 60 amino acids to about 1000 amino acids, about 70 amino acids to about 1000 amino acids, about 75 amino acids to about 1000 amino acids, about 80 amino acids to about 1000 amino acids, about 90 amino acids to about 1000 amino acids, about 100 amino acids to about 1000 amino acids, about 5 amino acids to about 900 amino acids, about 10 amino acids to about 900 amino acids, about 15 amino acids to about 900 amino acids, about 20 amino acids to about 900 amino acids, about 25 amino acids to about 900 amino acids, about 30 amino acids to about 900 amino acids, about 40 amino acids to about 900 amino acids, about 50 amino acids to about 900 amino acids, about 60 amino acids to about 900 amino acids, about 70 amino acids to about 900 amino acids, about 75 amino acids to about 900 amino acids, about 80 amino acids to about 900 amino acids, about 90 amino acids to about 900

amino acids, about 100 amino acids to about 900 amino acids, about 5 amino acids to about 800 amino acids, about 10 amino acids to about 800 amino acids, about 15 amino acids to about 800 amino acids, about 20 amino acids to about 800 amino acids, about 25 amino acids to about 800 amino acids, about 30 amino acids to about 800 amino acids, about 40 amino acids to about 800 amino acids, about 50 amino acids to about 800 amino acids, about 60 amino acids to about 800 amino acids, about 70 amino acids to about 800 amino acids, about 75 amino acids to about 800 amino acids, about 80 amino acids to about 800 amino acids, about 90 amino acids to about 800 amino acids, about 100 amino acids to about 800 amino acids, about 5 amino acids to about 700 amino acids, about 10 amino acids to about 700 amino acids, about 15 amino acids to about 700 amino acids, about 20 amino acids to about 700 amino acids, about 25 amino acids to about 700 amino acids, about 30 amino acids to about 700 amino acids, about 40 amino acids to about 700 amino acids, about 50 amino acids to about 700 amino acids, about 60 amino acids to about 700 amino acids, about 70 amino acids to about 700 amino acids, about 75 amino acids to about 700 amino acids, about 80 amino acids to about 700 amino acids, about 90 amino acids to about 700 amino acids, about 100 amino acids to about 700 amino acids, about 5 amino acids to about 600 amino acids, about 10 amino acids to about 600 amino acids, about 15 amino acids to about 600 amino acids, about 20 amino acids to about 600 amino acids, about 25 amino acids to about 600 amino acids, about 30 amino acids to about 600 amino acids, about 40 amino acids to about 600 amino acids, about 50 amino acids to about 600 amino acids, about 60 amino acids to about 600 amino acids, about 70 amino acids to about 600 amino acids, about 75 amino acids to about 600 amino acids, about 80 amino acids to about 600 amino acids, about 90 amino acids to about 600 amino acids, about 100 amino acids to about 600 amino acids, about 5 amino acids to about 500 amino acids, about 10 amino acids to about 500 amino acids, about 15 amino acids to about 500 amino acids, about 20 amino acids to about 500 amino acids, about 25 amino acids to about 500 amino acids, about 30 amino acids to about 500 amino acids, about 40 amino acids to about 500 amino acids, about 50 amino acids to about 500 amino acids, about 60 amino acids to about 500 amino acids, about 70 amino acids to about 500 amino acids, about 75 amino acids to about 500 amino acids, about 80 amino acids to about 500 amino acids, about 90 amino acids to about 500 amino acids, about 100 amino acids to about 500 amino acids, about 5 amino acids to about 400 amino acids, about 10 amino acids to about 400 amino acids, about 15 amino acids to about 400 amino acids, about 20 amino acids to about 400 amino acids, about 25 amino acids to about 400

amino acids, about 30 amino acids to about 400 amino acids, about 40 amino acids to about 400 amino acids, about 50 amino acids to about 400 amino acids, about 60 amino acids to about 400 amino acids, about 70 amino acids to about 400 amino acids, about 75 amino acids to about 400 amino acids, about 80 amino acids to about 400 amino acids, about 90 amino acids to about 400 amino acids, about 100 amino acids to about 400 amino acids, about 5 amino acids to about 300 amino acids, about 10 amino acids to about 300 amino acids, about 15 amino acids to about 300 amino acids, about 20 amino acids to about 300 amino acids, about 25 amino acids to about 300 amino acids, about 30 amino acids to about 300 amino acids, about 40 amino acids to about 300 amino acids, about 50 amino acids to about 300 amino acids, about 60 amino acids to about 300 amino acids, about 70 amino acids to about 300 amino acids, about 75 amino acids to about 300 amino acids, about 80 amino acids to about 300 amino acids, about 90 amino acids to about 300 amino acids, about 100 amino acids to about 300 amino acids, about 5 amino acids to about 200 amino acids, about 10 amino acids to about 200 amino acids, about 15 amino acids to about 200 amino acids, about 20 amino acids to about 200 amino acids, about 25 amino acids to about 200 amino acids, about 30 amino acids to about 200 amino acids, about 40 amino acids to about 200 amino acids, about 50 amino acids to about 200 amino acids, about 60 amino acids to about 200 amino acids, about 70 amino acids to about 200 amino acids, about 75 amino acids to about 200 amino acids, about 80 amino acids to about 200 amino acids, about 90 amino acids to about 200 amino acids, about 100 amino acids to about 200 amino acids, about 5 amino acids to about 100 amino acids, about 10 amino acids to about 100 amino acids, about 15 amino acids to about 100 amino acids, about 20 amino acids to about 100 amino acids, about 25 amino acids to about 100 amino acids, about 30 amino acids to about 100 amino acids, about 40 amino acids to about 100 amino acids, about 50 amino acids to about 100 amino acids, about 60 amino acids to about 100 amino acids, about 70 amino acids to about 100 amino acids, about 75 amino acids to about 100 amino acids, about 80 amino acids to about 100 amino acids, or about 90 amino acids to about 100 amino acids.

[0285] In some aspects, the nucleocapsid protein comprises the CTD of a nucleocapsid protein of a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) or any fragments or variants thereof. In other aspects, the nucleocapsid protein comprises amino acids 210-419 of the nucleocapsid protein of a β-coronavirus, such as, for example, SARS-CoV or SARS-CoV-2, or any fragments or variants thereof. In yet another aspect, the nucleocapsid protein comprises

amino acids 210-419 from a human SARS-CoV-2 (See, for example, SEQ ID NO:2 which is also shown in underlining and italics in SEQ ID NO:2 in FIG. 4). In still yet another aspect, the nucleocapsid protein has the sequence of:

MAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKRTAT KAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGME VTPSGTWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQAL PQRQKKQQTVTLLPAADLDDFSKQLQQSMSSADSTQA (SEQ ID NO:1) or fragment or variant thereof. In yet another aspect, the nucleocapsid protein has the sequence of SEQ ID NO:24. SEQ ID NO:24 is a variant of SEQ ID NO:1 where the serine shown above in SEQ ID NO:1 in bold and underlining at position 26 is replaced with a phenylalanine (also known as S235F in the full context of the nucleocapsid protein, and here, because SEQ ID NO:24 is residues 210 to 419 of nucleocapsid protein, this would be S26F of the fragment of SEQ ID NO:24). A "fragment" of SEQ ID NO:1 refers to a protein or polypeptide that comprises a part that is less than the entirety of SEQ ID NO:1. A fragment of SEQ ID NO:1 can comprise from about 5 to about 200 contiguous amino acids. In another aspect, a fragment of SEQ ID NO:1 comprises at least about 5 contiguous amino acids of SEQ ID NO:1, at least about 10 contiguous amino acids of SEQ ID NO:1, at least about 15 contiguous amino acids of SEQ ID NO:1, at least about 20 contiguous amino acids of SEQ ID NO:1, at least about 25 contiguous amino acids of SEQ ID NO:1, at least about 30 contiguous amino acids of SEQ ID NO:1, at least about 35 contiguous amino acids of SEQ ID NO:1, at least about 40 contiguous amino acids of SEQ ID NO:1, at least about 45 contiguous amino acids of SEQ ID NO:1, at least about 50 contiguous amino acids of SEQ ID NO:1, at least about 55 contiguous amino acids of SEQ ID NO:1, at least about 60 contiguous amino acids of SEQ ID NO:1, at least about 65 contiguous amino acids of SEQ ID NO:1, at least about 70 contiguous amino acids of SEQ ID NO:1, at least about 75 contiguous amino acids of SEQ ID NO:1, at least about 80 contiguous amino acids of SEQ ID NO:1, at least about 85 contiguous amino acids of SEQ ID NO:1, at least about 90 contiguous amino acids of SEQ ID NO:1, at least about 95 contiguous amino acids of SEQ ID NO:1, at least 100 contiguous amino acids of SEQ ID NO:1, at least about 105 contiguous amino acids of SEQ ID NO:1, at least about 110 contiguous amino acids of SEQ ID NO:1, at least about 115 contiguous amino acids of SEQ ID NO:1, at least about 120 contiguous amino acids of SEQ ID NO:1, at least about 125 contiguous amino acids of SEQ ID NO:1, at least about 130 contiguous

amino acids of SEQ ID NO:1, at least about 135 contiguous amino acids of SEQ ID NO:1, at least about 140 contiguous amino acids of SEQ ID NO:1, at least about 145 contiguous amino acids of SEQ ID NO:1, at least about 150 contiguous amino acids of SEQ ID NO:1, at least about 55 contiguous amino acids of SEQ ID NO:1, at least about 160 contiguous amino acids of SEQ ID NO:1, at least about 165 contiguous amino acids of SEQ ID NO:1, at least about 170 contiguous amino acids of SEQ ID NO:1, at least about 175 contiguous amino acids of SEQ ID NO:1, at least about 180 contiguous amino acids of SEQ ID NO:1, at least about 85 contiguous amino acids of SEQ ID NO:1, at least about 190 contiguous amino acids of SEQ ID NO:1, at least about 95 contiguous amino acids of SEQ ID NO:1, at least 200 contiguous amino acids of SEQ ID NO:1 or at least about 205 contiguous amino acids of SEQ ID NO:1.

[0286] In some aspects, the at least one isolated polypeptide is a fusion protein comprising at least all or at least a portion of at least one β-coronavirus (such as SARS-CoV or SARS-CoV-2) isolated nucleocapsid protein or variant thereof. In another aspect, the fusion protein may comprise all or at least portion (e.g., at least 5 amino acids or more) of the NTD of a nucleocapsid protein from one strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) is operably linked, fused or grafted directly or indirectly (such as through one or more linking peptide sequences and/or HIS tags) to all or at least a portion (at least 5 amino acids or more) of a NTD from the same or different strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) (e.g., a Nc-NTD/NTD fusion protein or peptide)

[0287] In another aspect, the fusion protein may comprise all or at least portion (e.g., at least 5 amino acids or more) of the CTD of a nucleocapsid protein from one strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) operably linked, fused or grafted directly or indirectly (such as through one or more linking peptide sequences and/or HIS tags) to all or at least a portion (at least 5 amino acids or more) of a CTD of a nucleocapsid protein from the same or different strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) (e.g., a Nc-CTD/CTD fusion protein or peptide). In another aspect, the fusion protein may comprise all or at least portion (e.g., at least 5 amino acids or more) of the NTD of a nucleocapsid protein from one strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) operably linked, fused or grafted directly or indirectly (such as through one or more linking peptide sequences and/or HIS tags) to all or at least a portion (at least 5 amino acids or more) of a CTD of a nucleocapsid protein from the same or different strain

of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) (e.g., an eNc-CTD fusion protein or peptide or epitope grafted CTD fusion protein peptide).

[0288] For example, FIG. 3 provides a diagram illustrating the structure of a fusion protein comprising at least 5 amino acids of the N-terminal domain (NTD) of a nucleocapsid protein from a β-coronavirus (such as SARS-CoV or SARS-CoV-2) operably linked or grafted on to at least 5 amino acids of the C-terminal domain (CTD) of a nucleocapsid protein the same or different β-coronavirus, such as SARS-CoV or SARS-CoV-2 (such as through one or more linking peptide sequences and/or HIS tags). In some aspects, this fusion protein can comprise a methionine initiator residue operably linked or grafted (such as, for example, through one or more linking peptide sequences and/or HIS tags) on to amino acids 5 to 25 (optionally, residues 4 to 25) of SEQ ID NO:14 or amino acids 5 to 24 (optionally, residues 4 to 24) of SEQ ID NO:2 operably linked or grafted on to amino acids 211 to 419 of SEQ ID NO:2 (or analogous region from SEQ ID NO:14). Other variations are possible. For example, in other aspects, the fusion protein can comprise an epitope for the monoclonal antibody CR3018 (described in van den Brink et al., *J. Virol.* 79(3):1635-1644 (February 2005) and U.S. Patent No. 7,696,330, each of which is herein incorporated by referenced) fused at the N-terminal end of the CTD ("epitope-grafted CTD" fusion protein or peptide). In this example, a fusion protein comprising methionine as the starting amino acid operably linked or grafted on to an epitope (such as, for example, through one or more linking peptide sequences and/or HIS tags) comprising at least amino acids RNAPRITFG (SEQ ID NO:6) or RSAPRITFG (SEQ ID NO:7), which in turn, are operably linked or grafted (such as, for example, through one or more linking peptide sequences and/or HIS tags) on to the N-terminal end of the CTD, e.g., amino acids 211 to 419 of SEQ ID NO:2 (or the analogous region of SEQ ID NO:14). In some aspects, there may be one or more additional sequences operably linked or grafted (such as, for example, through one or more linking peptide sequences and/or HIS tags) on to the starting amino acid methionine, such as, for example, GPQNQ (SEQ ID NO:21) or GPQSNQ (SEQ ID NO:22), which in turn, is operably linked or grafted (such as, for example, through one or more linking peptide sequences and/or HIS tags) on to an epitope comprising at least amino acids RNAPRITFG (SEQ ID NO:6) or RSAPRITFG (SEQ ID NO:7), which furthermore, are operably linked or grafted (such as, for example, through one or more linking peptide sequences and/or HIS tags) on to the N-terminal end of the CTD, e.g., amino acids 211 to 419 of SEQ ID NO:2 (or the analogous region from

SEQ ID NO:14), , and optionally, furthermore may include the sequence GPTDST (SEQ ID NO:23). It is well known that in some proteins the initiator residue methionine is cleaved off by an enzyme since it may not be present in the mature polypeptide/protein. In another aspect, the fusion protein comprises the initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2. In still yet another aspect, the fusion protein comprises the initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2. In yet another aspect, the fusion protein comprises the following amino acid sequences in the order recited: (a) an initiator amino acid methionine; (b) amino acid sequence GPQNQ (SEQ ID NO:21); (c) amino acid sequence RSAPRITFG (SEQ ID NO:7); (d) amino acid sequence GPTDST (SEQ ID NO:23); and (e) amino acids 211 to 419 of SEQ ID NO:2. In still yet another aspect, the fusion protein comprises the following amino acid sequences in the order recited: (a) an initiator amino acid methionine; (b) amino acid sequence GPQSNQ (SEQ ID NO:22); (c) amino acid sequence RSAPRITFG (SEQ ID NO:7); (d) amino acid sequence GPTDST (SEQ ID NO:23); and (e) amino acids 211 to 419 of SEQ ID NO:2.

[0289] Alternatively, the fusion protein may comprise all or at least portion (e.g., at least 5 amino acids or more) of a nucleocapsid protein (NTD and/or CTD) from one strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) operably linked, fused or grafted directly or indirectly (such as through one or more linking peptide sequences) to all or at least a portion (at least 5 amino acids or more) of a spike protein (e.g., such as, for example, all or a portion of the RBD of a spike protein (see, for example, SEQ ID NOS: 15-18 or any fragments or variants thereof)) from the same or different strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). Also, one of skill in the art would understand that a consensus sequence for any β-coronavirus (such as SARS-CoV or SARS-CoV-2) could be substituted with any strain specific variations (e.g., SEQ ID NOS: 2 and 14).

[0290] In some aspects, the recombinant antigen of the at least one first specific binding partner (e.g., capture reagent) comprises all or at least a portion of at least one β-coronavirus (such as SARS-CoV or SARS-CoV-2) isolated polypeptide or variant thereof from a spike protein or variant thereof. The spike protein comprises S1 and S2 polypeptides. The S1 polypeptide contains the receptor binding domain (RBD) of the protein, while the S2 polypeptide

forms the stalk of the spike molecule. SEQ ID NO: 15 in FIG. 4 provides the amino acid sequence of the spike protein from SARS-CoV-2. The RBD can be found at amino acids 319 to 542 of SEQ ID NO:15 (FIG. 4). SEQ ID NO:16 in FIG. 4 provides the amino acid sequence of the spike protein from SARS-CoV. The RBD can be found at amino acids 306 to 528 of SEQ ID NO:16 (FIG. 4).

[0291] In some aspects, an isolated SARS-CoV-2 variant polypeptide can comprise one or more substitutions in one or more of the following amino acid positions within SEQ ID NO:15 as shown below in Table C.

[0292] Table C

		Strain							
	Country	**Ohio**	**Ohio**	**Canada**	**Brazil**	**Brazil**	**USCF**	**UK**	**SA**
Protein	**Mutation**	**COH.26G/ 677H**	**COH.20G/ 501Y**	**L00311131**	**P.1**	**B.1.1.28**	**UC301-3**	**B.1.1.7**	**501Y.V2**
Spike	N501Y		X		X		X	X	X
Spike	K417N								X
Spike	K417T				X				
Spike	E484K				X	X			X
Spike	L452R								
Spike	S477N			X					
Spike	A570D								
Spike	P681H								
Spike	T716I								
Spike	S982A								
Spike	D1118H								

[0293] In some aspects, the isolated SARS-CoV-2 variant polypeptide can comprise one or more substitutions in one or more of the amino acid positions within SEQ ID NO:2 as shown in Table B and/or one or more substitutions in one or more of the amino acid positions within SEQ ID NO:15 as shown in Table C.

[0294] In other aspects, the isolated SARS-CoV-2 variant polypeptide can comprise one or more substitutions and/or deletions in one or more of the following amino acid positions within SEQ ID NO:15: (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484

(E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); (7) replacing alanine with aspartic acid at amino acid position 570 (A570D); (8) replacing proline with histidine at amino acid position 681 (P681H); (9) replacing aspartic acid with glycine at amino acid position 614 (D614G); (10) replacing threonine with isoleucine at amino acid position 716 (T716I); (11) replacing serine with alanine at amino acid position 982 (S982A); (12) replacing aspartic acid with histidine at amino acid position 1118 (D1118H); (13) a deletion of histidine (H) at amino acid position 69; (14) a deletion of valine (V) at amino acid position 70; (15) a deletion of tyrosine (Y) at amino acid position 144; or (16) any combinations of (1)-(15), either alone or combined with any other substitutions and/or deletions in SEQ ID NO:15 other than those recited in (1)-(15).

[0295] In some aspects, an isolated SARS-CoV-2 variant polypeptide can comprise one or more substitutions in one or more of the following amino acid positions within SEQ ID NO:25: (i) replacing lysine with asparagine at position 417 (K417N); (ii) replacing glutamic acid with lysine at position 484 (E484K); (iii) replacing asparagine with tyrosine at position 501 (N501Y); or (iv) any combinations of (i) – (iii). In some aspects, the variant polypeptide can comprise one or more deletions, such as a deletion of histidine (H) and/or valine (V) at amino acids 69-70 of SEQ ID NO:25 and/or tyrosine (Y) at amino acid 144 of SEQ ID NO:25. In some aspects, the variant polypeptide can comprise (1) one or more substitutions in one or more of the following amino acid positions in SEQ ID NO:25: (i) replacing lysine with asparagine at position 417 (K417N); (ii) replacing glutamic acid with lysine at position 484 (E484K); (iii) replacing asparagine with tyrosine at position 501 (N501Y); (iv) any combinations of (i) – (iii) and (2) one or more deletions, such as a deletion of histidine (H) and/or valine (V) at amino acids 69-70 of SEQ ID NO:25 and/or tyrosine (Y) at amino acid 144 of SEQ ID NO:25.

[0296] In other aspects, the isolated SARS-CoV-2 variant polypeptide can comprise one or more substitutions and/or deletions in one or more positions of amino acids 319 to 542 of SEQ ID NO:15: (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any

combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319 to 542 of SEQ ID NO:15 other than those recited in (1)-(6).

[0297] In some aspects, the spike protein or variant thereof of the at least one first specific binding partner can have a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554,

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1114, 1115, 1116, 1117, 1118, 1119, 1120, 1121, 1122, 1123, 1124, 1125, 1126, 1127, 1128, 1129, 1130, 1131, 1132, 1133, 1134, 1135, 1136, 1137, 1138, 1139, 1140, 1141, 1142, 1143, 1144, 1145, 1146, 1147, 1148, 1149, 1150, 1151, 1152, 1153, 1154, 1155, 1156, 1157, 1158, 1159, 1160, 1161, 1162, 1163, 1164, 1165, 1166, 1167, 1168, 1169, 1170, 1171, 1172, 1173, 1174, 1175, 1176, 1177, 1178, 1179, 1180, 1181, 1182, 1183, 1184, 1185, 1186, 1187, 1188, 1189, 1190, 1191, 1192, 1193, 1194, 1195, 1196, 1197, 1198, 1199, 1200, 1201, 1202, 1203, 1204, 1205, 1206, 1207, 1208, 1209, 1210, 1211, 1212, 1213, 1214, 1215, 1216, 1217, 1218, 1219, 1220, 1221, 1222, 1223, 1224, 1225, 1226, 1227, 1228, 1229, 1230, 1231, 1232, 1233, 1234, 1235, 1236, 1237, 1238, 1239, 1240, 1241, 1242, 1243, 1244, 1245, 1246, 1247, 1248, 1249, 1250, 1251, 1252, 1253, 1254, 1255, 1256, 1257, 1258, 1259, 1260, 1261, 1262, 1263, 1264, 1265, 1266, 1267, 1268, 1269, 1270, 1272, 1272, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1286, 1287, 1288, 1289, 1290, 1291, 1292, 1293, 1294, 1295, 1296, 1297, 1298, 1299, 1300, 1301, 1302, 1303, 1304, 1305, 1306, 1307, 1308, 1309, 1310, 1311, 1312, 1313, 1314, 1315, 1316, 1317, 1318, 1319, 1320, 1321, 1322, 1323, 1324, 1325, 1326, 1327, 1328, 1329, 1330, 1331, 1332, 1333, 1334, 1335, 1336, 1337, 1338, 1339, 1340, 1341, 1342, 1343, 1344, 1345, 1346, 1347, 1348, 1349, 1350, 1351, 1352, 1353, 1354, 1355, 1356, 1357, 1358, 1359, 1360, 1361, 1362, 1363, 1364, 1365, 1366, 1367, 1368, 1369, 1370, 1371, 1372, 1373, 1374, 1375, 1376, 1377, 1378, 1379, 1380, 1381, 1382, 1383, 1384, 1385, 1386, 1387, 1388, 1389, 1390, 1391, 1392, 1393, 1394, 1395, 1396, 1397, 1398, 1399, 1400, 1401, 1402, 1403, 1404, 1405, 1406, 1407, 1408, 1409, 1410, 1411, 1412, 1413, 1414, 1415, 1416, 1417, 1418, 1419, 1420, 1421, 1422, 1423, 1424, 1425, 1426, 1427, 1428, 1429, 1430, 1431, 1432, 1433, 1434, 1435, 1436, 1437, 1438, 1439, 1440, 1441, 1442, 1443, 1444, 1445, 1446, 1447, 1448, 1449, 1450, 1451, 1452, 1453, 1454, 1455, 1456, 1457, 1458, 1459, 1460, 1461, 1462, 1463, 1464, 1465, 1466, 1467, 1468, 1469, 1470, 1471, 1472, 1473, 1474, 1475, 1476, 1477, 1478, 1479, 1480, 1481, 1482, 1483, 1484, 1485, 1486, 1487, 1488, 1489, 1490, 1491, 1492, 1493, 1494, 1495, 1496, 1497, 1498, 1499 or 1500 amino acids.

[0298] In some aspects, the spike protein or variant thereof of the at least one first specific binding partner can have a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98,

99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687,

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[0306] In some aspects, the spike protein or variant thereof of the at least one first specific binding partner can have a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193,

194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, or 600 amino acids.

[0307] In some aspects, the spike protein or variant thereof of the at least one first specific binding partner can have a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174,

175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, or 500 amino acids.

[0308] In some aspects, the spike protein or variant thereof of the at least one first specific binding partner can have a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250,

251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399 or 400 amino acids.

[0309] In some aspects, the spike protein or variant thereof of the at least one first specific binding partner can have a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249 or 250 amino acids.

[0310] In another aspect, the spike or variant thereof used of the first specific binding partner can have a length of about 5 amino acids to about 1500 amino acids, about 10 amino acids to about 1500 amino acids, about 15 amino acids to about 1500 amino acids, about 20 amino acids to about 1500 amino acids, about 25 amino acids to about 1500 amino acids, about 30 amino acids to about 1500 amino acids, about 40 amino acids to about 1500 amino acids, about 50 amino acids to about 1500 amino acids, about 60 amino acids to about 1500 amino acids, about 70 amino acids to about 1500 amino acids, about 75 amino acids to about 1500 amino acids, about 80 amino acids to about 1500 amino acids, about 90 amino acids to about 1500 amino

acids, about 100 amino acids to about 1500 amino acids, about 5 amino acids to about 1400 amino acids, about 10 amino acids to about 1400 amino acids, about 15 amino acids to about 1400 amino acids, about 20 amino acids to about 1400 amino acids, about 25 amino acids to about 1400 amino acids, about 30 amino acids to about 1400 amino acids, about 40 amino acids to about 1400 amino acids, about 50 amino acids to about 1400 amino acids, about 60 amino acids to about 1400 amino acids, about 70 amino acids to about 1400 amino acids, about 75 amino acids to about 1400 amino acids, about 80 amino acids to about 1400 amino acids, about 90 amino acids to about 1400 amino acids, about 100 amino acids to about 1400 amino acids, about 5 amino acids to about 1300 amino acids, about 10 amino acids to about 1300 amino acids, about 15 amino acids to about 1300 amino acids, about 20 amino acids to about 1300 amino acids, about 25 amino acids to about 1300 amino acids, about 30 amino acids to about 1300 amino acids, about 40 amino acids to about 1300 amino acids, about 50 amino acids to about 1300 amino acids, about 60 amino acids to about 1300 amino acids, about 70 amino acids to about 1300 amino acids, about 75 amino acids to about 1300 amino acids, about 80 amino acids to about 1300 amino acids, about 90 amino acids to about 1300 amino acids, about 100 amino acids to about 1300 amino acids, about 5 amino acids to about 1200 amino acids, about 10 amino acids to about 1200 amino acids, about 15 amino acids to about 1200 amino acids, about 20 amino acids to about 1200 amino acids, about 25 amino acids to about 1200 amino acids, about 30 amino acids to about 1200 amino acids, about 40 amino acids to about 1200 amino acids, about 50 amino acids to about 1200 amino acids, about 60 amino acids to about 1200 amino acids, about 70 amino acids to about 1200 amino acids, about 75 amino acids to about 1020 amino acids, about 80 amino acids to about 1200 amino acids, about 90 amino acids to about 1200 amino acids, about 100 amino acids to about 1200 amino acids, about 5 amino acids to about 1100 amino acids, about 10 amino acids to about 1100 amino acids, about 15 amino acids to about 1100 amino acids, about 20 amino acids to about 1100 amino acids, about 25 amino acids to about 1100 amino acids, about 30 amino acids to about 1100 amino acids, about 40 amino acids to about 1100 amino acids, about 50 amino acids to about 1100 amino acids, about 60 amino acids to about 1100 amino acids, about 70 amino acids to about 1100 amino acids, about 75 amino acids to about 1100 amino acids, about 80 amino acids to about 1100 amino acids, about 90 amino acids to about 1100 amino acids, about 100 amino acids to about 1100 amino acids, about 5 amino acids to about 1000 amino acids, about 10 amino acids to about

1000 amino acids, about 15 amino acids to about 1000 amino acids, about 20 amino acids to about 1000 amino acids, about 25 amino acids to about 1000 amino acids, about 30 amino acids to about 1000 amino acids, about 40 amino acids to about 1000 amino acids, about 50 amino acids to about 1000 amino acids, about 60 amino acids to about 1000 amino acids, about 70 amino acids to about 1000 amino acids, about 75 amino acids to about 1000 amino acids, about 80 amino acids to about 1000 amino acids, about 90 amino acids to about 1000 amino acids, about 100 amino acids to about 1000 amino acids, about 5 amino acids to about 900 amino acids, about 10 amino acids to about 900 amino acids, about 15 amino acids to about 900 amino acids, about 20 amino acids to about 900 amino acids, about 25 amino acids to about 900 amino acids, about 30 amino acids to about 900 amino acids, about 40 amino acids to about 900 amino acids, about 50 amino acids to about 900 amino acids, about 60 amino acids to about 900 amino acids, about 70 amino acids to about 900 amino acids, about 75 amino acids to about 900 amino acids, about 80 amino acids to about 900 amino acids, about 90 amino acids to about 900 amino acids, about 100 amino acids to about 900 amino acids, about 5 amino acids to about 800 amino acids, about 10 amino acids to about 800 amino acids, about 15 amino acids to about 800 amino acids, about 20 amino acids to about 800 amino acids, about 25 amino acids to about 800 amino acids, about 30 amino acids to about 800 amino acids, about 40 amino acids to about 800 amino acids, about 50 amino acids to about 800 amino acids, about 60 amino acids to about 800 amino acids, about 70 amino acids to about 800 amino acids, about 75 amino acids to about 800 amino acids, about 80 amino acids to about 800 amino acids, about 90 amino acids to about 800 amino acids, about 100 amino acids to about 800 amino acids, about 5 amino acids to about 700 amino acids, about 10 amino acids to about 700 amino acids, about 15 amino acids to about 700 amino acids, about 20 amino acids to about 700 amino acids, about 25 amino acids to about 700 amino acids, about 30 amino acids to about 700 amino acids, about 40 amino acids to about 700 amino acids, about 50 amino acids to about 700 amino acids, about 60 amino acids to about 700 amino acids, about 70 amino acids to about 700 amino acids, about 75 amino acids to about 700 amino acids, about 80 amino acids to about 700 amino acids, about 90 amino acids to about 700 amino acids, about 100 amino acids to about 700 amino acids, about 5 amino acids to about 600 amino acids, about 10 amino acids to about 600 amino acids, about 15 amino acids to about 600 amino acids, about 20 amino acids to about 600 amino acids, about 25 amino acids to about 600 amino acids, about 30 amino acids to about 600 amino acids, about 40 amino acids to about 600 amino acids,

about 50 amino acids to about 600 amino acids, about 60 amino acids to about 600 amino acids, about 70 amino acids to about 600 amino acids, about 75 amino acids to about 600 amino acids, about 80 amino acids to about 600 amino acids, about 90 amino acids to about 600 amino acids, about 100 amino acids to about 600 amino acids, about 5 amino acids to about 500 amino acids, about 10 amino acids to about 500 amino acids, about 15 amino acids to about 500 amino acids, about 20 amino acids to about 500 amino acids, about 25 amino acids to about 500 amino acids, about 30 amino acids to about 500 amino acids, about 40 amino acids to about 500 amino acids, about 50 amino acids to about 500 amino acids, about 60 amino acids to about 500 amino acids, about 70 amino acids to about 500 amino acids, about 75 amino acids to about 500 amino acids, about 80 amino acids to about 500 amino acids, about 90 amino acids to about 500 amino acids, about 100 amino acids to about 500 amino acids, about 5 amino acids to about 400 amino acids, about 10 amino acids to about 400 amino acids, about 15 amino acids to about 400 amino acids, about 20 amino acids to about 400 amino acids, about 25 amino acids to about 400 amino acids, about 30 amino acids to about 400 amino acids, about 40 amino acids to about 400 amino acids, about 50 amino acids to about 400 amino acids, about 60 amino acids to about 400 amino acids, about 70 amino acids to about 400 amino acids, about 75 amino acids to about 400 amino acids, about 80 amino acids to about 400 amino acids, about 90 amino acids to about 400 amino acids, about 100 amino acids to about 400 amino acids, about 5 amino acids to about 300 amino acids, about 10 amino acids to about 300 amino acids, about 15 amino acids to about 300 amino acids, about 20 amino acids to about 300 amino acids, about 25 amino acids to about 300 amino acids, about 30 amino acids to about 300 amino acids, about 40 amino acids to about 300 amino acids, about 50 amino acids to about 300 amino acids, about 60 amino acids to about 300 amino acids, about 70 amino acids to about 300 amino acids, about 75 amino acids to about 300 amino acids, about 80 amino acids to about 300 amino acids, about 90 amino acids to about 300 amino acids, about 100 amino acids to about 300 amino acids, about 5 amino acids to about 200 amino acids, about 10 amino acids to about 200 amino acids, about 15 amino acids to about 200 amino acids, about 20 amino acids to about 200 amino acids, about 25 amino acids to about 200 amino acids, about 30 amino acids to about 200 amino acids, about 40 amino acids to about 200 amino acids, about 50 amino acids to about 200 amino acids, about 60 amino acids to about 200 amino acids, about 70 amino acids to about 200 amino acids, about 75 amino acids to about 200 amino acids, about 80 amino acids to about 200 amino acids, about 90 amino acids to about 200 amino acids,

about 100 amino acids to about 200 amino acids, about 5 amino acids to about 100 amino acids, about 10 amino acids to about 100 amino acids, about 15 amino acids to about 100 amino acids, about 20 amino acids to about 100 amino acids, about 25 amino acids to about 100 amino acids, about 30 amino acids to about 100 amino acids, about 40 amino acids to about 100 amino acids, about 50 amino acids to about 100 amino acids, about 60 amino acids to about 100 amino acids, about 70 amino acids to about 100 amino acids, about 75 amino acids to about 100 amino acids, about 80 amino acids to about 100 amino acids, or about 90 amino acids to about 100 amino acids.

[0311] In some aspects, the spike protein comprises the RBD of a spike protein of a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) or any fragments or variants thereof. In other aspect, the spike protein comprises amino acids 319-542 of SEQ ID NO:15 or amino acids 306 to 528 of SEQ ID NO:16. In yet another aspect, the RBD protein has the sequence of RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFK CYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWN SNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGF QPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFX_{aa} (SEQ ID NO:17) or a fragment or variant thereof, wherein X_{aa}, is absent or present, and, if present, is an N or S.

[0312] A "fragment" of SEQ ID NO:17 refers to a protein or polypeptide that comprises a part that is less than the entirety of SEQ ID NO:17. A fragment of SEQ ID NO:17 can comprise from about 5 to about 200 contiguous amino acids. In another aspect, a fragment of SEQ ID NO:17 comprises at least about 5 contiguous amino acids of SEQ ID NO:17, at least about 10 contiguous amino acids of SEQ ID NO:17, at least about 15 contiguous amino acids of SEQ ID NO:17, at least about 20 contiguous amino acids of SEQ ID NO:17, at least about 25 contiguous amino acids of SEQ ID NO:17, at least about 30 contiguous amino acids of SEQ ID NO:17, at least about 35 contiguous amino acids of SEQ ID NO:17, at least about 40 contiguous amino acids of SEQ ID NO:17, at least about 45 contiguous amino acids of SEQ ID NO:17, at least about 50 contiguous amino acids of SEQ ID NO:17, at least about 55 contiguous amino acids of SEQ ID NO:17, at least about 60 contiguous amino acids of SEQ ID NO:17, at least about 65 contiguous amino acids of SEQ ID NO:17, at least about 70 contiguous amino acids of SEQ ID NO:17, at least about 75 contiguous amino acids of SEQ ID NO:17, at least about 80 contiguous amino acids of SEQ ID NO:17, at least about 85 contiguous amino acids of SEQ ID NO:17, at

least about 90 contiguous amino acids of SEQ ID NO:17, at least about 95 contiguous amino acids of SEQ ID NO:17, at least 100 contiguous amino acids of SEQ ID NO:17, at least about 105 contiguous amino acids of SEQ ID NO:17, at least about 110 contiguous amino acids of SEQ ID NO:17, at least about 115 contiguous amino acids of SEQ ID NO:17, at least about 120 contiguous amino acids of SEQ ID NO:17, at least about 125 contiguous amino acids of SEQ ID NO:17, at least about 130 contiguous amino acids of SEQ ID NO:17, at least about 135 contiguous amino acids of SEQ ID NO:17, at least about 140 contiguous amino acids of SEQ ID NO:17, at least about 145 contiguous amino acids of SEQ ID NO:17, at least about 150 contiguous amino acids of SEQ ID NO:17, at least about 55 contiguous amino acids of SEQ ID NO:17, at least about 160 contiguous amino acids of SEQ ID NO:17, at least about 165 contiguous amino acids of SEQ ID NO:17, at least about 170 contiguous amino acids of SEQ ID NO:17, at least about 175 contiguous amino acids of SEQ ID NO:17, at least about 180 contiguous amino acids of SEQ ID NO:17, at least about 85 contiguous amino acids of SEQ ID NO:17, at least about 190 contiguous amino acids of SEQ ID NO:17, at least about 95 contiguous amino acids of SEQ ID NO:17, at least 200 contiguous amino acids of SEQ ID NO:17 or at least about 205 contiguous amino acids of SEQ ID NO:17.

[0313] In yet another aspect, the RBD protein has the sequence of RVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTF FSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFMG CVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPDGKPCTPPALNCYWP LNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIKNQCVNF X_{aa} (SEQ ID NO:18) or a fragment or variant thereof, wherein X_{aa} is either absent or present, and, if present, is a N or S. A "fragment" of SEQ ID NO:18 refers to a protein or polypeptide that comprises a part that is less than the entirety of SEQ ID NO:18. A fragment of SEQ ID NO:18 can comprise from about 5 to about 200 contiguous amino acids. In another aspect, a fragment of SEQ ID NO:18 comprises at least about 5 contiguous amino acids of SEQ ID NO:18, at least about 10 contiguous amino acids of SEQ ID NO:18, at least about 15 contiguous amino acids of SEQ ID NO:18, at least about 20 contiguous amino acids of SEQ ID NO:18, at least about 25 contiguous amino acids of SEQ ID NO:18, at least about 30 contiguous amino acids of SEQ ID NO:18, at least about 35 contiguous amino acids of SEQ ID NO:18, at least about 40 contiguous amino acids of SEQ ID NO:18, at least about 45 contiguous amino acids of SEQ ID NO:18, at least

about 50 contiguous amino acids of SEQ ID NO:18, at least about 55 contiguous amino acids of SEQ ID NO:18, at least about 60 contiguous amino acids of SEQ ID NO:18, at least about 65 contiguous amino acids of SEQ ID NO:18, at least about 70 contiguous amino acids of SEQ ID NO:18, at least about 75 contiguous amino acids of SEQ ID NO:18, at least about 80 contiguous amino acids of SEQ ID NO:18, at least about 85 contiguous amino acids of SEQ ID NO:18, at least about 90 contiguous amino acids of SEQ ID NO:18, at least about 95 contiguous amino acids of SEQ ID NO:18, at least 100 contiguous amino acids of SEQ ID NO:18, at least about 105 contiguous amino acids of SEQ ID NO:18, at least about 110 contiguous amino acids of SEQ ID NO:18, at least about 115 contiguous amino acids of SEQ ID NO:18, at least about 120 contiguous amino acids of SEQ ID NO:18, at least about 125 contiguous amino acids of SEQ ID NO:18, at least about 130 contiguous amino acids of SEQ ID NO:18, at least about 135 contiguous amino acids of SEQ ID NO:18, at least about 140 contiguous amino acids of SEQ ID NO:18, at least about 145 contiguous amino acids of SEQ ID NO:18, at least about 150 contiguous amino acids of SEQ ID NO:18, at least about 55 contiguous amino acids of SEQ ID NO:18, at least about 160 contiguous amino acids of SEQ ID NO:18, at least about 165 contiguous amino acids of SEQ ID NO:18, at least about 180 contiguous amino acids of SEQ ID NO:18, at least about 185 contiguous amino acids of SEQ ID NO:18, at least about 180 contiguous amino acids of SEQ ID NO:18, at least about 85 contiguous amino acids of SEQ ID NO:18, at least about 190 contiguous amino acids of SEQ ID NO:18, at least about 95 contiguous amino acids of SEQ ID NO:18, at least 200 contiguous amino acids of SEQ ID NO:18 or at least about 205 contiguous amino acids of SEQ ID NO:18.

[0314] In some aspects, the at least one isolated polypeptide is a fusion protein comprising at least all or at least a portion of at least one β-coronavirus (such as SARS-CoV or SARS-CoV-2) isolated spike protein or variant thereof. In another aspect, the fusion protein may comprise all or at least portion (e.g., at least 5 amino acids or more) of the S1 polypeptide, S2 polypeptide, and/or RBD of a spike protein from one strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) operably linked, fused or grafted directly or indirectly (such as through one or more linking peptide sequences and/or HIS tags) to all or at least a portion (at least 5 amino acids or more) of a S1 polypeptide, S2 polypeptide, and/or RBD from the same or different strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). By way of example, in one aspect, the fusion protein may comprise all or at least a portion (e.g., at least 5 amino acids or more) of the RBD of a spike

protein from one strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) operably linked, fused or grafted directly or indirectly (such as through one or more linking peptide sequences and/or HIS tags) to all or at least a portion (at least 5 amino acids or more) of RBD of a spike protein from the same or different strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). In another aspect, the fusion protein may comprise all or at least portion (e.g., at least 5 amino acids or more) of the NBD of a spike protein from one strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) operably linked, fused or grafted directly or indirectly (such as through one or more linking peptide sequences and/or HIS tags) to all or at least a portion (at least 5 amino acids or more) of a S1 or S2 polypeptide of a spike protein from the same or different strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). Other variations are possible. For example, in other aspects, the fusion protein can comprise an epitope for the monoclonal antibody CR3022 (described in U.S. Patent No. 8,106,170, ter Meulen, et al., *PLOS Medicine*, 3(7):1071-1079 (July 2006) and Yuan et a., *Science*, published on-line on April 3, 2020 (10.1126/science.abb7269) the contents of which are herein incorporated by reference) fused at the N-terminal end of the RBD (also referred to herein as an "epitope-grafted RBD" fusion protein or peptide). In this example, a fusion protein comprising methionine as the starting amino acid can be operably linked or grafted on to an epitope (such as, for example, through one or more linking peptide sequences and/or HIS tags) comprising at least amino acids YNST (SEQ ID NO:8), YNSA (SEQ ID NO:9), DDFM (SEQ ID NO:10), DDFT (SEQ ID NO:11), FSTFKCYGVSATK (SEQ ID NO:12), FSTFKCYGVSPTK (SEQ ID NO:13) ATSTGNYNYKYRYLRHGKLR (SEQ ID NO:19) and/or YTTTGIGYQPYRVVLSFEL (SEQ ID NO:20) which in turn, are operably linked or grafted (such as, for example, through one or more linking peptide sequences and/or HIS tags) on to the N-terminal end of the RBD, e.g., amino acids 319-541 of SEQ ID NO:15 or amino acids 306 to 527 of SEQ ID NO:16 (or any analogous region of SEQ ID NOS: 15 or 16). It is well known that in some proteins the initiator residue methionine is cleaved off by an enzyme since it may not be present in the mature polypeptide/protein.

[0315] In some aspects the at least one first specific binding partner (e.g., capture reagent) comprises all or at least one portion of at least one isolated polypeptide or variant thereof from a nucleocapsid protein (e.g., NTD and/or CTD) or variant thereof from one strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) and all or at least a portion of at least one isolated

polypeptide or variant thereof from a spike protein (e.g., S1 polypeptide, RBD and/or S2 polypeptide) or variant thereof from the same or different strain of β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). The nucleocapsid protein or variant thereof and spike protein or variant thereof used in said binding member can have the lengths as described previously herein.

At least one Second Specific Binding Partner

[0316] The at least one second specific binding partner comprising at least detectable label can be either at least one recombinant antigen or at least one antibody. In some aspects, the at least one second specific binding partner is at least one recombinant antigen which comprises all or at least a portion of at least one isolated polypeptide from (a) a nucleocapsid protein or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) a spike protein or a variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); or (c) a nucleocapsid protein and spike protein or a variant of a nucleocapsid protein and/or a spike protein from a β-coronavirus (such as SARS-CoV or SARS-CoV-2), which can be from the same β-coronavirus (such as SARS-CoV or SARS-CoV-2) or from a different β-coronavirus (such as SARS-CoV or SARS-CoV-2). The isolated polypeptides used in the recombinant antigens described previously herein for the at least one second specific binding partner can also be used with the second specific binding partner (e.g., derived from the same materials, have the same lengths, etc). Moreover, the isolated polypeptides used in the recombinant antigen of the second specific binding partner can be the same or different than the isolated polypeptides used in the recombinant antigen of the first specific binding partner.

[0317] Alternatively, the at least one second specific binding partner can comprise at least one antibody that specifically binds to the anti-β-coronavirus antibody (e.g., analyte) contained in the at least one first specific binding partner-anti-β-coronavirus antibody complex. The antibody used as the at least one second specific binding partner is not critical and can be a polyclonal antibody, a monoclonal antibody, a humanized antibody, a chimeric antibody, a fully human antibody, a bispecific antibody or a multi-specific antibody. In one aspect, the antibody used as at least one second specific binding partner can be an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM (e.g., anti-human-IgM IgG) antibody or an anti-species IgG (e.g., anti-human-IgG IgG) and anti-species IgM (e.g., anti-human-IgM IgG) antibody. For example, in some aspects, the antibody used as the at least one second specific binding partner

can be a mouse IgG antibody, a mouse IgM antibody, a mouse IgG and mouse IgM antibody, a rat IgG antibody, a rat IgM antibody, a rat IgG and rat IgM antibody, a rabbit IgG antibody, a rabbit IgM antibody, a rabbit IgG and rabbit IgM antibody, a goat IgG antibody, a goat IgM antibody, a goat IgG and goat IgM antibody, a sheep IgG antibody, a sheep IgM antibody, a sheep IgG and sheep IgM antibody, a non-human primate IgG antibody, a non-human primate IgM antibody, a non-human primate IgG and non-human primate IgM antibody, a human IgG antibody, a human IgM antibody or a human IgG and human IgM antibody. In one aspect, the at least one second specific binding partner can be anti-human IgG (mouse monoclonal) antibody available in the ARCHITECT®/Alinity® I Rubella IgG assay (Abbott Laboratories, Abbott Park, IL), although any other commercially available anti-species IgG (e.g., anti-human-IgG IgG) antibody can be used. In another aspect, the at least one second specific binding partner can be the murine anti-human IgM antibody available in the ARCHITECT®/Alinity® I Rubella IgM assay (Abbott Laboratories, Abbott Park, IL) , although any other commercially available anti-species IgG (e.g., anti-human-IgG IgG) antibody can be used. In some further aspects, the method comprises at least a third specific binding partner which is an anti-species IgG (e.g., anti-human-IgG IgG) and/or anti-species IgM (e.g., anti-human-IgM IgG) antibody and at least a fourth specific binding partner which is an anti-species IgG (e.g., anti-human-IgG IgG) and/or anti-species IgM (e.g., anti-human-IgM IgG) antibody.

[0318] The β-coronavirus (such as SARS-CoV or SARS-CoV-2) antibody captured in the first specific binding partner-anti-β-coronavirus antibody-second specific binding partner complex can be an anti-β-coronavirus IgG antibody, an anti-β-coronavirus IgM antibody, or an anti-β-coronavirus IgG and anti-β-coronavirus IgM antibody. In one aspect, the antibody analyte is an anti-SARS-CoV IgG antibody, an anti-SARS-CoV IgM antibody, an anti-SARS-CoV-2 IgG antibody and/or an anti-SARS-CoV-2 IgM antibody. For example, in some aspects, the methods described herein involve detecting at least one anti-SARS-CoV-2 IgG antibody in at least one biological sample obtained from a subject wherein the at least one first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID

NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2 and specifically binds to at least one anti-SARS-CoV-2 IgG antibody and the second specific binding partner is at least one anti-species IgG (e.g., anti-human-IgG IgG) antibody. In yet other aspects, the methods described herein involve detecting at least one anti-SARS-CoV-2 IgM antibody in at least one biological sample obtained from a subject wherein the at least one first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2 and specifically binds to at least one anti-SARS-CoV-2 IgM antibody and the second specific binding partner is at least one anti-species IgM (e.g., anti-human-IgM IgG) antibody.

[0319] In yet other aspects, the methods described herein involve detecting at least one anti-SARS-CoV-2 IgM antibody in at least one biological sample obtained from a subject wherein the at least one first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17 and specifically binds to at least one anti-SARS-CoV-2 IgM antibody, and the second specific binding partner is at least one anti-species IgM (e.g., anti-human-IgM IgG) antibody. In still yet other aspects, the methods described herein involve detecting at least one anti-SARS-CoV-2 IgG antibody in at least one biological sample obtained from a subject wherein the at least one first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17 and specifically binds to at least one anti-SARS-CoV-2 IgG antibody, and the second specific binding partner is at least one anti-species IgG (e.g., anti-human-IgG IgG) antibody.

[0320] In yet other aspects, the methods described herein involve determining the presence of or amount, level and/or concentration (e.g., quantitating or semi-quantitating) of at least one anti-SARS-CoV-2 IgG antibody in at least one biological sample obtained from a subject wherein the at least one first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15

or SEQ ID NO:17 and specifically binds to at least one anti-SARS-CoV-2 IgG antibody, and the second specific binding partner is at least one anti-species IgG (e.g., anti-human-IgG IgG) antibody. When determining the presence of or amount, level and/or concentration of at least one anti-SARS-CoV-2 IgG antibody in a biological sample, the dynamic range of the method can be improved by diluting the biological sample either prior to or before performing the method. While not wishing to be bound by any theory, it is conceivable that dilution of the biological sample prior to (i.e., or before) performing the method serves to prevent saturation of antigen binding sites on a solid support where an antibody or other molecule comprising antigen binding sites is employed for capture. In some aspects, the dilution occurs prior to performing the methods herein, e.g., as a separate dilution step. In other aspects, the dilution occurs during the performance of the method, e.g., where an automated instrument is employed this may be done on the instrument itself. For example, the biological sample can be diluted about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 10-fold, about 11-fold, about 12-fold, about 13-fold, about 15-fold, about 16-fold, about 17-fold, about 18-fold, about 19-fold, about 20-fold, about 21-fold, about 22-fold, about 23-fold, about 24-fold, about 25-fold, about 26-fold, about 27-fold, about 28-fold, about 29-fold, about 30-fold, about 31-fold, about 32-fold, about 33-fold, about 34-fold, about 35-fold, about 36-fold, about 37-fold, about 38-fold, about 39-fold, about 40-fold, about 41-fold, about 42-fold, about 43-fold, about 44-fold, about 45-fold, about 46-fold, about 47-fold, about 48-fold, about 49-fold, about 50-fold, about 100-fold, or greater prior to use (either before performing the method or during the performance of the method). In certain aspects, the biological sample is diluted about 1-fold. In other aspects, the biological sample is diluted about 5-fold. In yet other aspects, the biological sample is diluted about 10-fold. In still yet further aspects, the biological sample is diluted about 15-fold. In yet other aspects, the biological sample is diluted about 20-fold. In still yet further aspects, the biological sample is diluted about 25-fold. In yet further aspects the biological sample is diluted about 30-fold. In still yet further aspects, the biological sample is diluted about 35-fold.

[0321] In some aspects, the method described herein can be used for the qualitative and quantative determination of IgG antibodies to SARS-CoV-2 in a biological sample, such as, for use an an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. In other aspects, the methods described herein are used for the quantating or semi-quantitating of IgG antibodies to SARS-CoV-2 in a biological sample. In

these aspects, the method described herein is capable of quantifying about 95%, about 96%, about 97%, about 98% or about 99% of anti-SARS-CoV-2 IgG antibodies within an EMI with automated dilution. In yet other aspects, the method quantifies about 99% of anti-SARS-CoV-2 IgG antibodies within an EMI with automated dilution. In still further aspects, the method quantifies about 87%, about 88%, about 89%, about 90%, about 91%, about 92% or about 93% of anti-SARS-CoV-2 IgG antibodies within an ULMI with no dilution. In yet further aspects, the method quantifies about 91% of anti-SARS-CoV-2 IgG antibodies within an ULMI with no dilution. In still a further aspect, the method quantifies about 98% of anti-SARS-CoV-2 IgG antibodies within an ULMI with no dilution. In still yet further aspects, the ULMI of the methods of the present invention are about 2.0 times higher, about 2.5 times higher, about 3.0 times higher, about 4.0 times higher, about 5.0 times higher, about 10 times higher, about 15 times higher, about 20 times higher, or about 25 times higher than other methods known in the art. In some aspects, the ULMI of the methods of the present invention are about 2.7 times higher than other methods known in the art (unpublished data). In still further aspects, the ULMI is about 22 times higher than other methods known in the art (unpublished data). In still other aspects, the method described herein can be used as an aid in the diagnosis of SARS-CoV-2 infection in conjunction with clinical presentation and other laboratory tests. The method can also be used as an aid in evaluating immune status of SARS-CoV-2 infected individuals and to monitor antibody response to individuals that have received one or more SARS-CoV-2 vaccines.

[0322] In yet other aspects, the methods described herein involve determining the presence of or amount, level and/or concentration (e.g., quantitating or semi-quantitating) of at least one anti-SARS-CoV-2 IgG antibody in at least one biological sample obtained from a subject wherein the at least one first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17 and specifically binds to at least one anti-SARS-CoV-2 IgG antibody, and the second specific binding partner is at least one anti-species IgG (e.g., anti-human-IgG IgG) antibody. When determining the amount, level and/or concentration of at least one anti-SARS-CoV-2 IgG antibody in a biological sample, the dynamic range of the method optionally can be improved by diluting the biological sample either prior to or before performing the method. While not wishing to be bound by any theory, it is conceivable that dilution of the biological sample prior to (i.e., or before) performing the method serves to prevent saturation of antigen

binding sites on one or more solid supports (e.g., where an antibody or other molecule comprising antigen binding sites is employed for capture). In some aspects, the dilution occurs prior to performing the methods herein, e.g., as a separate dilution step. In other aspects, the dilution occurs during the performance of the method, e.g., where an automated instrument is employed this may be done on the instrument itself. In some aspects, the dilution is performed by mixing about 10 μL (microliter) of the biological sample (e.g., original biological sample) and about 290 μL of at least one buffer (such as for example, an assay buffer, a wash buffer, etc.) to form a biological sampling mixture (e.g., for a total volume of 300 μL). 10 μL of the total volume of the biological sampling mixture (e.g., about 0.033 μL of the original biological sample (10 μL/300 μL)) is removed and then used or employed in the performance of the method (0.033 μL x 10 μL = 0.33 μL of biological sample). In some aspects, the method described herein is capable of quantifying about 95%, about 96%, about 97%, about 98% or about 99% of anti-SARS-CoV-2 IgG antibodies within an EMI with automated dilution. In yet other aspects, the method quantifies about 99% of anti-SARS-CoV-2 IgG antibodies within an EMI with automated dilution. In other aspects, the method quantifies about 87%, about 88%, about 89%, about 90%, about 91%, about 92% or about 93% of anti-SARS-CoV-2 IgG antibodies within an ULMI with no dilution. In yet further aspect, the method quantifies about 91% of anti-SARS-CoV-2 IgG antibodies within an ULMI with no dilution. Additionally, in some aspects, the method described herein can be used for the qualitative and quantitative determination of IgG antibodies to SARS-CoV-2 in human serum in plasma. In other aspects, the method described herein can be used as an aid in the diagnosis of SARS-CoV-2 infection in conjunction with clinical presentation and other laboratory tests. The method can also be used as an aid in evaluating immune status of SARS-CoV-2 infected individuals and to monitor antibody response to individuals that have received one or more SARS-CoV-2 vaccines.

[0323] In still yet other aspects of the methods described herein, the methods further comprise detecting at least one anti-SARS-CoV-2 IgG neutralizing antibody, such as, for an example, at least one anti-SARS-CoV-2 IgG neutralizing antibody. Specifically, in this aspect, the methods described herein have been found to demonstrate high qualitative agreement with a plaque reduction neutralization assay. Specifically, increasing amounts of anti-SARS-CoV-2 IgG antibodies detected by the methods described herein have been found to be associated with increasing amounts of anti-SARS-CoV-2 neutralizing antibodies (e.g., anti-SARS-CoV-2 IgG

neutralizing antibodies). In this aspect, levels of anti-SARS-CoV-2 IgG antibodies of at least about 4100 AU/mL (at least about 582 BAU/mL), about 4120 AU/mL (about 585 BAU/mL), about 4130 AU/mL (about 586 BAU/mL), about 4140 AU/mL (about 587 BAU/mL), about 4150 AU/mL (about 589 BAU/mL), about 4160 AU/mL (about 590 BAU/mL), about 4170 AU/mL (about 592 BAU/mL), about 4180 AU/mL (about 593 BAU/mL), about 4190 AU/mL (about 594 BAU/mL), or about 4200 AU/mL (about 596 BAU/mL) have been determined to correlate with neutralization. For example, levels of anti-SARS-CoV-2 IgG antibodies of at least 4160 AU/mL (about 590 BAU/mL) used as a cut-off or threshold have been found to correspond to an about 0.95 probability of obtaining a plaque reduction neutralization test ID50 at a 1:250 dilution. Additionally, the probability profile of the methods described herein correspond to high titer levels in the plaque reduction neutralization assay such that there is a high probability of the levels of anti-SARS-CoV-2 IgG antibodies determined by the methods being at or above the levels of anti-SARS-CoV-2 IgG neutralizing antibodies determined in the plaque reduction neutralization assay (See, Example 9). Finally, in still a further aspect the methods described herein have been found to demonstrate high qualitative agreement with an ACE2 binding inhibition assay (See, Example 10).

[0324] In further aspects, the methods described herein employ a small volume of biological sample (e.g., which can be obtained via dilution as described in the immediately preceding paragraph). The small volume of biological sample used in the method is from about 0.30 µL to about 0.40 µL. In some aspects, the small volume of biological sample used in the method is at least about 0.30 µL, at least about 0.31 µL, at least about 0.32 µL, at least about 0.33 µL, at least about 0.34 µL, at least about 0.35 µL, at least about 0.36 µL, at least about 0.37 µL, at least about 0.38 µL, at least about 0.39 µL, or at least about 0.40 µL.

[0325] In some aspects, when the method uses only a first specific binding partner and a second specific binding partner, either specific binding partner can be immobilized on a solid support. For example in some aspects, the first specific binding partner can be immobilized on the solid support. In other aspects, when more than two specific binding partners are used in the method (e.g., a first specific binding partner, a second specific binding partner, a third specific binding partner, a fourth specific binding partner, any or all of the binding members can be immobilized on solid support).

[0326] In some aspects, the method further comprises a third specific binding partner, such as a second capture reagent (e.g., second, third or fourth recombinant antigen) or a second, third, or fourth detection reagent (e.g., recombinant antigen and/or antibody). In some aspects, the method further comprises (i) at least one first specific binding partner comprising at least one isolated polypeptide from a C-terminal domain of a nucleocapsid protein from a β-coronavirus (such as SARS-CoV or SARS-CoV-2) wherein said first specific binding partner specifically binds to (a) an IgG antibody; (b) an IgM antibody; or (b) both an IgG and IgM antibody; and (ii) at least one second specific binding partner comprising isolated polypeptide from a receptor binding domain (RBD) of a spike protein from a β-coronavirus (such as SARS-CoV or SARS-CoV-2), wherein said second specific binding partner specifically binds to (a) an IgG antibody; and (b) an IgM antibody; or (b) both an IgG and IgM antibody. For example, in one aspect, the method involves (i) at least one first specific binding partner comprising at least one isolated polypeptide from a C-terminal domain of a nucleocapsid protein from SARS-CoV-2 (e.g., SEQ ID NO:1), wherein said first specific binding partner specifically binds to an IgG antibody; (ii) at least one second specific binding partner comprising an isolated polypeptide from a receptor binding domain (RBD) of a spike protein from SARS-CoV-2 (e.g., amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17), wherein said second specific binding partner specifically binds to an IgM antibody; and (iii) at least one third specific binding partner comprising a detectable label. In some aspects, the at least one first specific binding partner and the at least one second specific binding partner may be immobilized on the same solid support. In other aspects, the at least one first specific binding partner and the at least one second specific binding partner may be immobilized on different solid supports. In other aspects, when the at least one first specific binding partner and at least one second specific binding partner are immobilized on the same solid support, the amount or ratio of at least one first specific binding partner and at least one second specific binding partner can be optimized using routine techniques known in the art to achieve the desired sensitivity and specificity for the IgG, IgM and/or IgG and IgM antibodies to be detected or determined (e.g., amount or concentration determined).

[0327] In yet another aspect, the method involves (i) at least one first specific binding partner comprising at least one isolated polypeptide from a C-terminal domain of a nucleocapsid protein from SARS-CoV-2 (e.g., SEQ ID NO:1), wherein said first specific binding partner specifically binds to an IgG antibody; (ii) at least one second specific binding partner comprising an isolated

polypeptide from a receptor binding domain (RBD) of a spike protein from SARS-CoV-2 (e.g., amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17), wherein said second specific binding partner specifically binds to an IgM antibody; (iii) at least one third specific binding partner comprising a detectable label; and (iv) at least one fourth specific binding partner comprising a detectable label. In some aspects, the at least one first specific binding partner and the at least one second specific binding partner may be immobilized on the same solid support.

[0328] In other aspects, the at least one first specific binding partner and the at least one second specific binding partner may be immobilized on different solid supports. In other aspects, when the at least one first specific binding partner and at least one second specific binding partner are immobilized on the same solid support, the amount or ratio of at least one first specific binding partner and at least one second specific binding partner can be optimized using routine techniques known in the art to achieve the desired sensitivity and specificity for the IgG, IgM and/or IgG and IgM antibodies to be detected or determined (e.g., amount or concentration determined). In some aspects, the detectable label used for the third specific binding partner and the fourth specific binding partner can be the same label or can be a different label.

[0329] In some embodiments, the biological sample is diluted or undiluted either prior to or during the performance of the method. In some aspects, the dilution occurs prior to performing the methods. In other aspects, the dilution occurs during the performance of the method. In one aspect, the sample can be from about 1 to about 25 microliters, about 1 to about 24 microliters, about 1 to about 23 microliters, about 1 to about 22 microliters, about 1 to about 21 microliters, about 1 to about 20 microliters, about 1 to about 18 microliters, about 1 to about 17 microliters, about 1 to about 16 microliters, about 15 microliters or about 1 microliter, about 2 microliters, about 3 microliters, about 4 microliters, about 5 microliters, about 6 microliters, about 7 microliters, about 8 microliters, about 9 microliters, about 10 microliters, about 11 microliters, about 12 microliters, about 13 microliters, about 14 microliters, about 15 microliters, about 16 microliters, about 17 microliters, about 18 microliters, about 19 microliters, about 20 microliters, about 21 microliters, about 22 microliters, about 23 microliters, about 24 microliters or about 25 microliters. In some embodiments, the sample is from about 1 to about 150 microliters or less or from about 1 to about 25 microliters or less. Alternatively, in another aspect, the biological sample can be diluted about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, about

6-fold, about 10-fold, about 11-fold, about 12-fold, about 13-fold, about 15-fold, about 16-fold, about 17-fold, about 18-fold, about 19-fold, about 20-fold, about 21-fold, about 22-fold, about 23-fold, about 24-fold, about 25-fold, about 26-fold, about 27-fold, about 28-fold, about 29-fold, about 30-fold, about 31-fold, about 32-fold, about 33-fold, about 34-fold, about 35-fold, about 36-fold, about 37-fold, about 38-fold, about 39-fold, about 40-fold, about 41-fold, about 42-fold, about 43-fold, about 44-fold, about 45-fold, about 46-fold, about 47-fold, about 48-fold, about 49-fold, about 50-fold, about 100-fold, or greater prior to use (either before performing the method or during the performance of the method).

In some aspects, the methods described herein can be performed without dilution and the ULMI can be from about 15,500 to about 40,000 AU/mL and/or the EMI can be from about 40,000 to about 80,000 AU/mL. In yet other aspects, the methods can be performed without dilution and the ULMI can be from about 15,500 to about 25,000 AU/mL and/or the EMI can be from about 25,000 to about 50,000 AU/mL (See, FIG. 13). In still further aspects, the methods described herein can be performed without dilution and the ULMI can be from about 7.1 to about 5680 BAU/mL. In other words, the methods herein can be employed to detect SARS-CoV-2 IgG at lower levels (e.g., from about 7.1 to about 5680 BAU/mL) without a need for further dilution.

[0330] EMI and ULMI optionally can be assessed, e.g., on an Abbott ARCHITECT® automated analyzer.

[0331] Other methods of detection include the use of or can be adapted for use on a nanopore device or nanowell device, e.g., for single molecule detection. Examples of nanopore devices are described in PCT International Application WO 2016/161402, which is hereby incorporated by reference in its entirety. Examples of nanowell device are described in PCT International Application WO 2016/161400, which is hereby incorporated by reference in its entirety. Other devices and methods appropriate for single molecule detection also can be employed.

[0332] The nature of methods described herein is not critical and the test can be any assay known in the art such as, for example, immunoassays, point-of-care assays, clinical chemistry assay, protein immunoprecipitation, immunoelectrophoresis, chemical analysis, SDS-PAGE and Western blot analysis, or protein immunostaining, electrophoresis analysis, a protein assay, a competitive binding assay, a lateral flow assay, a functional protein assay, or chromatography or spectrometry methods, such as high-performance liquid chromatography (HPLC) or liquid chromatography–mass spectrometry (LC/MS). Also, the assay can be employed in a clinical

chemistry format such as would be known by one of ordinary skill in the art. Such assays are described in further detail herein in Sections 9-14. It is known in the art that the values (e.g., reference levels, cutoffs, thresholds, specificities, sensitivities, concentrations of calibrators and/or controls etc.) used in an assay that employs specific sample type (e.g., such as an immunoassay that utilizes serum or a point-of-care device (that uses a point-of-care assay (e.g., which may be an immunoassay)) that employs whole blood) can be extrapolated to other assay formats using known techniques in the art, such as assay standardization. For example, one way in which assay standardization can be performed is by applying a factor to the calibrator employed in the assay to make the sample concentration read higher or lower to get a slope that aligns with the comparator method. Other methods of standardizing results obtained on one assay to another assay are well known and have been described in the literature (See, for example, David Wild, *Immunoassay Handbook*, 4th edition, chapter 3.5, pages 315-322, the contents of which are herein incorporated by reference).

SARS-CoV-2 Variants

[0333] It is known and expected that mutations are present or will occur within proteins such as the nucleocapsid and spike proteins of SARS-CoV-2. As described herein, these variants can be used in the methods, improved methods, kits and systems for detection of SARS-CoV-2. The methods, improved methods, kits and systems described herein also are used for detecting the presence of or determining an amount of anti-SARS-CoV-2 antibodies directed against either variant nucleocapsid or variant spike proteins (e.g., for detecting the presence of or determining an amount of anti-SARS-CoV-2 IgG antibody, anti-SARS-CoV-2 IgG antibody, or anti-SARS-CoV-2 IgG neutralizing antibody, where the antibody specifically binds to a variant nucleocapsid or variant spike protein). An example of a variant SARS-CoV-2 spike protein includes those described as occurring in a distinct phylogenetic cluster named lineage B.1.1.7, which includes a variant of the RBD of spike protein that comprises one or more amino acid substitutions including an amino acid substitution replacing asparagine with tyrosine at position 501 within amino acids 319 to 542 of SEQ ID NO:15 (also known as N501Y, and, as set out out in SEQ ID NO:25) (e.g., reported by Rambaut et al., Dec. 2020, https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563). Other examples of variant SARS-CoV-2 spike proteins include those

described as occurring in a phylogenetic lineage named 501Y.V2, which includes N501Y as well as other mutations, namely, one or more amino substitutions and/or deletions within amino acids 319 to 542 of SEQ ID NO:15 including replacing lysine with asparagine at amino acid position 417 (K417N); replacing lysine with threonine at amino acid position 417 (K417T); replacing leucine with arginine at amino acid position 452 (L452R); replacing serine with asparagine at amino acid position 477 (S477N); replacing glutamic acid with lysine at amino acid position 484 (E484K); or any combinations thereof and as set out in SEQ ID NO:25 (e.g., reported by Tegally et al. Dec. 22, 2200, medRxiv preprint doi: https://doi.org/10.1101/2020.12.21.202486400. Rambaut et al. also describe variant SARS-CoV-2 nucleocapsid proteins, including a variant of the nucleocapsid protein that comprises an amino acid substitution replacing serine with phenylalanine at position 235 within amino acids 210 to 419 of SEQ ID NO:2 (also known as S235F). Additionally, other substitutions and/or deletions in one or more positions of amino acids 210-419 of SEQ ID NO:2 include one or more of replacing methionine with isoleucine at amino acid position 234 (M234I); replacing lysine with asparagine at amino acid position 373 (K373N); replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); replacing alanine with threonine at amino acid position 376 (A376T) or any combinations thereof.

[0334] Thus, provided herein are methods wherein the anti-SARS-CoV-2 antibody detected or determined specifically binds to: a) a variant of the nucleocapsid protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 210-419 of SEQ ID NO:2 such as (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210 to 419 of SEQ ID NO:2 other than those recited in of (1)-(5); or SEQ ID NO:24; b) a variant of the RBD of a spike protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 319-542 of SEQ ID NO:15: (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6)

replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or SEQ ID NO:25; or c) a variant a variant of the nucleocapsid protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 210-419 of SEQ ID NO:2 such as (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210 to 419 of SEQ ID NO:2 other than those recited in of (1)-(5); or SEQ ID NO:24; and a variant of the RBD of a spike protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 319-542 of SEQ ID NO:15: (1) replacing lysine with asparagine at positions 417 (K417N); (2) replacing lysine with threonine at position 417 (K417T); (3) replacing leucine with arginine at position 452 (L452R); (4) replacing serine with asparagine at position 477 (S477N); (5) replacing glutamic acid with lysine at position 484 (E484K); (6) replacing asparagine with tyrosine at position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or SEQ ID NO:25.

[0335] Provided herein also are kits wherein the anti-β-coronavirus antibody detected or determined using the kits is an anti-SARS-CoV-2 antibody that specifically binds to: a) a variant of the nucleocapsid protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 210-419 of SEQ ID NO:2 such as (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in of (1)-(5); or SEQ ID NO:24; b) a variant of the RBD of a spike protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 319-542 of SEQ ID NO:15: (1) replacing lysine with asparagine at

amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or SEQ ID NO:25; or c) a variant a variant of the nucleocapsid protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 210-419 of SEQ ID NO:2 such as (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in of (1)-(5); or SEQ ID NO:24; and a variant of the RBD of a spike protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 319-542 of SEQ ID NO:15: (1) replacing lysine with asparagine at positions 417 (K417N); (2) replacing lysine with threonine at position 417 (K417T); (3) replacing leucine with arginine at position 452 (L452R); (4) replacing serine with asparagine at position 477 (S477N); (5) replacing glutamic acid with lysine at position 484 (E484K); (6) replacing asparagine with tyrosine at position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or SEQ ID NO:25.

[0336] Further provided herein are systems where the anti-β-coronavirus antibody detected by such systems is an anti-SARS-CoV-2 antibody that specifically binds to: a) a variant of the nucleocapsid protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 210-419 of SEQ ID NO:2 such as (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of

(1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210 to 419 of SEQ ID NO:2 other than those recited in of (1)-(5); or SEQ ID NO:24; b) a variant of the RBD of a spike protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 319-542 of SEQ ID NO:15: (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or SEQ ID NO:25; or c) a variant a variant of the nucleocapsid protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 210-419 of SEQ ID NO:2 such as (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210 to 419 of SEQ ID NO:2 other than those recited in of (1)-(5); or SEQ ID NO:24; and a variant of the RBD of a spike protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 319-542 of SEQ ID NO:15: (1) replacing lysine with asparagine at positions 417 (K417N); (2) replacing lysine with threonine at position 417 (K417T); (3) replacing leucine with arginine at position 452 (L452R); (4) replacing serine with asparagine at position 477 (S477N); (5) replacing glutamic acid with lysine at position 484 (E484K); (6) replacing asparagine with tyrosine at position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or SEQ ID NO:25.

[0337] Also in the improved methods described herein, the anti-β-coronavirus antibody detected is an anti-SARS-CoV-2 antibody that specifically binds to: a a) a variant of the nucleocapsid protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 210-419 of SEQ ID NO:2 such as (1) replacing serine with

phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210 to 419 of SEQ ID NO:2 other than those recited in of (1)-(5); or SEQ ID NO:24; b) a variant of the RBD of a spike protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 319-542 of SEQ ID NO:15: (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or SEQ ID NO:25; or c) a variant a variant of the nucleocapsid protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 210-419 of SEQ ID NO:2 such as (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210 to 419 of SEQ ID NO:2 other than those recited in of (1)-(5); or SEQ ID NO:24; and a variant of the RBD of a spike protein that comprises one or more substitutions and/or deletions in one or more positions of amino acids 319-542 of SEQ ID NO:15: (1) replacing lysine with asparagine at positions 417 (K417N); (2) replacing lysine with threonine at position 417 (K417T); (3) replacing leucine with arginine at position 452 (L452R); (4) replacing serine with asparagine at position 477 (S477N); (5) replacing glutamic acid with lysine at position 484 (E484K); (6) replacing asparagine with tyrosine at position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or SEQ ID NO:25.

3. Predicting Outcome In Subjects that are or have been Infected with a β-Coronavirus (such as SARS-CoV or SARS-CoV-2)

[0338] In another aspect, the present disclosure relates to methods of predicting the outcome in a subject that is or was infected with a β-coronavirus, such as SARS-CoV or SARS-CoV-2. Specifically, in some aspects, the present disclosure relates to methods of predicting the outcome in a subject identified according to the methods described above in Section 2 as having a β-coronavirus (e.g., β-coronavirus antigen).

[0339] In some aspects, the method involves predicting the outcome in a subject that is or was infected with SARS-CoV-2. In this aspect, the method involves determining, within about the first ten days after the onset of one or more symptoms of SARS-CoV-2 (symptoms of SARS-CoV-2, include one or more of fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, and/or diarrhea), whether a subject has seroconverted to anti-SARS-CoV-2 IgG antibodies before, after, or at the same time as seroconversion to anti-SARS-CoV-2 IgM antibodies. In some aspects, the subjects have been hospitalized for symptoms of SARS-CoV-2.

[0340] Specifically, subjects found to seroconvert to anti-SARS-CoV-2 IgG antibodies before or prior to seroconversion to anti-SARS-CoV-2 IgM antibodies within about the first ten days after the onset of one or more symptoms of SARS-CoV-2 were found more likely than not to have an unfavorable outcome (e.g., death, etc.). Subjects in found to seroconvert to anti-SARS-CoV-2 IgM antibodies before or prior to seroconversion to anti-SARS-CoV-2 IgG antibodies or seroconverted at the same time to anti-SARS-CoV-2 IgG and anti-SARS-CoV-2 IgM antibodies were found to have a favorable outcome. Such favorable outcome includes surviving infection with SARS-CoV-2. The methods used to detect anti-SARS-CoV IgG and anti-SARS-CoV IgM antibodies for predicting the (favorable or unfavorable) outcome of a subject are not critical and can be any method known in the art, including those described above in Section 2.

[0341] In one aspect, the method involves obtaining one or more biological samples and detecting the presence of an anti-SARS-CoV-2 IgG antibody and an anti-SARS-CoV-2 IgM antibody within about the first ten days (e.g., about ten, about eleven, about twelve, about 13 or about 14 days) after onset of one or more symptoms of SARS-CoV-2. The anti-SARS-CoV-2

IgG antibody and the anti-SARS-CoV-2 IgM antibody can be detected in the same biological sample or in different biological samples, simultaneously or sequentially, on the same day or on different days after the onset of one or more symptoms of SARS-CoV-2 (e.g., such as one day after the onset of symptoms, two days after the onset of one or more symptoms, three days after the onset of one or more symptoms, four days after the onset of one or more symptoms, five days after the onset of one or more symptoms, six days after the onset of one or more symptoms, seven days after the onset of one or more symptoms, eight days after the onset of one or more symptoms, nine days after the onset of one or more symptoms, and/or ten days after the onset of one or more symptoms). Additionally, multiple samples can be obtained from the subject within about the first ten days after onset of one or more symptoms (e.g., such as on day one after the onset of one or more symptoms, day three after the onset of one or more sympteoms, and day six after the onset of one or more symptoms, etc.).

[0342] Regardless of when a biological sample is obtained within about the first ten days (e.g., about ten, about eleven, about twelve, about 13 or about 14 days) after onset of one or more symptoms of SARS-CoV-2, once anti-SARS-CoV-2 IgG antibody and/or anti-SARS-CoV-2 IgM antibody is detected, a determination is made which of the anti-SARS-CoV-2 IgG or anti-SARS-CoV-2 IgM antibodies appeared first in the subject. If it is determined that anti-SARS-COV-2 IgG antibody appeared first in the subject prior to the appearance of anti-SARS-COV-2 IgM antibody, then it is predicted that the subject is more likely to have an unfavorable outcome (e.g., death). If it is determined that anti-SARS-COV-2 IgG antibody appeared first in the subject at the same time or after the appearance of anti-SARS-CoV-2 IgM antibody, then it is predicted that the subject is more likely to have a favorable outcome (e.g., survive the SARS-CoV-2 infection). By way of example, if no anti-SARS-CoV-2 IgG or IgM antibodies are detected in a biological sample obtained from a subject on day one after onset of one or more symptoms SARS-CoV-2 (e.g., such as using the methods described above in Section 2), but anti-SARS-CoV-2 IgM antibodies and no SARS-CoV-2 IgG antibodies are detected in subsequent biological sample obtained from the same subject on day four after the onset of one or more symptoms of SARS-CoV-2, then it would be predicted that the subject would more likely than not have a favorable outcome (e.g., survive) from the SARS-CoV-2. By way of another example, if no anti-SARS-CoV-2 IgG or IgM antibodies are detected in a biological sample obtained from a subject on day one after onset of one or more symptoms SARS-CoV-2 (e.g., such as using the methods

described above in Section 2), no anti-SARS-CoV-2 IgG or IgM antibodies are detected in a biological sample obtained from a subject on day three after onset of one or more symptoms SARS-CoV-2 but anti-SARS-CoV-2 IgM antibodies and SARS-CoV-2 IgG antibodies are detected in subsequent biological sample obtained from the same subject on day six after the onset of one or more symptoms of SARS-CoV-2, then it would be predicted that the subject would more likely than not have a favorable outcome (e.g., survive) from the SARS-CoV-2. By way of yet another example, if no anti-SARS-CoV-2 IgG or IgM antibodies are detected in a biological sample obtained from a subject on day three after onset of one or more symptoms SARS-CoV-2 (e.g., such as using the methods described above in Section 2), but anti-SARS-CoV-2 IgG antibodies and no SARS-CoV-2 IgM antibodies are detected in subsequent biological sample obtained from the same subject on day eight after the onset of one or more symptoms of SARS-CoV-2, it would be predicted that the subject would more likely than not have an unfavorable outcome (e.g., death) from the SARS-CoV-2.

[0343] In another aspect, the present disclosure relates to a method of predicting outcome in a subject infected with or suspected of having been infected with SARS-CoV-2 by determining the signal-to-cutoff (S/CO) ratio of at least one anti-SARS-CoV-2 IgM antibody in at least one biological sample obtained from the subject at least ten days (e.g., about ten, about eleven, about twelve, about 13 or about 14 days) after onset of symptoms of SARS-CoV-2. The method used to determine the signal-to-cutoff ratio in the method described herein is not critical and can include the methods described above in Section 2. The biological sample obtained from the subject can be obtained at least ten days after the onset of symptoms of SARS-CoV-2, at least about 11 days after the onset of symptoms of SARS-CoV-2, about 12 days after the onset of symptoms of SARS-CoV-2, about 13 days after the onset of symptoms of SARS-CoV-2, about 14 days after the onset of symptoms of SARS-CoV-2, about 15 days after the onset of symptoms of SARS-CoV-2, about 16 days after the onset of symptoms of SARS-CoV-2, about 17 days after the onset of symptoms of SARS-CoV-2, about 18 days after the onset of symptoms of SARS-CoV-2, about 19 days after the onset of symptoms of SARS-CoV-2, about 20 days after the onset of symptoms of SARS-CoV-2, about 21 days after the onset of symptoms of SARS-CoV-2, about 22 days after the onset of symptoms of SARS-CoV-2, about 23 days after the onset of symptoms of SARS-CoV-2, about 24 days after the onset of symptoms of SARS-CoV-2, about 25 days after the onset of symptoms of SARS-CoV-2, about 26 days after the onset of

symptoms of SARS-CoV-2, about 27 days after the onset of symptoms of SARS-CoV-2, about 28 days after the onset of symptoms of SARS-CoV-2, about 29 days after the onset of symptoms of SARS-CoV-2 and/or about 30 days after the onset of symptoms of SARS-CoV-2.

[0344] In this aspect, the method involves obtaining a S/CO ratio of at least one anti-SARS-CoV-2 IgM antibody. If the S/CO ratio of the anti-SARS-IgM antibody is determined to be equal to or greater than about 10 S/CO, it would be predicted that the subject would more likely than not have an unfavorable outcome (e.g., death, etc.) from the SARS-CoV-2. In some aspects, if the S/CO ratio is determined to be between about 10 S/CO to about 15 S/CO, it would be predicted that the subject would more likely than not have an unfavorable outcome (e.g, death, etc.).

4. Predicting Whether Subjects Suffering from a β-Coronavirus (such as SARS-CoV or SARS-CoV-2) Infection are at Risk of Experiencing a Cytokine Storm and/or Acute Respiratory Distress Syndrome (ARDS)

[0345] In another aspect, the present disclosure relates to methods of predicting whether a subject who has been infected with a β-coronavirus, such as SARS-CoV or SARS-CoV-2, is at risk of experiencing or developing at least one of a cytokine storm, acute respiratory distress syndrome (ARDS) or both a cytokine storm and acute respiratory distress syndrome. Specifically, in some aspects, the present disclosure relates to methods of predicting whether a subject that has been infected with a β-coronavirus (such as SARS-CoV or SARS-CoV-2) will or will not develop or experience at least one of a cytokine storm, ARDS or a combination of a cytokine storm and ARDS by detecting the presence or amount of at least one anti-β-coronavirus antibody (such as an anti-SARS-CoV or anti-SARS-CoV-2 antibody) using the methods described in Section 2.

[0346] Many subjects diagnosed with SARS-CoV-2 recover: (1) within about 7 days after the onset of symptoms and after experiencing mild to moderate disease (e.g., mild upper respiratory disease); or (2) within about 14 days after the onset of symptoms and after experiencing localized pneumonia and disseminated inflammation. It has been found that biological samples obtained from these subjects contain detectable levels (e.g., meaning the presence of) or amounts of anti-SARS-CoV-2 antibodies, such as anti-SARS-CoV-2 IgM antibodies, anti-SARS-CoV-2 IgG antibodies or anti-SARS-CoV-2 IgM antibodies and anti-SARS-CoV-2 IgG antibodies within about the first 14 days after the onset of symptoms (e.g., within about 1 day after the onset of symptoms, about 2 days after the onset of symptoms, about 3 days after the onset of

symptoms, about 4 days after the onset of symptoms, about 5 days after the onset of symptoms, about 6 days after the onset of symptoms, about 7 days after the onset of symptoms, about 8 days after the onset of symptoms, about 9 days after the onset of symptoms, about 10 days after the onset of symptoms, about 11 days after the onset of symptoms, about 12 days after the onset of symptoms, about 13 days after the onset of symptoms or about 14 days after the onset of symptoms). In contrast, it has been found that biological samples obtained from subjects that experience at least one of a cytokine storm, ARDS, or a combination of a cytokine storm or ARDS do not contain any detectable levels (e.g., lack the presence of) or amounts of anti-SARS-CoV-2 antibodies, such anti-SARS-CoV-2 IgM antibodies, anti-SARS-CoV-2 IgG antibodies or anti-SARS-CoV-2 IgM antibodies and anti-SARS-CoV-2 IgG antibodies within about 14 days days (e.g., about 14 days, about 15 days, about 16 days, about 17 days, about 18 days, about 19 days, about 20 days, about 21 days, about 22 days, about 23 days, about 24 days, about 25 days, about 26 days, about 27 days, about 28 days, about 29 days, about 30 days, about 31 days, about 32 days, about 33 days, about 34 days, about 35 days, etc.) after the onset of symptoms.

[0347] In some aspects, the methods of the present disclosure involve predicting whether a subject that has been infected with a β-coronavirus (such as SARS-CoV or SARS-CoV-2) is more likely than not at risk of experiencing or developing at least one of a cytokine storm, ARDS or a combination of a cytokine storm and ARDS by detecting the presence or amount of at least one anti-β-coronavirus antibody (such as an anti-SARS-CoV or anti-SARS-CoV-2 antibody). Specifically, the method involves obtaining at least one biological sample from a subject diagnosed with a β-coronavirus (such as SARS-CoV or SARS-CoV-2) within a critical time of between about 14 days to about 35 days after the onset of systems and detecting the presence or amount of at least one anti-β-coronavirus antibody (such as, at least one anti-SARS-CoV or anti-SARS-CoV-2 antibody, for example, an anti-SARS-CoV-2 IgM antibody, an anti-SARS-CoV-2 IgG antibody) or a combination of an anti-SARS-COV-2 IgM antibody and an anti-SARS-CoV-2 IgG antibody) using the methods described in Section 2. In some aspects, the critical time is about 14 days after the onset of symptoms. In yet another aspect, the critical time is about 15 days after the onset of symptoms. In still yet another aspect, the critical time is about 16 days after the onset of symptoms. In still a further aspect, the critical time is about 17 days after the onset of symptoms. In still a further aspect, the critical time is about 18 days after the onset of symptoms. In still a further aspect, the critical time is about 19 days after the onset of symptoms.

In still a further aspect, the critical time is about 20 days after the onset of symptoms. In still a further aspect, the critical time is about 21 days after the onset of symptoms. In still a further aspect, the critical time is about 22 days after the onset of symptoms. In still a further aspect, the critical time is about 23 days after the onset of symptoms. In still a further aspect, the critical time is about 24 days after the onset of symptoms. In still a further aspect, the critical time is about 25 days after the onset of symptoms. In still a further aspect, the critical time is about 26 days after the onset of symptoms. In still a further aspect, the critical time is about 27 days after the onset of symptoms. In still a further aspect, the critical time is about 28 days after the onset of symptoms. In still a further aspect, the critical time is about 29 days after the onset of symptoms. In still a further aspect, the critical time is about 30 days after the onset of symptoms. In still a further aspect, the critical time is about 31 days after the onset of symptoms. In still a further aspect, the critical time is about 32 days after the onset of symptoms. In still a further aspect, the critical time is about 33 days after the onset of symptoms. In still a further aspect, the critical time is about 34 days after the onset of symptoms. In still a further aspect, the critical time is about 35 days after the onset of symptoms.If no anti-β-coronavirus antibodies (such as any anti-SARS-CoV or anti-SARS-CoV-2 antibodies) are detected in the biological sample at any point during the critical time (e.g., within about 14 days to about 35 days after the onset of symptoms), then the subject more likely than not will develop or experience at least one of a cytokine storm, ARDS, or a combination of cytokine storm and ARDS. If the presence or an amount at least one anti-β-coronavirus antibody (such as an anti-SARS-CoV or anti-SARS-CoV-2 antibody) is detected in the biological sample at any point during the critical time (e.g., within about 14 days to about 35 days after the onset of symptoms), then the subject more likely than not will not develop or experience (e.g., fail to develop or experience) at least one of a cytokine storm, ARDS, or a combination of a cytokine storm and ARDS. Subjects determined to be at risk of developing or experiencing at least one of a cytokine storm, ARDS or a combination of a cytokine storm and ARDS, can be treated pursuant to the methods described in Section 5.

5. Treatment and Monitoring of Subjects Identified as having a β-Coronavirus (such as SARS-CoV or SARS-CoV-2) and/or a β-Coronavirus (such as SARS-CoV or SARS-CoV-2) Antibody or Predicted More Likely than not to have an Unfavorable Outcome as a Result of Infection with a β-Coronavirus (such as SARS-CoV or SARS-CoV-2)

[0348] A subject identified according to the methods described above as having a β-coronavirus (e.g., β-coronavirus antigen, such as a SARS-CoV antigen or SARS-CoV-2 antigen)

and/or having at least one anti-β-coronavirus antibody (such as at least one anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) and/or having a certain amount, concentration and/or level of at least one anti-β-coronavirus antibody (such as at least one anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) and/or predicted to have a favorable or unfavorable outcome may be treated, monitored (e.g., anti-β-coronavirus IgG and/or IgM antibody levels monitored in the subject), treated and monitored and/or monitored and treated using routine techniques known in the art. In some aspects, the methods described herein further include treating the subject (e.g., such as a human) identified as having at least one anti-β-coronavirus antibody (such as at least one anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) and/or having a certain amount, concentration and/or level of at least one anti-β-coronavirus antibody (such as at least one anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) in one or more biological samples obtained from the subject.

[0349] The treatment can take a variety of forms depending on whether or not the subject is asymptomatic or experiencing mild, moderate or severe infection with a β-coronavirus, such as SARS-CoV or SARS-CoV-2. For example, subjects experiencing mild infection with SARS-CoV-2, will experience a fever, cough (with or without sputum production), anorexia, malaise, muscle pain, sore throat, dyspnea, nasal congestion, headache, diarrhea, nausea, and vomiting or any combination thereof. Subjects experiencing a moderate infection will experience a fever greater than 100.4°F that lasts for several days, chills, shortness of breath, lethargy, or any combination thereof. Such subjects may be suffering from pneumonia. Subjects experiencing severe infection will experience trouble breathing, persistant pain or pressure in the chest, confusion, inability to rouse, bluish lips or face, or any combination thereof. Such subjects may be suffering from severe pneumonia.

[0350] If the subject is asymptomatic or has mild symptoms, the subject may be treated with rest and sleep, by keeping warm, ingesting fluids (e.g., remaining hydrated) minimizing social interaction with other subjects (e.g., remain isolated or quarantined, such as, for example, at home), or any combination thereof. Additionally, the subject can be monitored to see if symptoms arise and/or worsen.

[0351] Subjects with moderate or severe symptoms of infection with a a β-coronavirus, such as SARS-CoV or SARS-CoV-2, may be treated with one or more drugs, vaccines, convalescent plasma therapy (e.g., receiving plasma from blood taken from a subject that has survived an

infection with a β-coronavirus, such as SARS-CoV or SARS-CoV-2), or respiratory support or assistance (e.g., receiving supplemental oxygen through a nasal cannula, nasal progns, face mask, or non-invasive or invasive (e.g. intubation) ventilation) or combinations thereof. Examples of one or more drugs that can be used to treat a subject include, but are not limited to, remdesivir, hydroxychloroquine, chloroquine or combinations thereof. Subjects receiving any of the aforementioned treatment can also further be monitored using routine techniques known in the art.

[0352] In other aspects, a subject may be monitored prior to being treated for a β-coronavirus, such as SARS-CoV or SARS-CoV-2. Such monitoring involves detecting, analyzing and/or interpreting changes in the subject's anti-β-coronavirus IgG and/or IgM antibody levels over the course of time. For example, depending on a subject's β-coronavirus IgM antibody level, a subject may be monitored prior to receiving any treatment to gauge whether the subject's immune system is able to fight the virus on its own without any treatment intervention. During the course of the monitoring, if the subject's β-coronavirus (such as SARS-CoV or SARS-CoV-2) IgM antibody levels increase, treatment can be commenced. Likewise, during treatment, a subject's β-coronavirus (such as SARS-CoV or SARS-CoV-2) IgM and IgG levels can be monitored. If during treatment the subject's β-coronavirus (such as SARS-CoV or SARS-CoV-2) IgM antibody levels remain high and β-coronavirus IgG levels remain low, the subject can be continued to be treated for the β-coronavirus and continued to monitored until such time that the subject's β-coronavirus (such as SARS-CoV or SARS-CoV-2) IgM antibody levels have lowered and β-coronavirus (such as SARS-CoV or SARS-CoV-2) IgG levels increased.

6. Vaccinations

[0353] In another embodiment, the present disclosure relates to use of the methods described above in Section 2 in connection with at least one vaccinations and/or re-vaccinations (e.g., further vaccinations) of a subject against one or more β-coronaviruses (e.g., SARS-CoV or SARS-CoV-2). In some aspects, the methods described in Section 2 are used to detect the presence or determine the amount of at least one anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibodies or anti-SARS-CoV-2 antibodies) and/or at least one anti-β-coronavirus neutralizing antibody (e.g., an anti-SARS-CoV neutralizing antibody or SARS-CoV-2 neutralizing antibody (e.g., an anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) in at least one biological sample

obtained from a subject to determine whether or not the subject should or can be administered at least one vaccine (e.g., such as a first or initial vaccine, one or more further or additional vaccines, etc.) against at least one β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). For example, in some aspects, the subject tested may: (1) be naïve (meaning the subject does not have any immunity or lacks immunologic immunity to a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2)) and not previously vaccinated against a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2); (2) be naïve and previously vaccinated against a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2); (3) be currently infected with a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) and exhibiting no or mild symptoms and not previously vaccinated against a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2); (4) be currently infected with a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) and exhibiting no or mild symptoms and previously vaccinated against a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2); (5) have been previously infected with a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2), recovered and not previously vaccinated against a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2); or (6) have been previously infected with a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2), recovered and previously vaccinated against a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). The above method can be performed regardless of the variation in timing and/or severity of prior infection with a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). Using the methods described in Section 2, if: (1) at least one anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or SARS-CoV-2 antibody) is detected or an amount determined in the biological sample; or (2) an amount of (a) at least one anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or SARS-CoV-2 antibody); or (b) at least one anti-β-coronavirus neutralizing antibody (e.g., an anti-SARS-CoV neutralizing antibody or anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) sufficient to impart immunity is determined in the biological sample, the subject may need to wait for a period of time (e.g., 30 days, 60 days, 90 days, etc.) to be administered at least one vaccine (whether the vaccine to be administered is the first dose of the vaccine, a second (e.g., booster) dose of the vaccine, a third (e.g., booster) dose of the vaccine, etc.) against a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). Alternatively, if: (1) at least one anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) is not detected or no amount is determined in the biological sample; or (2) the amount of (a) at

least one anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or SARS-CoV-2 antibody); or (b) at least one anti-β-coronavirus neutralizing antibody (e.g., an anti-SARS-CoV neutralizing antibody or anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) determined in the biological sample is insufficient to impart immunity, then at least one vaccine can be administered to the subject. In some aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV neutralizing antibody or anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) is from at least about 100 BAU/mL to about 490 BAU/mL; at least about 110 BAU/mL to about 490 BAU/mL; at least about 200 BAU/mL to about 490 BAU/mL; at least from about 300 BAU mL to about 490 BAU/mL; at least about 400 BAU mL to about 490 BAU/mL; at least about 100 BAU/mL; at least about 110 BAU/mL; at least about 120 BAU/mL; at least about 130 BAU/mL; at least about 140 BAU/mL; at least about 150 BAU/mL; at least about 160 BAU/mL; at least about 170 BAU/mL; at least about 180 BAU/mL; at least about 190 BAU/mL; at least about 200 BAU/mL; at least about 210 BAU/mL; at least about 220 BAU/mL; at least about 230 BAU/mL; at least about 240 BAU/mL; at least about 250 BAU/mL; at least about 260 BAU/mL; at least about 270 BAU/mL; at least about 280 BAU/mL; at least about 290 BAU/mL; at least about 300 BAU/mL; at least about 300 BAU/mL; at least about 310 BAU/mL; at least about 320 BAU/mL; at least about 330 BAU/mL; at least about 340 BAU/mL; at least about 350 BAU/mL; at least about 360 BAU/mL; at least about 370 BAU/mL; at least about 380 BAU/mL; at least about 390 BAU/mL; at least about 400 BAU/mL; at least about 400 BAU/mL; at least about 410 BAU/mL; at least about 420 BAU/mL; at least about 430 BAU/mL; at least about 440 BAU/mL; at least about 450 BAU/mL; at least about 460 BAU/mL; at least about 470 BAU/mL; at least about 480 BAU/mL; or at least about 490 BAU/mL.

[0354] In some aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody (e.g., an anti-SARS-CoV-2 IgG antibody)) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV neutralizing antibody or anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart

immunity to a subject (e.g., human) is from at least about 500 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 500 BAU/mL; from at least about 510 BAU/mL; at least about 520 BAU/mL; at least about 530 BAU/mL; at least about 540 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL; at least about 560 BAU/mL; at least about 570 BAU/mL; at least about 580 BAU/mL; at least about 590 BAU/mL; at least about 600 BAU/mL; at least about 610 BAU/mL; at least about 620 BAU/mL; at least about 630 BAU/mL; at least about 639 BAU/mL; at least about 640 BAU/mL; or at least about 650 BAU/mL.

[0355] In other aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV neutralizing antibody or anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) is from about 580 BAU/mL to about 640 BAU/mL. In other aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV neutralizing antibody or anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) is from about 600 BAU/mL to about 640 BAU/mL. In still yet other aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV neutralizing antibody or anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) is from at least about 100 BAU/mL to about 490 BAU/mL; at least about 110 BAU/mL to about 490 BAU/mL; at least about 200 BAU/mL to about 490 BAU/mL; at least from about 300 BAU mL to about 490 BAU/mL; at least about 400 BAU mL to about 490 BAU/mL; at least about 100 BAU/mL; at least about 110 BAU/mL; at least about 120 BAU/mL; at least about 130 BAU/mL; at least about 140 BAU/mL; at least about 150 BAU/mL; at least about 160 BAU/mL; at least about 170 BAU/mL; at least about 180 BAU/mL; at least about 190 BAU/mL; at least about 200 BAU/mL; at least about 210 BAU/mL; at least about 220 BAU/mL; at least about 230 BAU/mL; at least

about 240 BAU/mL; at least about 250 BAU/mL; at least about 260 BAU/mL; at least about 270 BAU/mL; at least about 280 BAU/mL; at least about 290 BAU/mL; at least about 300 BAU/mL; at least about 300 BAU/mL; at least about 310 BAU/mL; at least about 320 BAU/mL; at least about 330 BAU/mL; at least about 340 BAU/mL; at least about 350 BAU/mL; at least about 360 BAU/mL; at least about 370 BAU/mL; at least about 380 BAU/mL; at least about 390 BAU/mL; at least about 400 BAU/mL; at least about 400 BAU/mL; at least about 410 BAU/mL; at least about 420 BAU/mL; at least about 430 BAU/mL; at least about 440 BAU/mL; at least about 450 BAU/mL; at least about 460 BAU/mL; at least about 470 BAU/mL; at least about 480 BAU/mL; or at least about 490 BAU/mL.

[0356] In still yet other aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV neutralizing antibody or anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) is from at least about 500 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 500 BAU/mL; from at least about 510 BAU/mL; at least about 520 BAU/mL; at least about 530 BAU/mL; at least about 540 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL; at least about 560 BAU/mL; at least about 570 BAU/mL; at least about 580 BAU/mL; at least about 590 BAU/mL; at least about 600 BAU/mL; at least about 610 BAU/mL; at least about 620 BAU/mL; at least about 630 BAU/mL; at least about 639 BAU/mL; at least about 640 BAU/mL; or at least about 650 BAU/mL.

[0357] In still yet other aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV neutralizing antibody or anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) is at least about 590 BAU/mL, at least about 600 BAU/mL, at least about 610 BAU/mL, at least about 620 BAU/mL, at least about 630 BAU/mL, at least about 631 BAU/mL, at least about 632 BAU/mL, at least about 633 BAU/mL, at least about 634 BAU/mL, at least about 635 BAU/mL, at least about 636 BAU/mL, at least about 637 BAU/mL, at least about 638 BAU/mL, at least about 639 BAU/mL, at least about 640 BAU/mL, at least about 641 BAU/mL, at least about 642

BAU/mL, at least about 643 BAU/mL, at least about 644 BAU/mL or at least about 645 BAU/mL.

[0358] In some aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV neutralizing antibody or anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) appears to be at least from about 4000 to about 4700 AU/mL, e.g., about 4500 AU/mL (unpublished studies done by Abbott Laboratories, Abbott Park, IL using the CE-marked Abbott anti-S SARS-CoV-2 IgG II Quantitative antibody assay (available for use on Abbott's ARCHITECT® and Alinity i™ platforms)) or from about 568 BAU/mL to about 667 BAU/mL. The measure AU/mL can be converted to the WHO International standard of binding antibody unit per mL (BAU/mL) using the following formula: BAU/mL=0.142 x AU/mL (e.g., about 4500 AU/mL is about 639 BAU/mL (4500 AU/mL x 0.142 = 639 BAU/mL)).

[0359] In another aspect, the methods described in Section 2 are used to detect the presence or determine the amount of at least one anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibodies or anti-SARS-CoV-2 antibodies) in at least one biological sample obtained from the subject within a time frame after the subject is administered at least one vaccine for a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) in order to: (1) determine whether or not the subject should be administered at least one further vaccination (e.g. receive one or more boosters) against a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2); and/or (2) monitor the subject following the admistration of at least one vaccine for a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). The above method can be performed regardless of the variation in timing and/or severity of prior infection with a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). In some aspects, the method involves obtaining the biological sample within a time frame after the subject has been administered at least one vaccine for a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). The time frame after the subject has been administered at least one vaccine for a β-coronavirus (e.g., receive SARS-CoV or SARS-CoV-2) can be at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, at least 15 days, at least 16 days, at least 17 days, at least 18 days, at least 19 days, at least 20 days,

at least 21 days, at least 22 days, at least 23 days, at least 24 days, at least 25 days, at least 26 days, at least 27 days, at least 28 days, at least 29 days, at least 30 days, at least 31 days, at least 32 days, at least 33 days, at least 34 days, at least 35 days, at least 36 days, at least 37 days, at least 38 days, at least 39 days, at least 40 days, at least 41 days, at least 42 days, at least 43 days, at least 44 days, at least 45 days, at least 46 days, at least 47 days, at least 48 days, at least 49 days, at least 50 days, etc. In some aspects, the biological sample is obtained within about 7 to about 21 days after the subject has been administered at least one vaccine for a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). Additionally, in yet further aspects, the monitoring of the subject involves monitoring for post-vaccine symptomology or side-effects after a subject receives one or more vaccines (e.g., such as after a first dose of a vaccine for SARS-CoV-2, a second dose of a vaccine for SARS-CoV-2, etc.) for a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). For example, as shown in Example 10, subjects previously infected with a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) after receipt of a first (e.g., initial) dose of a vaccine for a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) may experience more post-vaccine symptoms or side effects (e.g., one or more of fatigue or malaise, headache, dizziness, or lightheadedness, fever or chills, muscle, bone, joint or nerve symptoms, nausea, vomiting, diarrhea, or other digestive symptoms, sleep changes, swollen lymph node, skin/nail or face changes, eye, ear, mouth or throat changes, cought, chest or breathing symptoms and/or memory or mood changes) than a subject who has not previously been infected with a β-coronavirus (e.g., SARS-CoV or SARS-CoV-2).

[0360] Using the methods described in Section 2, if: (1) at least one anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or SARS-CoV-2 antibody) is not detected or no amount determined in the biological sample; (2) at least one anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV neutralizing antibody or anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) is not detected or no amount determined in the biological sample; or (3) the amount of (a) at least one anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or SARS-CoV-2 antibody); or (b) at least one anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV neutralizing antibody or anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) determined in the biological sample is insufficient to

impart immunity, then at least one further vaccines (e.g., one or more boosters) can be administered to the subject. Alternatively, if: (1) at least one anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) is detected or an amount determined in the biological sample; (2) at least one anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV neutralizing antibody or anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) is detected or an amount determined in the biological sample; or (3) the amount of (a) at least one anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or SARS-CoV-2 antibody); or (b) at least one anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV IgG neutralizing antibody or anti-SARS-CoV-2 IgG neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) is sufficient to impart immunity is determined in the biological sample, then at least one further vaccines should not be administered to the subject.

[0361] In some aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV IgG neutralizing antibody or anti-SARS-CoV-2 IgG neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) is from about 500 BAU/mL to about 650 BAU/mL. In other aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV IgG neutralizing antibody or anti-SARS-CoV-2 IgG neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) is from about 550 BAU/mL to about 640 BAU/mL. In other aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV IgG neutralizing antibody or anti-SARS-CoV-2 IgG neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) is from about 580 BAU/mL to about 640 BAU/mL. In other aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus

neutralizing antibody (e.g., anti-SARS-CoV IgG neutralizing antibody or anti-SARS-CoV-2 IgG neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) is from about 600 BAU/mL to about 640 BAU/mL.

[0362] In still yet other aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV IgG neutralizing antibody or anti-SARS-CoV-2 IgG neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) is from at least about 100 BAU/mL to about 490 BAU/mL; at least about 110 BAU/mL to about 490 BAU/mL; at least about 200 BAU/mL to about 490 BAU/mL; at least from about 300 BAU mL to about 490 BAU/mL; at least about 400 BAU mL to about 490 BAU/mL; at least about 100 BAU/mL; at least about 110 BAU/mL; at least about 120 BAU/mL; at least about 130 BAU/mL; at least about 140 BAU/mL; at least about 150 BAU/mL; at least about 160 BAU/mL; at least about 170 BAU/mL; at least about 180 BAU/mL; at least about 190 BAU/mL; at least about 200 BAU/mL; at least about 210 BAU/mL; at least about 220 BAU/mL; at least about 230 BAU/mL; at least about 240 BAU/mL; at least about 250 BAU/mL; at least about 260 BAU/mL; at least about 270 BAU/mL; at least about 280 BAU/mL; at least about 290 BAU/mL; at least about 300 BAU/mL; at least about 300 BAU/mL; at least about 310 BAU/mL; at least about 320 BAU/mL; at least about 330 BAU/mL; at least about 340 BAU/mL; at least about 350 BAU/mL; at least about 360 BAU/mL; at least about 370 BAU/mL; at least about 380 BAU/mL; at least about 390 BAU/mL; at least about 400 BAU/mL; at least about 400 BAU/mL; at least about 410 BAU/mL; at least about 420 BAU/mL; at least about 430 BAU/mL; at least about 440 BAU/mL; at least about 450 BAU/mL; at least about 460 BAU/mL; at least about 470 BAU/mL; at least about 480 BAU/mL; or at least about 490 BAU/mL.

[0363] In still yet other aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV IgG neutralizing antibody or anti-SARS-CoV-2 IgG neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) is from at least about 500 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL

to about 650 BAU/mL; at least about 500 BAU/mL; from at least about 510 BAU/mL; at least about 520 BAU/mL; at least about 530 BAU/mL; at least about 540 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL; at least about 560 BAU/mL; at least about 570 BAU/mL; at least about 580 BAU/mL; at least about 590 BAU/mL; at least about 600 BAU/mL; at least about 610 BAU/mL; at least about 620 BAU/mL; at least about 630 BAU/mL; at least about 639 BAU/mL; at least about 640 BAU/mL; or at least about 650 BAU/mL.

[0364] In still yet other aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV IgG neutralizing antibody or anti-SARS-CoV-2 IgG neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) is at least about 590 BAU/mL, at least about 600 BAU/mL, at least about 610 BAU/mL, at least about 620 BAU/mL, at least about 630 BAU/mL, at least about 631 BAU/mL, at least about 632 BAU/mL, at least about 633 BAU/mL, at least about 634 BAU/mL, at least about 635 BAU/mL, at least about 636 BAU/mL, at least about 637 BAU/mL, at least about 638 BAU/mL, at least about 639 BAU/mL, at least about 640 BAU/mL, at least about 641 BAU/mL, at least about 642 BAU/mL, at least about 643 BAU/mL, at least about 644 BAU/mL or at least about 645 BAU/mL. In some aspects, the amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV IgG neutralizing antibody or anti-SARS-CoV-2 IgG neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) appears to be at least from about 4000 AU/mL to about 4500 AU/mL (unpublished studies done by Abbott Laboratories, Abbott Park, IL using the CE-marked Abbott anti-S SARS-CoV-2 IgG II Quantitative antibody assay (available for use on Abbott's ARCHITECT® and Alinity i™ platforms)). The measure AU/mL can be converted to the WHO International standard of binding antibody unit per mL (BAU/mL) using the following formula: BAU/mL=0.142 x AU/mL (e.g., about 4000 AU/mL is about 568 BAU/mL (4000 AU/mL x 0.142 = 568 BAU/mL) and about 4500 AU/mL is about 639 BAU/mL (4500 AU/mL x 0.142 = 639 BAU/mL)).

7. Methods for Determining Immune Status in Subjects with SARS-CoV-2

[0365] In another embodiment, the present disclosure relates to use of the methods described above in Section 2 for determining the immune status of a subject to SARS-CoV-2. In some aspects, the method involves determining the presence of (e.g., which can qualitative, semi-quantitative or quantitative) or an amount, level or concentration of (e.g., quantitating or semi-quantitating) at least one anti-SARS-CoV-2 IgG antibody, at least one anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG neutralizing antibody) or at least one anti-SARS-CoV-2 IgG antibody and at least one anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG neutralizing antibody) in at least one biological sample obtained from the subject using the methods described in Section 2. Such a subject optionally has been infected or is suspected of being infected with SARS-CoV-2. Alternatively, optionally, the subject has had an unknown, limited or no potential exposure to SARS-CoV-2 but determination of immune status is desired nonetheless. After the amount of at least one anti-SARS-CoV-2 IgG antibody, at least one anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG neutralizing antibody) or at least one anti-SARS-CoV-2 IgG antibody and at least one anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG neutralizing antibody) in the biological sample is determined, the immune status of the subject is determined. In some aspects, if the amount of at least one anti-SARS-CoV-2 IgG antibody, at least one anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG neutralizing antibody) or at least one anti-SARS-CoV-2 IgG antibody and at least one anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG neutralizing antibody) determined in the biological sample is from at least about 550 BAU/mL to about 650 BAU/mL or from at least about 100 BAU/mL to about 490 BAU/mL, the subject is considered to have or possess immunity to SARS-CoV-2. If the amount of at least one anti-SARS-CoV-2 IgG antibody, at least one anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG neutralizing antibody) or at least one anti-SARS-CoV-2 IgG antibody and at least one anti-SARS-CoV-2 neutralizing antibody (e.g., anti-SARS-CoV-2 IgG neutralizing antibody) determined in the biological sample obtained from the subject is not from at least about 550 BAU/mL to about 650 BAU/mL or from at least about 100 BAU/mL to about 490 BAU/mL, the subject is not considered to have or possess immunity to SARS-CoV-2.

[0366] The methods for determining the immune status of subject can be performed at any time regardless or irrespective of the (a) subject's prior infection history with SARS-CoV-2 (e.g.,

in some aspects, the subject may never have been infected with or potentially exposed to SARS-CoV-2, in other aspects, the subject may have been infected one or more times with SARS-CoV-2), and/or vaccination history with SARS-CoV-2 (e.g., in some aspects, the subject may never have been vaccinated against SARS-CoV-2, in other aspects, the subject may have been vaccinated one or more times against SARS-CoV-2); and/or (b) whether there is any knowledge of the subject's prior infection and/or vaccination history with SARS-CoV-2 (e.g., there may be full, no, or limited information). There is no limit on the number of times the method for determining the SARS-CoV-2 immune status of a subject is performed. For example, the method may be performed once (e.g., such as if the subject is planning to travel and its status is required for such travel). Alternatively, the method can be performed once or more times a week, month, and/or year.

[0367] The immune status of subject determined according to the methods described herein can be communicated to the subject directly from a clinician (e.g., doctor, nurse, etc.) or their representative or agent using any means (e.g., in person, via telephone, mail delivery (e.g., postal service), etc.), via text or e-mail messages, or by the use a mobile phone application. The means for communicating communicating the immune status to the subject is not critical and can use any routine techniques or emerging technologies known in the art.

8. Preparation/Productions Methods for Recombinant Antigens and Antibodies for use as Specific Binding Partners

a. Recombinant antigens

[0368] *Synthetic Production of Isolated Nucleocapsid and/or Spike Polypeptides from a β-Coronavirus*

[0369] Once sequenced, polypeptides, such as (a) a nucleocapsid protein, fragment or variant thereof of a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) a spike protein, fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2), can be synthesized using methods known

in the art, such as, for example, exclusive solid phase synthesis, partial solid phase synthesis, fragment condensation, and classical solution synthesis. See, e.g., Merrifield, *J. Am. Chem. Soc.* 85: 2149 (1963). On a solid phase, the synthesis typically begins from the C-terminal end of the peptide using an alpha-amino protected resin. A suitable starting material can be prepared, for instance, by attaching the required alpha-amino acid to a chloromethylated resin, a hydroxymethyl resin, or a benzhydrylamine resin. One such chloromethylated resin is sold under the tradename BIO-BEADS SX-1 by Bio Rad Laboratories (Richmond, Calif.), and the preparation of the hydroxymethyl resin is described by Bodonszky et al., *Chem. Ind.* (London) 38: 1597 (1966). The benzhydrylamine (BHA) resin has been described by Pietta and Marshall, *Chem. Comm* 650 (1970) and is commercially available from Beckman Instruments, Inc. (Palo Alto, Calif.) in the hydrochloride form. Automated peptide synthesizers are commercially available, as are services that make peptides to order.

[0370] Thus, the polypeptides can be prepared by coupling an alpha-amino protected amino acid to the chloromethylated resin with the aid of, for example, cesium bicarbonate catalyst, according to the method described by Gisin, *Hely. Chim. Acta.* 56: 1467 (1973). After the initial coupling, the alpha-amino protecting group is removed by a choice of reagents including trifluoroacetic acid (TFA) or hydrochloric acid (HCl) solutions in organic solvents at room temperature.

[0371] Suitable alpha-amino protecting groups include those known to be useful in the art of stepwise synthesis of peptides. Examples of alpha-amino protecting groups are: acyl type protecting groups (e.g., formyl, trifluoroacetyl, and acetyl), aromatic urethane type protecting groups (e.g., benzyloxycarbonyl (Cbz) and substituted Cbz), aliphatic urethane protecting groups (e.g., t-butyloxycarbonyl (Boc), isopropyloxycarbonyl, and cyclohexyloxycarbonyl), and alkyl type protecting groups (e.g., benzyl and triphenylmethyl). Boc and Fmoc are preferred protecting groups. The side chain protecting group remains intact during coupling and is not split off during the deprotection of the amino-terminus protecting group or during coupling. The side chain protecting group must be removable upon the completion of the synthesis of the final peptide and under reaction conditions that will not alter the target peptide.

[0372] After removal of the alpha-amino protecting group, the remaining protected amino acids are coupled stepwise in the desired order. An excess of each protected amino acid is

generally used with an appropriate carboxyl group activator such as dicyclohexylcarbodiimide (DCC) in solution, for example, in methylene chloride and dimethyl formamide (DMF) mixtures.

[0373] After the desired amino acid sequence has been completed, the desired peptide is decoupled from the resin support by treatment with a reagent, such as TFA or hydrogen fluoride (HF), which not only cleaves the peptide from the resin, but also cleaves all remaining side chain protecting groups. When the chloromethylated resin is used, HF treatment results in the formation of the free peptide acids. When the benzhydrylamine resin is used, HF treatment results directly in the free peptide amide. Alternatively, when the chloromethylated resin is employed, the side chain protected peptide can be decoupled by treatment of the peptide resin with ammonia to give the desired side chain protected amide or with an alkylamine to give a side chain protected alkylamide or dialkylamide. Side chain protection is then removed in the usual fashion by treatment with hydrogen fluoride to give the free amides, alkylamides, or dialkylamides.

[0374] These and other solid phase peptide synthesis procedures are well-known in the art. Such procedures are also described by Stewart and Young in *Solid Phase Peptide Syntheses* (2nd Ed., Pierce Chemical Company, 1984).

[0375] *Recombinant Production of Isolated Nucleocapsid and/or Spike Polypeptides from a β-Coronavirus (such as SARS-CoV or SARS-CoV-2)*

[0376] All or a portion of a (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment, variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2) can be recombinantly produced using methods known in the art. For example, an isolated or purified nucleic acid molecule comprising a nucleotide sequence encoding the polypeptide can be expressed in a host cell, and the polypeptide can be isolated. The isolated or purified nucleic acid molecule can comprise a nucleotide sequence encoding a (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof

that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2 . In one aspect, the isolated or purified nucleic acid can comprise a nucleotide sequence encoding a nucleocapsid protein having the amino acid sequence of SEQ ID NO:1 or a fragment or variant thereof. In another aspect, the isolated or purified nucleic acid can comprise a nucleotide sequence encoding a RBD from a spike protein having the amino acid sequence of SEQ ID NOS: 17 or 18. The isolated or purified nucleic acid molecule can be a vector.

[0377] The isolated nucleic acid can be synthesized with an oligonucleotide synthesizer, for example. One of ordinary skill in the art will readily appreciate that, due to the degeneracy of the genetic code, more than one nucleotide sequence can encode a given amino acid sequence. In this regard, a nucleotide sequence encoding an amino acid sequence that is substantially identical to an amino acid sequence of a SEQ ID NO. specified herein can be used. Codons, which are favored by a given host cell, preferably are selected for recombinant production. A nucleotide sequence encoding the amino acid sequence of a specified SEQ ID NO. can be combined with other nucleotide sequences using polymerase chain reaction (PCR), ligation, or ligation chain reaction (LCR) to encode a mutated truncated nucleocapsid and/or spike polypeptide. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly. Once assembled, the nucleotide sequence encoding (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2) can be inserted into a vector, operably linked to control sequences as necessary for expression in a given host cell, and introduced (such as by transformation or transfection) into a host cell. The nucleotide sequence can be further manipulated (for example, linked to one or more nucleotide sequences encoding additional immunoglobulin domains, such as additional constant regions) and/or expressed in a host cell.

[0378] Although not all vectors and expression control sequences may function equally well to express a polynucleotide sequence of interest and not all hosts function equally well with the same expression system, it is believed that those skilled in the art will be able to make a selection among these vectors, expression control sequences, optimized codons, and hosts for use in the present disclosure without any undue experimentation. For example, in selecting a vector, the host must be considered because the vector must be able to replicate in it or be able to integrate into the chromosome. The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as antibiotic markers, should also be considered. In selecting an expression control sequence, a variety of factors also can be considered. These include, but are not limited to, the relative strength of the sequence, its controllability, and its compatibility with the nucleotide sequence encoding (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2), particularly with regard to potential secondary structures. Hosts should be selected by consideration of their compatibility with the chosen vector, their codon usage, their secretion characteristics, their ability to fold the polypeptide correctly, their fermentation or culture requirements, their ability (or lack thereof) to glycosylate the protein, and the ease of purification of the products encoded by the nucleotide sequence, etc.

[0379] The recombinant vector can be an autonomously replicating vector, namely, a vector existing as an extrachromosomal entity, the replication of which is independent of chromosomal replication (such as a plasmid). Alternatively, the vector can be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

[0380] The vector is preferably an expression vector in which the polynucleotide sequence encoding the (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof

that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2), is operably linked to additional segments required for transcription of the polynucleotide sequence. The vector is typically derived from plasmid or viral DNA. A number of suitable expression vectors for expression in the host cells mentioned herein are commercially available or described in the literature. Useful expression vectors for eukaryotic hosts, include, but are not limited to, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus. Specific vectors include pcDNA3.1 (+)\Hyg (Invitrogen Corp., Carlsbad, Calif.) and pCI-neo (Stratagene, La Jolla, Calif.). Examples of expression vectors for use in yeast cells include, but are not limited to, the 2μ plasmid and derivatives thereof, the POT1 vector (see, e.g., U.S. Patent No. 4,931,373), the pJSO37 vector (described in Okkels, *Ann New York Acad. Sci.* 782: 202-207 (1996)) and pPICZ A, B or C (Invitrogen). Examples of expression vectors for use in insect cells include, but are not limited to, pVL941, pBG311 (Cate et al., *Cell* 45: 685-698 (1986)), and pBluebac 4.5 and pMelbac (both of which are available from Invitrogen).

[0381] Other vectors that can be used allow the nucleotide sequence encoding the (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2), to be amplified in copy number. Such amplifiable vectors are well-known in the art. These vectors include, but are not limited to, those vectors that can be amplified by dihydrofolate reductase (DHFR) amplification (see, for example, U.S. Patent No. 4,470,461 and Kaufinan et al., *Mol. Cell. Biol.* 2: 1304-1319 (1982)) and glutamine synthetase (GS) amplification (see, for example, U.S. Patent No. 5,122,464 and EP Patent Application Publication No. 0 338 841).

[0382] The recombinant vector can further comprise a nucleotide sequence enabling the vector to replicate in the host cell in question. An example of such a sequence for use in a mammalian host cell is the SV40 origin of replication. Suitable sequences enabling the vector to

replicate in a yeast cell are the yeast plasmid 2μ replication genes REP 1-3 and origin of replication.

[0383] The vector can also comprise a selectable marker, namely, a gene or polynucleotide, the product of which complements a defect in the host cell, such as the gene coding for DHFR or the Schizosaccharomyces pombe TPI gene (see, e.g., Russell, *Gene* 40: 125-130 (1985)), or one which confers resistance to a drug, such as ampicillin, kanamycin, tetracycline, chloramphenicol, neomycin, hygromycin or methotrexate. For filamentous fungi, selectable markers include, but are not limited to, amdS, pyrG, arcB, niaD and sC.

[0384] Also present in the vector are "control sequences," which are any components that are necessary or advantageous for the expression of the (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2). Each control sequence can be native or foreign to the nucleotide sequence encoding the (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2) . Such control sequences include, but are not limited to, a leader, a polyadenylation sequence, a propeptide sequence, a promoter, an enhancer or an upstream activating sequence, a signal peptide sequence, and a transcription terminator. At a minimum, the control sequences include at least one promoter operably linked to the polynucleotide sequence encoding the (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any

protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2).

[0385] By "operably linked" is meant the covalent joining of two or more nucleotide sequences, by means of enzymatic ligation or otherwise, in a configuration relative to one another such that the normal function of the sequences can be performed. For example, a nucleotide sequence encoding a presequence or secretory leader is operably linked to a nucleotide sequence for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the nucleotide sequences being linked are contiguous and, in the case of a secretory leader, contiguous and in the same reading frame. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, then synthetic oligonucleotide adaptors or linkers can be used, in conjunction with standard recombinant DNA methods.

[0386] A wide variety of expression control sequences can be used in the context of the present disclosure. Such useful expression control sequences include the expression control sequences associated with structural genes of the foregoing expression vectors as well as any sequence known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. Examples of suitable control sequences for directing transcription in mammalian cells include the early and late promoters of SV40 and adenovirus, for example, the adenovirus 2 major late promoter, the MT-1 (metallothionein gene) promoter, the human cytomegalovirus immediate-early gene promoter (CMV), the human elongation factor 1α (EF-1α) promoter, the Drosophila minimal heat shock protein 70 promoter, the Rous Sarcoma Virus (RSV) promoter, the human ubiquitin C (UbC) promoter, the human growth hormone terminator, SV40 or adenovirus E1b region polyadenylation signals and the Kozak consensus sequence (Kozak, *J. Mol. Biol.* 196: 947-50 (1987)).

[0387] In order to improve expression in mammalian cells a synthetic intron can be inserted in the 5' untranslated region of a polynucleotide sequence encoding the antibody or a fragment thereof. An example of a synthetic intron is the synthetic intron from the plasmid pCI-Neo (available from Promega Corporation, Madison, Wis.).

[0388] Examples of suitable control sequences for directing transcription in insect cells include, but are not limited to, the polyhedrin promoter, the P10 promoter, the baculovirus immediate early gene 1 promoter, the baculovirus 39K delayed-early gene promoter, and the SV40 polyadenylation sequence.

[0389] Examples of suitable control sequences for use in yeast host cells include the promoters of the yeast α-mating system, the yeast triose phosphate isomerase (TPI) promoter, promoters from yeast glycolytic genes or alcohol dehydrogenase genes, the ADH2-4-c promoter and the inducible GAL promoter.

[0390] Examples of suitable control sequences for use in filamentous fungal host cells include the ADH3 promoter and terminator, a promoter derived from the genes encoding *Aspergillus oryzae* TAKA amylase triose phosphate isomerase or alkaline protease, an *A. niger* α-amylase, *A. niger* or *A. nidulas* glucoamylase, *A. nidulans* acetamidase, *Rhizomucor miehei* aspartic proteinase or lipase, the TPI1 terminator, and the ADH3 terminator.

[0391] The polynucleotide sequence encoding the truncated DBP, mutated truncated DBP, or fusion protein of either of the foregoing may or may not also include a polynucleotide sequence that encodes a signal peptide. The signal peptide is present when the (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2) is to be secreted from the cells in which it is expressed. Such signal peptide, if present, should be one recognized by the cell chosen for expression of the polypeptide. The signal peptide can be homologous or heterologous to the (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2) or can be homologous or heterologous to the host cell, i.e., a signal

peptide normally expressed from the host cell or one which is not normally expressed from the host cell. Accordingly, the signal peptide can be prokaryotic, for example, derived from a bacterium, or eukaryotic, for example, derived from a mammalian, insect, filamentous fungal, or yeast cell.

[0392] The presence or absence of a signal peptide will, for example, depend on the expression host cell used for the production of the (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2). For use in filamentous fungi, the signal peptide can conveniently be derived from a gene encoding an *Aspergillus sp.* amylase or glucoamylase, a gene encoding a *Rhizomucor miehei* lipase or protease or a *Humicola lanuginosa* lipase. For use in insect cells, the signal peptide can be derived from an insect gene (see, e.g., PCT International Application WO 90/05783), such as the lepidopteran Manduca sexta adipokinetic hormone precursor (see, e.g., U.S. Patent No. 5,023,328), the honeybee melittin (Invitrogen), ecdysteroid UDP glucosyltransferase (egt) (Murphy et al., *Protein Expression and Purification* 4: 349-357 (1993), or human pancreatic lipase (hpl) (*Methods in Enzymology* 284: 262-272 (1997)).

[0393] Specific examples of signal peptides for use in mammalian cells include murine Ig kappa light chain signal peptide (Coloma, *J. Imm Methods* 152: 89-104 (1992)). Suitable signal peptides for use in yeast cells include the α-factor signal peptide from *S. cerevisiae* (see, e.g., U.S. Patent No. 4,870,008), the signal peptide of mouse salivary amylase (see, e.g., Hagenbuchle et al., *Nature* 289: 643-646 (1981)), a modified carboxypeptidase signal peptide (see, e.g., Valls et al., *Cell* 48: 887-897 (1987)), the yeast BAR1 signal peptide (see, e.g., PCT International Application WO 87/02670), and the yeast aspartic protease 3 (YAPS) signal peptide (see, e.g., Egel-Mitani et al., *Yeast* 6: 127-137 (1990)).

[0394] In view of the above, the above-described isolated or purified nucleic acid molecule, which can be a vector, can be introduced into a host cell as described herein below. Accordingly, a host cell comprising the isolated or purified nucleic acid molecule is provided.

[0395] Any suitable host can be used to produce the (a) nucleocapsid protein,a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2), including bacteria, fungi (including yeasts), plant, insect, mammal or other appropriate animal cells or cell lines, as well as transgenic animals or plants. A preferred host cell is a Chinese hamster ovary (CHO) cell. Examples of bacterial host cells include, but are not limited to, gram-positive bacteria, such as strains of *Bacillus*, for example, *B. brevis* or *B. subtilis*, *Pseudomonas* or Streptomyces, or gram-negative bacteria, such as strains of *E. coli*. The introduction of a vector into a bacterial host cell can, for instance, be effected by protoplast transformation (see, for example, Chang et al., *Molec. Gen. Genet.* 168: 111-115 (1979)), using competent cells (see, for example, Young et al., *J. of Bacteriology* 81: 823-829 (1961), or Dubnau et al., *J. of Molec. Biol.* 56: 209-221 (1971)), electroporation (see, for example, Shigekawa et al., *Biotechniques* 6: 742-751 (1988)), or conjugation (see, for example, Koehler et al., *J. of Bacteriology* 169: 5771-5278 (1987)).

[0396] Examples of suitable filamentous fungal host cells include, but are not limited to, strains of *Aspergillus*, for example, *A. oryzae*, *A. niger*, or *A. nidulans*, *Fusarium* or *Trichoderma*. Fungal cells can be transformed by a process involving protoplast formation, transformation of the protoplasts, and regeneration of the cell wall using techniques known to those ordinarily skilled in the art. Suitable procedures for transformation of Aspergillus host cells are described in EP Patent Application No. 0 238 023 and U.S. Patent No. 5,679,543. Suitable methods for transforming *Fusarium* species are described by Malardier et al., Gene 78: 147-156 (1989), and PCT International Application WO 96/00787. Yeast can be transformed using the procedures described by Becker and Guarente, In Abelson, J. N. and Simon, M. I., editors, *Guide to Yeast Genetics and Molecular Biology, Methods in Enzymology* 194: 182-187, Academic Press, Inc., New York; Ito et al, *J. of Bacteriology* 153: 163 (1983); and Hinnen et al., *PNAS USA* 75: 1920 (1978).

[0397] Examples of suitable yeast host cells include strains of *Saccharomyces*, for example, *S. cerevisiae*, *Schizosaccharomyces*, *Klyveromyces*, *Pichia*, such as *P. pastoris* or *P. methanolica*,

Hansenula, such as *H. polymorpha* or *yarrowia*. Methods for transforming yeast cells with heterologous polynucleotides and producing heterologous polypeptides therefrom are disclosed by Clontech Laboratories, Inc, Palo Alto, Calif., USA (in the product protocol for the Yeastmaker™ Yeast Tranformation System Kit), and by Reeves et al., *FEMS Microbiology Letters* 99: 193-198 (1992), Manivasakam et al., *Nucleic Acids Research* 21: 4414-4415 (1993), and Ganeva et al., *FEMS Microbiology Letters* 121: 159-164 (1994).

[0398] Examples of suitable insect host cells include, but are not limited to, a *Lepidoptora* cell line, such as Spodoptera frugiperda (Sf9 or Sf21) or Trichoplusia ni cells (High Five) (see, e.g., U.S. Patent No. 5,077,214). Transformation of insect cells and production of heterologous polypeptides are well-known to those skilled in the art.

[0399] Examples of suitable mammalian host cells include Chinese hamster ovary (CHO) cell lines, simian (e.g., Green Monkey) cell lines (COS), mouse cells (for example, NS/O), baby hamster kidney (BHK) cell lines, human cells (such as human embryonic kidney (HEK) cells (e.g., HEK 293 cells (A.T.C.C. Accession No. CRL-1573)), myeloma cells that do not otherwise produce immunoglobulin protein, and plant cells in tissue culture. Preferably, the mammalian host cells are CHO cell lines and/or HEK (e.g., HEK 293) cell lines. Another preferred host cell is the B3.2 cell line (e.g., AbbVie, AbbVie Bioresearch Center, Worcester, Mass.), or another dihydrofolate reductase deficient ($DHFR^-$) CHO cell line (e.g., available from Invitrogen).

[0400] Methods for introducing exogenous polynucleotides into mammalian host cells include calcium phosphate-mediated transfection, electroporation, DEAE-dextran mediated transfection, liposome-mediated transfection, viral vectors and the transfection method described by Life Technologies Ltd, Paisley, UK using Lipofectamine™ 2000. These methods are well-known in the art and are described, for example, by Ausbel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, USA (1996). The cultivation of mammalian cells is conducted according to established methods, e.g., as disclosed in Jenkins, Ed., *Animal Cell Biotechnology, Methods and Protocols*, Human Press Inc. Totowa, N.J., USA (1999), and Harrison and Rae, *General Techniques of Cell Culture*, Cambridge University Press (1997).

[0401] In the production methods, cells are cultivated in a nutrient medium suitable for production of the (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof

that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2) using methods known in the art. For example, cells are cultivated by shake flask cultivation, small-scale or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or industrial fermenters performed in a suitable medium and under conditions allowing the (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2) to be expressed and/or isolated. The cultivation takes place in a suitable nutrient medium comprising carbon and nitrogen sources and inorganic salts, using procedures known in the art. Suitable media are available from commercial suppliers or can be prepared according to published compositions (e.g., in catalogues of the American Type Culture Collection). If the (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2) is secreted into the nutrient medium, it can be recovered directly from the medium. If the truncated DBP (or fusion protein thereof) or mutated truncated DBP (or fusion protein thereof) is not secreted, it can be recovered from cell lysates.

[0402] The resulting (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant

thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2) can be recovered by methods known in the art. For example, the (a) nucleocapsid protein,a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2) an be recovered from the nutrient medium by conventional procedures including, but not limited to, centrifugation, filtration, extraction, spray drying, evaporation, or precipitation.

[0403] The (a) nucleocapsid protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (b) spike protein, a fragment or variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); (c) any protein, fragment or variant thereof that binds to an anti-nucleocapsid antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2); and/or (d) any protein, fragment or variant thereof that binds to any anti-spike antibody or antibody variant thereof from a β-coronavirus (such as SARS-CoV or SARS-CoV-2) can be purified by a variety of procedures known in the art including, but not limited to, chromatography (such as, but not limited to, ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (such as, but not limited to, preparative isoelectric focusing), differential solubility (such as, but not limited to, ammonium sulfate precipitation), SDS-PAGE, or extraction (see, for example, Janson and Ryden, editors, Protein Purification, VCH Publishers, New York (1989)). In some aspects, the purification can be done in CHO and/or HEK cells using routine techniques known in the art. In other aspects, when purifying the RBD of spike, any monomeric RBD can be separated from any dimeric RBD using routine techniques known in the art such as, for example, affinity chromatography, gel fitration chromatography, ion-exchange chromatography, high-pressure liquid chromatography, etc. In some aspects, the purification is done using immobilized metal affinity chromatography (IMAC), such as, for example, as described in "Block et al., "Chapter 27 Immobilized-Metal Affinity Chromatography (IPAC): A Review", *Methods in* Enzymology, 463:439-473 (2009) and Spriestersbach et al., "Chapter One – Purification of His-Tagged

Proteins", *Methods in Enzymology*, 559:1-15 (2015), the contents of which are herein incorporated by reference.

b. Antibodies

[0404] Antibodies may be prepared by any of a variety of techniques, including those well known to those skilled in the art. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies via conventional techniques, or via transfection of antibody genes, heavy chains, and/or light chains into suitable bacterial or mammalian cell hosts, to allow for the production of antibodies, wherein the antibodies may be recombinant. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express the antibodies in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells is preferable, and most preferable in mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody.

[0405] Exemplary mammalian host cells for expressing the recombinant antibodies include Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described in Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77: 4216-4220 (1980)), used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp, *J. Mol. Biol.*, 159: 601-621 (1982), NS0 myeloma cells, COS cells, and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods. In some aspects, the purification of the antibodies can be done in CHO and/or HEK cells using routine techniques known in the art.

[0406] Host cells can also be used to produce functional antibody fragments, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure may be performed. For example, it may be desirable to transfect a host cell with DNA encoding functional fragments of either the light chain and/or the heavy chain of an antibody.

Recombinant DNA technology may also be used to remove some, or all, of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to the antigens of interest. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody (i.e., binds to one or more epitopes on a β-coronavirus (such as SARS-CoV or SARS-CoV-2), such as SARS-CoV or SARS-CoV-2) and the other heavy and light chain are specific for an antigen other than a human β-coronavirus (such as SARS-CoV or SARS-CoV-2), such as SARS-CoV or SARS-CoV-2) by crosslinking an antibody to a second antibody by standard chemical crosslinking methods.

[0407] In a preferred system for recombinant expression of an antibody, or antigen-binding portion thereof, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr-CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells, and recover the antibody from the culture medium. Still further, the method of synthesizing a recombinant antibody may be by culturing a host cell in a suitable culture medium until a recombinant antibody is synthesized. The method can further comprise isolating the recombinant antibody from the culture medium.

[0408] Methods of preparing monoclonal antibodies involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity. Such cell lines may be produced from spleen cells obtained from an immunized animal. The animal may be immunized with β-coronavirus, such as SARS-CoV or SARS-CoV-2) or a fragment (e.g., such as from the nucleocapsid and/or spike proteins) and/or variant thereof. The peptide used to immunize the animal may comprise amino acids encoding human Fc, for example the fragment crystallizable region or tail region of human antibody. The spleen cells may then be immortalized by, for

example, fusion with a myeloma cell fusion partner. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports that growth of hybrid cells, but not myeloma cells. One such technique uses hypoxanthine, aminopterin, thymidine (HAT) selection. Another technique includes electrofusion. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity may be used.

[0409] Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. Affinity chromatography is an example of a method that can be used in a process to purify the antibodies.

[0410] The proteolytic enzyme papain preferentially cleaves IgG molecules to yield several fragments, two of which (the F(ab) fragments) each comprise a covalent heterodimer that includes an intact antigen-binding site. The enzyme pepsin is able to cleave IgG molecules to provide several fragments, including the $F(ab')_2$ fragment, which comprises both antigen-binding sites.

[0411] The Fv fragment can be produced by preferential proteolytic cleavage of an IgM, and on rare occasions IgG or IgA immunoglobulin molecules. The Fv fragment may be derived using recombinant techniques. The Fv fragment includes a non-covalent VH::VL heterodimer including an antigen-binding site that retains much of the antigen recognition and binding capabilities of the native antibody molecule.

[0412] The antibody, antibody fragment, or derivative may comprise a heavy chain and a light chain complementarity determining region ("CDR") set, respectively interposed between a heavy chain and a light chain framework ("FR") set which provide support to the CDRs and define the spatial relationship of the CDRs relative to each other. The CDR set may contain three hypervariable regions of a heavy or light chain V region.

[0413] Other suitable methods of producing or isolating antibodies of the requisite specificity can be used, including, but not limited to, methods that select recombinant antibody from a peptide or protein library (e.g., but not limited to, a bacteriophage, ribosome, oligonucleotide, RNA, cDNA, yeast or the like, display library); e.g., as available from various commercial vendors such as Cambridge Antibody Technologies (Cambridgeshire, UK), MorphoSys (Martinsreid/Planegg, Del.), Biovation (Aberdeen, Scotland, UK) BioInvent (Lund, Sweden), using methods known in the art. See U.S. Patent Nos. 4,704,692; 5,723,323; 5,763,192; 5,814,476; 5,817,483; 5,824,514; 5,976,862. Alternative methods rely upon immunization of transgenic animals (*e.g.*, SCID mice, Nguyen et al. (1997) *Microbiol. Immunol.* 41:901-907; Sandhu et al.,*Crit. Rev. Biotechnol.* 16:95-118 (1996); Eren et al., *Immunol.* 93:154-161 (1998)) that are capable of producing a repertoire of human antibodies, as known in the art and/or as described herein. Such techniques, include, but are not limited to, ribosome display (Hanes et al. *Proc. Natl. Acad. Sci. USA*, 94:4937-4942 (1997); Hanes et al., *Proc. Natl. Acad. Sci. USA*, 95:14130-14135 (1998)); single cell antibody producing technologies (e.g., selected lymphocyte antibody method ("SLAM") (U.S. Patent No. 5,627,052, Wen et al., *J. Immunol.* 17:887-892 (1987); Babcook et al., *Proc. Natl. Acad. Sci. USA* 93:7843-7848 (1996)); gel microdroplet and flow cytometry (Powell et al., *Biotechnol.*. 8:333-337 (1990); One Cell Systems, (Cambridge, Mass).; Gray et al., *J. Imm. Meth.*. 182:155-163 (1995); Kenny et al., *Bio/Technol.*, 13:787-790) (1995); B-cell selection (Steenbakkers et al., *Molec. Biol. Reports* 19:125-134 (1994)).

[0414] An affinity matured antibody may be produced by any one of a number of procedures that are known in the art. For example, see Marks et al., *BioTechnology*, 10: 779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by Barbas et al., *Proc. Nat. Acad. Sci. USA*, 91: 3809-3813 (1994); Schier et al., *Gene*, 169: 147-155 (1995); Yelton *et al.*, *J. Immunol.*, 155: 1994-2004 (1995); Jackson et al., *J. Immunol.*, 154(7): 3310-3319 (1995); Hawkins et al., *J. Mol. Biol.*, 226: 889-896 (1992). Selective mutation at selective mutagenesis positions and at contact or hypermutation positions with an activity enhancing amino acid residue is described in U.S. Patent No. 6,914,128 B1.

[0415] Antibody variants can also be prepared using delivering a polynucleotide encoding an antibody to a suitable host such as to provide transgenic animals or mammals, such as goats, cows, horses, sheep, and the like, that produce such antibodies in their milk. These methods are

known in the art and are described for example in U.S. Patent Nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; and 5,304,489.

[0416] Antibody variants also can be prepared by delivering a polynucleotide to provide transgenic plants and cultured plant cells (*e.g.*, but not limited to tobacco, maize, and duckweed) that produce such antibodies, specified portions or variants in the plant parts or in cells cultured therefrom. For example, Cramer et al.,*Curr. Top. Microbiol. Immunol.* 240:95-118 (1999) and references cited therein, describe the production of transgenic tobacco leaves expressing large amounts of recombinant proteins, e.g., using an inducible promoter. Transgenic maize have been used to express mammalian proteins at commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. See, e.g., Hood et al., *Adv. Exp. Med. Biol.*, 464:127-147 (1999) and references cited therein. Antibody variants have also been produced in large amounts from transgenic plant seeds including antibody fragments, such as single chain antibodies (scFv's), including tobacco seeds and potato tubers. See, e.g., Conrad et al., *Plant Mol. Biol.* 38:101-109 (1998) and reference cited therein. Thus, antibodies can also be produced using transgenic plants, according to known methods.

[0417] Antibody derivatives can be produced, for example, by adding exogenous sequences to modify immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic. Generally, part or all of the non-human or human CDR sequences are maintained while the non-human sequences of the variable and constant regions are replaced with human or other amino acids.

[0418] Small antibody fragments may be diabodies having two antigen-binding sites, wherein fragments comprise a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (VH VL). See for example, EP 404,097; WO 93/11161; and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. See also, U.S. Patent No. 6,632,926 which is hereby incorporated by reference in its entirety and discloses antibody variants that have one or more amino acids inserted into a hypervariable region of the parent antibody and a binding affinity for a target antigen which is at least about two fold stronger than the binding affinity of the parent antibody for the antigen.

[0419] The antibody may be a linear antibody. The procedure for making a linear antibody is known in the art and described in Zapata et al., *Protein Eng.* 8(10):1057-1062 (1995). Briefly, these antibodies comprise a pair of tandem Fd segments (VH-CH1-VH-CH1) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

[0420] The antibodies may be recovered and purified from recombinant cell cultures by known methods including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography ("HPLC") can also be used for purification.

[0421] It may be useful to detectably label the antibody. Methods for conjugating antibodies to these agents are known in the art. For the purpose of illustration only, antibodies can be labeled with a detectable moiety such as a radioactive atom, a chromophore, a fluorophore, or the like. Such labeled antibodies can be used for diagnostic techniques, either in vivo, or in an isolated test sample. They can be linked to a cytokine, to a ligand, to another antibody. Suitable agents for coupling to antibodies to achieve an anti-tumor effect include cytokines, such as interleukin 2 (IL-2) and Tumor Necrosis Factor (TNF); photosensitizers, for use in photodynamic therapy, including aluminum (III) phthalocyanine tetrasulfonate, hematoporphyrin, and phthalocyanine; radionuclides, such as iodine-131 (131I), yttrium-90 (90Y), bismuth-212 (212Bi), bismuth-213 (213Bi), technetium-99m (99mTc), rhenium-186 (186Re), and rhenium-188 (188Re); antibiotics, such as doxorubicin, adriamycin, daunorubicin, methotrexate, daunomycin, neocarzinostatin, and carboplatin; bacterial, plant, and other toxins, such as diphtheria toxin, pseudomonas exotoxin A, staphylococcal enterotoxin A, abrin-A toxin, ricin A (deglycosylated ricin A and native ricin A), TGF-alpha toxin, cytotoxin from chinese cobra (naja naja atra), and gelonin (a plant toxin); ribosome inactivating proteins from plants, bacteria and fungi, such as restrictocin (a ribosome inactivating protein produced by Aspergillus restrictus), saporin (a ribosome inactivating protein from Saponaria officinalis), and RNase; tyrosine kinase inhibitors; ly207702 (a difluorinated purine nucleoside); liposomes containing anti cystic agents (*e.g.*, antisense oligonucleotides, plasmids which encode for toxins, methotrexate, etc.); and other antibodies or antibody fragments, such as F(ab).

[0422] Antibody production via the use of hybridoma technology, the selected lymphocyte antibody method (SLAM), transgenic animals, and recombinant antibody libraries is described in more detail below.

Monoclonal Antibodies Using Hybridoma Technology

[0423] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow *et al.*, Antibodies: *A Laboratory Manual*, second edition, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1988); Hammerling, *et al.*, *In Monoclonal Antibodies and T-Cell Hybridomas*, (Elsevier, N.Y., 1981). It is also noted that the term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[0424] Methods of generating monoclonal antibodies as well as antibodies produced by the method may comprise culturing a hybridoma cell secreting an antibody wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from an animal, e.g., a rat or a mouse, immunized with a β-coronavirus, such as SARS-CoV or SARS-CoV-2 (e.g., such as a human, mouse, rat, rabbit SARS-CoVor SARS-CoV-2), or a fragment or variant thereof (collectively referred to as a "β-coronavirus antigen") with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide. Briefly, rats can be immunized with a β-coronavirus (such as SARS-CoV or SARS-CoV-2) antigen. In a preferred embodiment, the β-coronavirus (such as SARS-CoV or SARS-CoV-2) antigen is administered with an adjuvant to stimulate the immune response. Such adjuvants include complete or incomplete Freund's adjuvant, RIBI (muramyl dipeptides) or ISCOM (immunostimulating complexes). Such adjuvants may protect the polypeptide from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. Preferably, if a polypeptide is being administered, the immunization schedule will involve two or more administrations of the polypeptide, spread out over several weeks; however, a single administration of the polypeptide may also be used.

[0425] After immunization of an animal with a β-coronavirus (such as SARS-CoV or SARS-CoV-2) antigen, antibodies and/or antibody-producing cells may be obtained from the animal. An anti- β-coronavirus (such as SARS-CoV or SARS-CoV-2) antibody-containing serum is obtained from the animal by bleeding or sacrificing the animal. The serum may be used as it is obtained from the animal, an immunoglobulin fraction may be obtained from the serum, or the anti-β-coronavirus antibodies may be purified from the serum. Serum or immunoglobulins obtained in this manner are polyclonal, thus having a heterogeneous array of properties.

[0426] Once an immune response is detected, e.g., antibodies specific for the antigen β-coronavirus (such as SARS-CoV or SARS-CoV-2) are detected in the rat serum, the rat spleen is harvested and splenocytes isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example, cells from cell line SP20 available from the American Type Culture Collection (ATCC, Manassas, Va., US). Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding to a β-coronavirus (such as, for example, SARS-CoV or SARS-CoV-2). Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing rats with positive hybridoma clones.

[0427] In another embodiment, antibody-producing immortalized hybridomas may be prepared from the immunized animal. After immunization, the animal is sacrificed and the splenic B cells are fused to immortalized myeloma cells as is well known in the art. See, *e.g.*, Harlow and Lane, supra. In a preferred embodiment, the myeloma cells do not secrete immunoglobulin polypeptides (a non-secretory cell line). After fusion and antibiotic selection, the hybridomas are screened using a β-coronavirus (such as SARS-CoV or SARS-CoV-2), or a portion thereof, or a cell expressing a β-coronavirus (such as SARS-CoV or SARS-CoV-2) or portion thereof. In a preferred embodiment, the initial screening is performed using an enzyme-linked immunosorbent assay (ELISA) or a radioimmunoassay (RIA), preferably an ELISA. An example of ELISA screening is provided PCT International Application WO 00/37504.

[0428] β-coronavirus antibody-producing hybridomas are selected, cloned, and further screened for desirable characteristics, including robust hybridoma growth, high antibody production, and desirable antibody characteristics. Hybridomas may be cultured and expanded *in vivo* in syngeneic animals, in animals that lack an immune system, *e.g.*, nude mice, or in cell

culture *in vitro*. Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art.

[0429] In a preferred embodiment, hybridomas are rat hybridomas. In another embodiment, hybridomas are produced in a non-human, non-rat species such as mice, sheep, pigs, goats, cattle, or horses. In yet another preferred embodiment, the hybridomas are human hybridomas, in which a human non-secretory myeloma is fused with a human cell expressing an anti-β-coronavirus antibody.

[0430] Antibody fragments that recognize specific epitopes may be generated by known techniques. For example, Fab and $F(ab')_2$ fragments may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce two identical Fab fragments) or pepsin (to produce an $F(ab')_2$ fragment). A $F(ab')_2$ fragment of an IgG molecule retains the two antigen-binding sites of the larger ("parent") IgG molecule, including both light chains (containing the variable light chain and constant light chain regions), the CH1 domains of the heavy chains, and a disulfide-forming hinge region of the parent IgG molecule. Accordingly, an $F(ab')_2$ fragment is still capable of crosslinking antigen molecules like the parent IgG molecule.

Monoclonal Antibodies Using SLAM

[0431] In another aspect, recombinant antibodies are generated from single, isolated lymphocytes using a procedure referred to in the art as the selected lymphocyte antibody method (SLAM), as described in U.S. Patent No. 5,627,052; PCT International Application WO 92/02551; and Babcook et al., *Proc. Natl. Acad. Sci. USA*, 93: 7843-7848 (1996). In this method, single cells secreting antibodies of interest, *e.g.*, lymphocytes derived from any one of the immunized animals are screened using an antigen-specific hemolytic plaque assay, wherein the β-coronavirus (such as SARS-CoV or SARS-CoV-2) antigen is coupled to sheep red blood cells using a linker, such as biotin, and used to identify single cells that secrete antibodies with specificity for a β-coronavirus (such as SARS-CoV or SARS-CoV-2). Following identification of antibody-secreting cells of interest, heavy- and light-chain variable region cDNAs are rescued from the cells by reverse transcriptase-PCR (RT-PCR) and these variable regions can then be expressed, in the context of appropriate immunoglobulin constant regions (e.g., human constant regions), in mammalian host cells, such as COS or CHO cells. The host cells transfected with the amplified immunoglobulin sequences, derived from in vivo selected lymphocytes, can then

undergo further analysis and selection in vitro, for example, by panning the transfected cells to isolate cells expressing antibodies to a β-coronavirus (such as SARS-CoV or SARS-CoV-2). The amplified immunoglobulin sequences further can be manipulated in vitro, such as by in vitro affinity maturation method. See, for example, PCT International Application WO 97/29131 and PCT International Application WO 00/56772.

Monoclonal Antibodies Using Transgenic Animals

[0432] In another embodiment, antibodies are produced by immunizing a non-human animal comprising some, or all, of the human immunoglobulin locus with a β-coronavirus (such as SARS-CoV or SARS-CoV-2) antigen. In an embodiment, the non-human animal is a XENOMOUSE® transgenic mouse, an engineered mouse strain that comprises large fragments of the human immunoglobulin loci and is deficient in mouse antibody production. See, e.g., Green et al., *Nature Genetics*, 7: 13-21 (1994) and U.S. Patent Nos. 5,916,771; 5,939,598; 5,985,615; 5,998,209; 6,075,181; 6,091,001; 6,114,598; and 6,130,364. See also. PCT International Application Nos. WO 91/10741; WO 94/02602; WO 96/34096; WO 96/33735; WO 98/16654; WO 98/24893; WO 98/50433; WO 99/45031; WO 99/53049; WO 00/09560; and WO 00/37504. The XENOMOUSE® transgenic mouse produces an adult-like human repertoire of fully human antibodies, and generates antigen-specific human monoclonal antibodies. The XENOMOUSE® transgenic mouse contains approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and x light chain loci. See Mendez et al., *Nature Genetics*, 15: 146-156 (1997), Green and Jakobovits, *J. Exp. Med.*, 188: 483-495 (1998), the disclosures of which are hereby incorporated by reference.

Monoclonal Antibodies Using Recombinant Antibody Libraries

[0433] *In vitro* methods also can be used to make the antibodies, wherein an antibody library is screened to identify an antibody having the desired β-coronavirus (such as SARS-CoV or SARS-CoV-2)-binding specificity. Methods for such screening of recombinant antibody libraries are well known in the art and include methods described in, for example, U.S. Patent No. 5,223,409 ; PCT International Application Nos. WO 92/18619 , WO 91/17271 , WO 92/20791 , WO 92/15679, WO 93/01288 , WO 92/01047 , WO 92/09690 ; Fuchs et al., Bio/Technology, 9: 1369-1372 (1991); Hay et al., *Hum. Antibod. Hybridomas*, 3: 81-85 (1992); Huse et al., *Science*, 246: 1275-1281 (1989); McCafferty et al., *Nature*, 348: 552-554 (1990); Griffiths et al., *EMBO*

J., 12: 725-734 (1993); Hawkins et al., J. Mol. Biol., 226: 889-896 (1992); Clackson et al., Nature, 352: 624-628 (1991); Gram et al., *Proc. Natl. Acad. Sci. USA*, 89: 3576-3580 (1992); Garrard et al., *Bio/Technology*, 9: 1373-1377 (1991); Hoogenboom et al., *Nucl. Acids Res.*, 19: 4133-4137 (1991); Barbas et al., *Proc. Natl. Acad. Sci. USA*, 88: 7978-7982 (1991); U.S. Patent Application Publication No. 2003/0186374; and PCT International Application WO 97/29131, the contents of each of which are incorporated herein by reference.

[0434] The recombinant antibody library may be from a subject immunized with a β-coronavirus (such as SARS-CoV or SARS-CoV-2) antigen. Alternatively, the recombinant antibody library may be from a naive subject, i.e., one who has not been immunized with a β-coronavirus (such as SARS-CoV or SARS-CoV-2) antigen, such as a human antibody library from a human subject who has not been immunized with a human β-coronavirus (such as SARS-CoV or SARS-CoV-2) antigen. Antibodies are selected by screening the recombinant antibody library with the peptide comprising human β-coronavirus (such as SARS-CoV or SARS-CoV-2) or fragment thereof to thereby select those antibodies that recognize the β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) of interest. Methods for conducting such screening and selection are well known in the art, such as described in the references in the preceding paragraph. To select antibodies having particular binding affinities, the art-known method of surface plasmon resonance can be used to select antibodies having the desired K_{off} rate constant. To select antibodies having a particular neutralizing activity for a β-coronavirus, such as SARS-CoV or SARS-CoV-2, such as those with a particular IC_{50}, standard methods known in the art for assessing the inhibition of β-coronavirus (such as SARS-CoV or SARS-CoV-2) activity may be used.

[0435] In one aspect, the disclosure pertains to an isolated antibody, or an antigen-binding portion thereof, that binds to an anti-species IgG (e.g., anti-human-IgG IgG), anti-species IgM (e.g., anti-human-IgM IgG) and/or anti-species IgG (e.g., anti-human-IgG IgG) or IgM antibody. In various embodiments, the antibody is a recombinant antibody or a monoclonal antibody. Methods for making anti-species antibodies, such as anti-species IgG (e.g., anti-human-IgG IgG) and/or IgM antibodies are well known in the art.

[0436] For example, antibodies can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. Such phage

can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv, or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies include those disclosed in Brinkmann et al., *J. Immunol. Methods*, 182: 41-50 (1995); Ames et al., J. Immunol. Methods, 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.*, 24: 952-958 (1994); Persic et al., Gene, 187: 9-18 (1997); Burton et al., Advances in *Immunology*, 57: 191-280 (1994); PCT International Application Nos. WO 92/01047, WO 90/02809, WO 91/10737, WO 92/01047, WO 92/18619, WO 93/11236, WO 95/15982, WO 95/20401, and U.S. Patent Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727, 5,733,743, and 5,969,108.

[0437] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies including human antibodies or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab', and $F(ab')_2$ fragments can also be employed using methods known in the art such as those disclosed in WO 92/22324; Mullinax et al., *BioTechniques*, 12(6): 864-869 (1992); Sawai et al., *Am. J. Reprod. Immunol.*, 34: 26-34 (1995); and Better et al., *Science*, 240: 1041-1043 (1988). Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patent Nos. 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology, 203: 46-88 (1991); Shu et al., *Proc. Natl. Acad. Sci. USA*, 90: 7995-7999 (1993); and Skerra et al., Science, 240: 1038-1041 (1988).

[0438] Alternative to screening of recombinant antibody libraries by phage display, other methodologies known in the art for screening large combinatorial libraries can be applied to the identification of antibodies. One type of alternative expression system is one in which the recombinant antibody library is expressed as RNA-protein fusions, as described in PCT International Application WO 98/31700 (Szostak and Roberts), and in Roberts and Szostak,

Proc. Natl. Acad. Sci. USA, 94: 12297-12302 (1997). In this system, a covalent fusion is created between an mRNA and the peptide or protein that it encodes by *in vitro* translation of synthetic mRNAs that carry puromycin, a peptidyl acceptor antibiotic, at their 3' end. Thus, a specific mRNA can be enriched from a complex mixture of mRNAs (e.g., a combinatorial library) based on the properties of the encoded peptide or protein, e.g., antibody, or portion thereof, such as binding of the antibody, or portion thereof, to the dual specificity antigen. Nucleic acid sequences encoding antibodies, or portions thereof, recovered from screening of such libraries can be expressed by recombinant means as described above (e.g., in mammalian host cells) and, moreover, can be subjected to further affinity maturation by either additional rounds of screening of mRNA-peptide fusions in which mutations have been introduced into the originally selected sequence(s), or by other methods for affinity maturation in vitro of recombinant antibodies, as described above. A preferred example of this methodology is PROfusion display technology.

[0439] In another approach, the antibodies can also be generated using yeast display methods known in the art. In yeast display methods, genetic methods are used to tether antibody domains to the yeast cell wall and display them on the surface of yeast. In particular, such yeast can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Examples of yeast display methods that can be used to make the antibodies include those disclosed in U.S. Patent No. 6,699,658 incorporated herein by reference.

Production of Recombinant Antibodies

[0440] Antibodies may be produced by any of a number of techniques known in the art. For example, expression from host cells, wherein expression vector(s) encoding the heavy and light chains is (are) transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection, and the like. Although it is possible to express the antibodies in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells is preferable, and most preferable in mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody.

[0441] Exemplary mammalian host cells for expressing the recombinant antibodies include Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described in Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77: 4216-4220 (1980), used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp, *J. Mol. Biol.*, 159: 601-621 (1982), NS0 myeloma cells, COS cells, and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods. In some aspects, the antibodies can be purified in CHO and/or HEK cells using routine techniques known in the art.

[0442] Host cells can also be used to produce functional antibody fragments, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure may be performed. For example, it may be desirable to transfect a host cell with DNA encoding functional fragments of either the light chain and/or the heavy chain of an antibody. Recombinant DNA technology may also be used to remove some, or all, of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to the antigens of interest. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody (i.e., binds to an IgG antibody, IgM antibody and/or IgG or IgM antibody) and the other heavy and light chain are specific for an antigen other than an IgG antibody, IgM antibody and/or an IgG and IgM antibody by crosslinking an antibody to a second antibody by standard chemical crosslinking methods.

[0443] In a preferred system for recombinant expression of an antibody, or antigen-binding portion thereof, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr-CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to

allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells, and recover the antibody from the culture medium. Still further, the disclosure provides a method of synthesizing a recombinant antibody by culturing a host cell in a suitable culture medium until a recombinant antibody is synthesized. The method can further comprise isolating the recombinant antibody from the culture medium.

Humanized Antibody

[0444] The humanized antibody may be an antibody or a variant, derivative, analog or portion thereof which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. The humanized antibody may be from a non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule.

[0445] As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. According to one aspect, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In some embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or of a heavy chain.

[0446] The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including without limitation IgG1, IgG2, IgG3, and IgG4. The humanized antibody may comprise sequences from more than one class or isotype, and particular constant domains may be selected to optimize desired effector functions using techniques well-known in the art.

[0447] The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, e.g., the donor antibody CDR or the consensus framework may be mutagenized by substitution, insertion and/or deletion of at least one amino acid residue so that the CDR or framework residue at that site does not correspond to either the donor antibody or the consensus framework. In one embodiment, such mutations, however, will not be extensive. Usually, at least 90%, at least 95%, at least 98%, or at least 99% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences. As used herein, the term "consensus framework" refers to the framework region in the consensus immunoglobulin sequence. As used herein, the term "consensus immunoglobulin sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related immunoglobulin sequences (See e.g., Winnaker, *From Genes to Clones* (Verlagsgesellschaft, Weinheim, Germany 1987)). In a family of immunoglobulins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence.

[0448] The humanized antibody may be designed to minimize unwanted immunological response toward rodent anti-human antibodies, which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. The humanized antibody may have one or more amino acid residues introduced into it from a source that is non-human. These non-human residues are often referred to as "import" residues, which are typically taken from a variable domain. Humanization may be performed by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. For example, see U.S. Patent No. 4,816,567, the contents of which are herein incorporated by reference. The humanized antibody may be a human antibody in which some hypervariable

region residues, and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies. Humanization or engineering of antibodies of the present disclosure can be performed using any known method, such as but not limited to those described in U.S. Patent Nos. 5,723,323; 5,976,862; 5,824,514; 5,817,483; 5,814,476; 5,763,192; 5,723,323; 5,766,886; 5,714,352; 6,204,023; 6,180,370; 5,693,762; 5,530,101; 5,585,089; 5,225,539; and 4,816,567.

[0449] The humanized antibody may retain high affinity for a β-coronavirus (such as SARS-CoV and SARS-CoV-2) and other favorable biological properties. The humanized antibody may be prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available. Computer programs are available that illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristics, such as increased affinity for β-coronavirus (such as SARS-CoV and SARS-CoV-2), is achieved. In general, the hypervariable region residues may be directly and most substantially involved in influencing antigen binding. As an alternative to humanization, human antibodies (also referred to herein as "fully human antibodies") can be generated. For example, it is possible to isolate human antibodies from libraries via PROfusion and/or yeast related technologies. It is also possible to produce transgenic animals (*e.g.*, mice that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. The humanized or fully human antibodies may be prepared according to the methods described in U.S. Patent Nos. 5,770,429; 5,833,985; 5,837,243; 5,922,845; 6,017,517; 6,096,311; 6,111,166; 6,270,765; 6,303,755; 6,365,116; 6,410,690; 6,682,928; and 6,984,720, the contents each of which are herein incorporated by reference.

9. Variations on Methods

[0450] The disclosed methods detect the presence or determine the amount or level of an anti-β-coronavirus antibody (such as an anti-SARS-CoV (IgG or IgM) antibody and an anti-SARS-CoV-2 (IgG or IgM) antibody) present in a biological sample as described herein. The methods may also be adapted in view of other methods for analyzing analytes. Examples of well-known variations include, but are not limited to, immunoassay, competitive inhibition immunoassay (e.g., forward and reverse), enzyme multiplied immunoassay technique (EMIT), a competitive binding assay, bioluminescence resonance energy transfer (BRET), one-step antibody detection assay, homogeneous assay, heterogeneous assay, capture on the fly assay, single molecule detection assay, lateral flow assay, etc.

a. Immunoassay

[0451] The analyte of interest, namely, an anti-β-coronavirus antibody (such as an anti-SARS-CoV (IgG or IgM) antibody and an anti-SARS-CoV-2 (IgG or IgM) antibody), may be analyzed using at least one first specific binding partner (e.g., at least one recombinant antigen) and at least one second specific binding partner (e.g., an antibody) in an immunoassay. The presence or amount of the analyte, namely, an anti-β-coronavirus antibody (such as an anti-SARS-CoV antibody or an anti-SARS-CoV-2 antibody), can be determined using the binding of the at least one first specific binding partner (e.g., at least one recombinant antigen) and at least one second specific binding partner to the analyte (e.g., an anti-β-coronavirus antibody (such as an anti-SARS-CoV antibody or an anti-SARS-CoV-2 antibody)). For example, at least recombinant antigen (e.g., at least one first specific binding partner) may specifically bind to the analyte (e.g., an anti-β-coronavirus antibody (such as anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody)). One or more antibodies or recombinant antigens labeled with at least one detectable label (e.g., second specific binding partner) can used to detect the presence of determine the amount of the analyte in the biological sample.

[0452] The presence or amount of the analyte (e.g., an anti-β-coronavirus antibody (such as an anti-SARS-CoV antibody or an anti-SARS-CoV-2 antibody)) present in a biological sample may be readily determined using an immunoassay. For example, in one aspect, one method that can be used is a chemiluminescent microparticle immunoassay, in particular one employing the ARCHITECT® automated analyzer (Abbott Laboratories, Abbott Park, IL), as an example.

Other methods that can be used include, for example, mass spectrometry, and immunohistochemistry (e.g., with sections from tissue biopsies). Additionally, methods of detection include those described in, for example, U.S. Patent Nos. 6,143,576; 6,113,855; 6,019,944; 5,985,579; 5,947,124; 5,939,272; 5,922,615; 5,885,527; 5,851,776; 5,824,799; 5,679,526; 5,525,524; and 5,480,792, each of which is hereby incorporated by reference in its entirety. Specific immunological binding of the antigen to an analyte (e.g., anti-β-coronavirus antibody) can be detected via direct labels, such as fluorescent or luminescent tags, metals and radionuclides attached to the antibody or via indirect labels, such as alkaline phosphatase or horseradish peroxidase.

[0453] The use of immobilized recombinant antigens (e.g., at least one first specific and/or second specific binding partner) and/or antibodies or antibody fragments thereof (e.g., at least one second specific binding partner) may be incorporated into the immunoassay. The recombinant antigens and/or antibodies may be immobilized onto a variety of supports, such as magnetic or chromatographic matrix particles, the surface of an assay plate (such as microtiter wells), pieces of a solid substrate material, and the like. An assay strip can be prepared by coating the antigen and/or antibody or plurality of antibodies in an array on a solid support. This strip can then be dipped into the test sample and processed quickly through washes and detection steps to generate a measurable signal, such as a colored spot.

[0454] A homogeneous format may be used. For example, after the biological sample is obtained from a subject, a mixture is prepared. The mixture contains the test sample being assessed for the analyte (e.g., anti-β-coronavirus antibody (such as an anti-SARS-CoV antibody or a anti-SARS-CoV-2 antibody)), a first specific binding partner, and a second specific binding partner. The order in which the test sample, the first specific binding partner, and the second specific binding partner are added to form the mixture is not critical. The test sample is simultaneously contacted with the first specific binding partner and the second specific binding partner. In some embodiments, the first specific binding partner and any anti-β-coronavirus antibody (such as anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) contained in the test sample may form a first specific binding partner-analyte (e.g., anti-β-coronavirus antibody)-complex and the second specific binding partner may form a first specific binding partner-analyte of interest (e.g., anti-β-coronavirus antibody (such as an anti-SARS-CoV antibody or an

anti-SARS-CoV-2 antibody))-second specific binding partner complex. Moreover, the second specific binding partner is labeled with or contains a detectable label as described above.

[0455] A heterogeneous format may be used. For example, after the biological sample is obtained from a subject, a first mixture is prepared. The mixture contains the biological sample being assessed for the analyte (anti-β-coronavirus antibody) and a first specific binding partner, wherein the first specific binding partner and any anti-β-coronavirus antibody contained in the biological sample form a first specific binding partner-analyte(anti-β-coronavirus antibody)-complex. The order in which the biological sample and the first specific binding partner are added to form the mixture is not critical.

[0456] The first specific binding partner (e.g., at least one recombinant antigen) may be immobilized on a solid phase. The solid phase used in the immunoassay (for the first specific binding partner and, optionally, the second specific binding partner) can be any solid phase known in the art, such as, but not limited to, a magnetic particle, a bead, a test tube, a microtiter plate, a cuvette, a membrane, a scaffolding molecule, a film, a filter paper, a disc, and a chip. In those embodiments where the solid phase is a bead, the bead may be a magnetic bead or a magnetic particle. Magnetic beads/particles may be ferromagnetic, ferrimagnetic, paramagnetic, superparamagnetic or ferrofluidic. Exemplary ferromagnetic materials include Fe, Co, Ni, Gd, Dy, CrO_2, MnAs, MnBi, EuO, and NiO/Fe. Examples of ferrimagnetic materials include $NiFe_2O_4$, $CoFe_2O_4$, Fe_3O_4 (or $FeO{\cdot}Fe_2O_3$). Beads can have a solid core portion that is magnetic and is surrounded by one or more non-magnetic layers. Alternately, the magnetic portion can be a layer around a non-magnetic core. The solid support on which the first specific binding partner is immobilized may be stored in dry form or in a liquid. The magnetic beads may be subjected to a magnetic field prior to or after contacting with the sample with a magnetic bead on which the first specific binding partner is immobilized.

[0457] After the mixture containing the first specific binding partner-analyte (e.g, anti-β-coronavirus antibody (such as an anti-SARS-CoV antibody or an anti-SARS-CoV-2 antibody)) complex is formed, any unbound analyte (e.g., anti-β-coronavirus antibody) is removed from the complex using any technique known in the art. For example, the unbound analyte (e.g., anti-β-coronavirus antibody) can be removed by washing. Desirably, however, the first specific binding partner is present in excess of any analyte (e.g., anti-β-coronavirus antibody (such as an anti-SARS-CoV antibody or an anti-SARS-CoV-2 antibody)) present in the test sample, such

that all analyte (e.g., anti-β-coronavirus antibody (such as an anti-SARS-CoV antibody or an anti-SARS-CoV-2 antibody)) that is present in the test sample is bound by the first specific binding partner.

[0458] After any unbound analyte (e.g., anti-β-coronavirus antibody (such as an anti-SARS-CoV antibody or an anti-SARS-CoV-2 antibody)) is removed, a second specific binding partner is added to the mixture to form a first specific binding partner-analyte of interest (e.g., anti-β-coronavirus antibody (such as an anti-SARS-CoV antibody or an anti-SARS-CoV-2 antibody))-second specific binding partner complex. Moreover, the second specific binding partner is labeled with or contains a detectable label as described above.

[0459] The use of recombinant antigens, immobilized antibodies or antibody fragments thereof may be incorporated into the immunoassay. The recombinant antigens or antibodies may be immobilized onto a variety of supports, such as magnetic or chromatographic matrix particles (such as a magnetic bead), latex particles or modified surface latex particles, polymer or polymer film, plastic or plastic film, planar substrate, the surface of an assay plate (such as microtiter wells), pieces of a solid substrate material, and the like. The recombinant antigen or antibody (antibodies) can be bound to the solid support by adsorption, by covalent bonding using a chemical coupling agent or by other means known in the art, provided that such binding does not interfere with the ability of the antigen or antibody to bind analyte (e.g., an anti-β-coronavirus antibody). An assay strip can be prepared by coating the antibody or plurality of antigens or antibodies in an array on a solid support. This strip can then be dipped into the test sample and processed quickly through washes and detection steps to generate a measurable signal, such as a colored spot.

b. Forward Competitive Inhibition Assay

[0460] In a forward competitive format, an aliquot of labeled analyte of interest (e.g., an anti-β-coronavirus antibody) having a fluorescent label, a tag attached with a cleavable linker, etc.) of a known concentration is used to compete with analyte of interest (e.g., anti-β-coronavirus antibody) in a biological sample for binding to recombinant antigen of interest or analyte of interest antibody.

[0461] In a forward competition assay, an immobilized specific binding partner (such as a recombinant antigen or antibody) can either be sequentially or simultaneously contacted with the biological sample and a labeled analyte of interest, labeled analyte of interest fragment or labeled

analyte of interest variant thereof. The analyte of interest, analyte of interest fragment or analyte of interest variant, can be labeled with any detectable label, including a detectable label comprised of tag attached with a cleavable linker. In this assay, the recombinant antigen or antibody can be immobilized on to a solid support. Alternatively, the antibody can be coupled to an antibody, such as an antispecies antibody, that has been immobilized on a solid support, such as a microparticle or planar substrate.

[0462] The labeled analyte of interest, the biological sample and the recombinant antigen or antibody are incubated at a pH of from about 4.5 to about 10.0, at a temperature of from about 2°C to about 45°C, and for a period from at least one (1) minute to about eighteen (18) hours, from about 2-6 minutes, from about 7-12 minutes, from a bout 5-15 minutes, or from about 3-4 minutes. Two different species of recombinant antigen or antibody-analyte of interest complexes may then be generated. Specifically, one of the recombinant antigen or antibody-analyte of interest complexes generated contains a detectable label (e.g., a fluorescent label, etc.) while the other recombinant antigen or antibody-analyte of interest complex does not contain a detectable label. The recombinant antigen or antibody-analyte of interest complex can be, but does not have to be, separated from the remainder of the biological sample prior to quantification of the detectable label. Regardless of whether the antigen or recombinant antibody-analyte of interest complex is separated from the remainder of the biological sample, the amount of detectable label in the recombinant antigen or antibody-analyte of interest complex is then quantified. The concentration of analyte of interest in the biological sample can then be determined as described above.

c. Reverse Competitive Inhibition Assay

[0463] In a reverse competition assay, an immobilized analyte of interest (e.g. an anti-β-coronavirus antibody) can either be sequentially or simultaneously contacted with a test sample and at least one labeled recombinant antigen or antibody.

[0464] The analyte of interest can be bound to a solid support, such as the solid supports discussed above.

[0465] The immobilized analyte of interest, biological sample and at least one labeled recombinant antigen or antibody are incubated under conditions similar to those described above. Two different species analyte of interest-recombinant antigen or antibody complexes are then generated. Specifically, one of the analyte of interest-recombinant antigen or antibody

complexes generated is immobilized and contains a detectable label (e.g., a fluorescent label, etc.) while the other analyte of interest-recombinant antigen or antibody complex is not immobilized and contains a detectable label. The non-immobilized analyte of interest-recombinant antigen or antibody complex and the remainder of the biological sample are removed from the presence of the immobilized analyte of interest-recombinant antigen or antibody complex through techniques known in the art, such as washing. Once the non-immobilized analyte of interest recombinant antigen or antibody complex is removed, the amount of detectable label in the immobilized analyte of interest-recombinant antigen or antibody complex is then quantified following cleavage of the tag. The concentration of analyte of interest in the test sample can then be determined by comparing the quantity of detectable label as described above.

d. One-Step Immunoassay or "Capture on the Fly" Assay

[0466] In a capture on the fly immunoassay, a solid substrate is pre-coated with an immobilization agent. The capture agent, the analyte (e.g., an anti-β-coronavirus antibody (such as an anti-SARS-CoV antibody or an anti-SARS-CoV-2 antibody)) and the detection agent are added to the solid substrate together, followed by a wash step prior to detection. The capture agent can bind the analyte (e.g., an anti-β-coronavirus antibody (such as an anti-SARS-CoV antibody or an anti-SARS-CoV-2 antibody)) and comprises a ligand for an immobilization agent. The capture agent and the detection agents may be antibodies, recombinant antigens or any other moiety capable of capture or detection as described herein or known in the art. The ligand may comprise a peptide tag and an immobilization agent may comprise an anti-peptide tag antibody. Alternately, the ligand and the immobilization agent may be any pair of agents capable of binding together so as to be employed for a capture on the fly assay (e.g., specific binding pair, and others such as are known in the art). More than one analyte may be measured. In some embodiments, the solid substrate may be coated with a recombinant antigen and the analyte to be analyzed is an antibody.

[0467] In certain other embodiments, in a one-step immunoassay or "capture on the fly", a solid support (such as a microparticle) pre-coated with an immobilization agent (such as biotin, streptavidin, etc.) and at least a first specific binding partner and a second specific binding partner (which function as capture and detection reagents, respectively) are used. The first specific binding partner comprises a ligand for the immobilization agent (for example, if the

immobilization agent on the solid support is streptavidin, the ligand on the first specific binding partner may be biotin) and also binds to the analyte of interest e.g., an anti-β-coronavirus antibody). The second specific binding partner comprises a detectable label and binds to an analyte of interest (e.g., an anti-β-coronavirus antibody). The solid support and the first and second specific binding partners may be added to a test sample (either sequentially or simultaneously). The ligand on the first specific binding partner binds to the immobilization agent on the solid support to form a solid support/first specific binding partner complex. Any analyte of interest present in the sample binds to the solid support/first specific binding partner complex to form a solid support/first specific binding partner/analyte complex. The second specific binding partner binds to the solid support/first specific binding partner/analyte complex and the detectable label is detected. An optional wash step may be employed before the detection. In certain embodiments, in a one-step assay more than one analyte may be measured. In certain other embodiments, more than two specific binding partners can be employed. In certain other embodiments, multiple detectable labels can be added. In certain other embodiments, multiple analytes of interest can be detected, or their amounts, levels or concentrations, measured, determined or assessed.

[0468] The use of a capture on the fly assay can be done in a variety of formats as described herein, and known in the art. For example, the format can be a sandwich assay such as described above, but alternately can be a competition assay, can employ a single specific binding partner, or use other variations such as are known.

e. Single Molecule Detection Assay

[0469] Single molecule detection assays and methods, such as the use of a nanopore device or nanowell device, can also be used. Examples of nanopore devices are described in PCT International Application WO 2016/161402, which is hereby incorporated by reference in its entirety. Examples of nanowell device are described in PCT International Application WO 2016/161400, which is hereby incorporated by reference in its entirety. Other devices and methods appropriate for single molecule detection can also be employed.

f. Lateral Flow Assays

[0470] Lateral flow assays are generally provided in a device comprising a lateral flow test strip (e.g., nitrocellulose or filter paper), a sample application area (e.g., sample pad), a test

results area (e.g., a test line), an optional control results area (e.g., a control line), and an analyte-specific binding partner that is bound to a detectable label (e.g., a colored particle or an enzyme detection system). See, e.g., U.S. Patent Nos. 6,485,982; 6,187,598; 5,622,871; 6,565,808; and 6,809,687; and U.S. Patent Publication No. 2004/0184954, each of which is incorporated herein by reference.

[0471] In some aspects, the present disclosure provides assays for detecting anti-β-coronavirus antibodies (such as anti-SARS-CoV (IgG or IgM) antibodies or anti-SARS-CoV-2 (IgG or IgM antibodies) in a sample. In some aspects, the technology relates to analytical devices that are suitable for use in the home, clinic, or hospital, and that are intended to give an analytical result that is rapid with minimum degree of skill and involvement from the user. In some aspects, use of the devices described herein involves methods in which a user performs a sequence of operations to provide an observable test result.

[0472] In some aspects, also provided is a test device comprising a reagent-impregnated test strip to provide a specific binding assay, e.g., an immunoassay. In some embodiments, a sample is applied to one portion of the test strip and is allowed to permeate through the strip material, usually with the aid of an eluting solvent such as water and/or a suitable buffer (e.g., optionally comprising a detergent). In so doing, the sample progresses into or through a detection zone in the test strip wherein a first specific binding partner (e.g., a recombinant antigen) for an analyte (e.g., anti-β-coronavirus antibodies (such as anti-SARS-CoV (IgG or IgM) antibodies or anti-SARS-CoV-2 (IgG or IgM antibodies)) suspected of being in the sample is immobilized. Analyte present in the sample can therefore become bound within the detection zone. The extent to which the analyte becomes bound in that zone can be determined with the aid of labelled reagents that can also be incorporated in the test strip or applied thereto subsequently.

[0473] In some aspects, the analytical test device comprises a hollow casing constructed of moisture-impervious solid material containing a dry porous carrier that communicates directly or indirectly with the exterior of the casing such that a liquid test sample can be applied to the porous carrier. In some aspects, the device also comprises a labelled specific binding partner for an analyte and the labelled specific binding partner is freely mobile within the porous carrier when in the moist state. In some aspects, the device comprises unlabeled specific binding partner for the same analyte and the unlabeled reagent is permanently immobilized in a detection zone on the carrier material and is therefore not mobile in the moist state. The relative positioning of

the labelled reagent and detection zone being such that liquid sample applied to the device can pick up labelled reagent and thereafter permeate into the detection zone and the device provides the extent (if any) to which the labelled reagent becomes in the detection zone to be observed.

[0474] Another aspect relates to a device that comprises a porous solid phase material carrying in a first zone a labelled reagent that is retained in the first zone while the porous material is in the dry state but is free to migrate through the porous material when the porous material is moistened, for example, by the application of an aqueous liquid sample suspected of containing the analyte. In some embodiments, the porous material comprises in a second zone, which is spatially distinct from the first zone, an unlabeled specific binding partner (e.g., a recombinant antigen and//or antibody) having specificity for the analyte and which is capable of participating with the labelled reagent in either a "sandwich" or a "competition" reaction. The unlabeled specific binding partner is firmly immobilized on the porous material such that it is not free to migrate when the porous material is in the moist state. In some aspects, the labelled reagent is a specific binding partner (e.g., a second specific binding partner) for the analyte. The labelled reagent, the analyte (if present), and the immobilized unlabeled specific binding partner (e.g., first specific binding partner) have specificities for different epitopes on the analyte and cooperate together in a reaction.

10. Samples and Controls

a. Test or Biological Sample

[0475] As used herein, "sample", "test sample", "biological sample" refer to fluid sample containing or suspected of containing an anti-β-coronavirus antibody, such as an anti-SARS-CoV (IgG and/or IgM) antibody or anti-SARS-CoV-2 (IgG and/or IgM) antibody. The sample may be derived from any suitable source. For example, in some aspects, the sample may be obtained, derived and/or extracted off of a face mask, swabbed from inside a ventilator, or from a breath sample, etc. In some cases, the sample may comprise a liquid, fluent particulate solid, or fluid suspension of solid particles. In some cases, the sample may be processed prior to the analysis described herein. For example, the sample may be separated or purified from its source prior to analysis; however, in certain embodiments, an unprocessed sample containing at least one anti-β-coronavirus antibody may be assayed directly. In a particular example, the source of an anti-β-coronavirus antibody is a mammalian (e.g., human) bodily substance (e.g., bodily fluid,

blood such as whole blood (including, for example, capillary blood, venous blood, etc.), serum, plasma, urine, saliva, sweat, an anal sample (such as anal swab specimen), sputum, semen, mucus (including nasal mucus), lacrimal fluid, lymph fluid, amniotic fluid, interstitial fluid, lower respiratory specimens such as, but not limited to, sputum, endotracheal aspirate or bronchoalveolar lavage, cerebrospinal fluid, feces, tissue, organ, one or more dried blood spots, or the like). Tissues may include, but are not limited to oropharyngeal specimens, nasopharyngeal specimens, skeletal muscle tissue, liver tissue, lung tissue, kidney tissue, myocardial tissue, brain tissue, bone marrow, cervix tissue, skin, etc. The sample may be a liquid sample or a liquid extract of a solid sample. In certain cases, the source of the sample may be an organ or tissue, such as a biopsy sample, which may be solubilized by tissue disintegration/cell lysis. Additionally, the sample can be a nasopharyngeal or oropharyngeal sample obtained using one or more swabs that, once obtained, is placed in a sterile tube containing a virus transport media (VTM) or universal transport media (UTM), for testing. In certain other cases, the sample can be a nasal mucus specimen. In still certain other cases, the sample can be an anal swab specimen.

[0476] A wide range of volumes of the fluid sample may be analyzed. In a few exemplary embodiments, the sample volume may be about 0.5 nL, about 1 nL, about 3 nL, about 0.01 μL, about 0.1 μL, about 1 μL, about 5 μL, about 10 μL, about 100 μL, about 1 mL, about 5 mL, about 10 mL, or the like. In some cases, the volume of the fluid sample is between about 0.01 μL and about 10 mL, between about 0.01 μL and about 1 mL, between about 0.01 μL and about 100 μL, or between about 0.1 μL and about 10 μL.

[0477] In some cases, the fluid sample may be diluted prior to use in an assay. For example, in embodiments where the source of anti-β-coronavirus antibody is a human body fluid (e.g., blood, serum), the fluid may be diluted with an appropriate solvent (e.g., a buffer such as PBS buffer). A fluid sample may be diluted about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 10-fold, about 11-fold, about 12-fold, about 13-fold, about 15-fold, about 16-fold, about 17-fold, about 18-fold, about 19-fold, about 20-fold, about 21-fold, about 22-fold, about 23-fold, about 24-fold, about 25-fold, about 26-fold, about 27-fold, about 28-fold, about 29-fold, about 30-fold, about 31-fold, about 32-fold, about 33-fold, about 34-fold, about 35-fold, about 36-fold, about 37-fold, about 38-fold, about 39-fold, about 40-fold, about 41-fold, about 42-fold, about 43-fold, about 44-fold, about 45-fold, about 46-fold, about 47-fold,

about 48-fold, about 49-fold, about 50-fold, about 100-fold, or greater, prior to use. In other cases, the fluid sample is not diluted prior to use in an assay. In some aspects, the diluent may optionally contain an antibody, such as an IgG antibody that is added to remove any IgG antibodies from the sample when the method involves detecting the presence of or measuring the amount or concentration of an IgM antibody.

[0478] In some cases, the sample may undergo pre-analytical processing or pre-treatment. Pre-analytical processing may offer additional functionality such as nonspecific protein removal and/or effective yet cheaply implementable mixing functionality. General methods of pre-analytical processing may include the use of electrokinetic trapping, AC electrokinetics, surface acoustic waves, isotachophoresis, dielectrophoresis, electrophoresis, or other pre-concentration techniques known in the art.

[0479] In some cases, pre-treatment may involve adding an antibody, such as an IgG and/or IgM antibody to the biological sample prior to the addition of the at least one first specific binding partner and/or at least one second specific binding partner. In some aspects, for an IgM assay, the pre-treatment can involve adding an anti-human IgG antibody to a biological sample prior to the addition of at least one first specific binding partner and/or at least one second specific binding partner in order to remove any IgG antibodies from the sample. In other aspects, for an IgG assay, the pre-treatment involves adding an anti-human IgM antibody to a biological sample prior to the addition of at least one first specific binding partner and/or at least one second specific binding partner in order to remove any IgM antibodies from the sample.

[0480] In some cases, the fluid sample may be concentrated prior to use in an assay. For example, in embodiments where the source of anti-β-coronavirus antibody is a human body fluid (e.g., blood, serum), the fluid may be concentrated by precipitation, evaporation, filtration, centrifugation, or a combination thereof. A fluid sample may be concentrated about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 10-fold, about 100-fold, or greater, prior to use.

b. Controls and Calibrators

[0481] It may be desirable to include a control (such as a positive and/or negative control, which are well known in the art). For example, a positive control can be any antibody or antibody fragment that binds to the first specific binding partner (e.g., recombinant antigen). In some aspects, the positive control can be any antibody or antibody fragment that binds to an

epitope or variant thereof in at least one nucleocapsid protein or at least one spike protein from a β-coronavirus (such as at least one SARS-CoV or SARS-CoV-2). In one aspect, the positive control can be any antibody or antibody fragment that binds to an epitope comprising at least amino acids RNAPRITFG (SEQ ID NO:6) or RSAPRITFG (SEQ ID NO:7) of the nucleocapsid protein of a β-coronavirus (such as SARS-CoV or SARS-CoV-2). An example of an antibody that binds to an epitope comprising SEQ ID NO:6 is monoclonal antibody CR3018 which is described in van den Brink et al., *J. Virol.* 79(3):1635-1644 (February 2005) and U.S. Patent No. 7,696,330, the contents of which are herein incorporated by reference. The scFv amino acid sequence for CR3018 as well as IgG heavy and light chains amino acid sequences are disclosed in U.S. Patent No. 7,696,330 (see, SEQ ID NOS: 96, 127 and 128, respectively)In still a futher aspect, any antibodies derived or constructed from monoclonal antibody CR3018 that bind to an epitope comprising SEQ ID NO: 6 can be used as positive control. Additionally, in other aspects, stable cell lines expressing CR3018 or any derivatives or variants thereof can be made using routine techniques known in the art, such as those described in Section 7 herein. Such stable cell lines can be used to provide the positive controls or calibrators for use in the methods described herein.

[0482] In another aspect, the positive control and/or calibrator can be any antibody or antibody fragment that binds to an epitope comprising at least amino acids YNST (SEQ ID NO:8), YNSA (SEQ ID NO:9), DDFM (SEQ ID NO:10), DDFT (SEQ ID NO:11), FSTFKCYGVSATK (SEQ ID NO:12), FSTFKCYGVSPTK (SEQ ID NO:13), ATSTGNYNYKYRYLRHGKLR (SEQ ID NO:19) and/or YTTTGIGYQPYRVVLSFEL (SEQ ID NO:20) of the receptor-binding domain on a spike protein of a β-coronavirus (such as SARS-CoV or SARS-CoV-2). An example of an antibody that binds to an epitope comprising at least one of SEQ ID NOS.: 8-13, 20 and/or 21 is monoclonal antibody CR3022 which is described in U.S. Patent No. 8,106,170, ter Meulen, et al., *PLOS Medicine*, 3(7):1071-1079 (July 2006) and Yuan et al., *Science*, published on-line on April 3, 2020 (10.1126/science.abb7269) the contents of which are herein incorporated by reference. Thus, in some aspects in the methods described herein, CR3022 can be used as a positive control. In yet other aspects of the methods described herein, CR3022 can be used as a calibrator. In still further aspects of the method described herein, CR3022 can be used as a positive control and as a calibrator. In still a futher aspect, any antibodies derived or constructed from monoclonal antibody CR3022 that bind to an epitope

comprising at least one of SEQ ID NOS. 8-13, 20 and/or 21 can be used as positive control and/or calibrator. The scFv amino acid sequence for CR3022 as well as IgG heavy and light chain amino acid sequences are described in U.S. Patent No. 8,106,170 (see, SEQ ID NOS:95, 6 and 10, respectively), herein incorporated by reference. U.S. Patent No. 8,106,170 also discloses the amino acid sequences for a fully generated immunoglobulin in SEQ ID NOS. 6 and 10. Additionally, in other aspects, stable cell lines expressing CR3022 or any derivatives or variants thereof can be made using routine techniques known in the art, such as those described in Section 7 herein. Such stable cell lines can be used to provide the positive controls or calibrators for use in the methods described herein.

[0483] In yet other aspects, the positive control and/or calibrator can be a pentameric IgG or IgM antibody or antibody fragment. In one aspect, the positive control and/or calibrator is a pentameric IgG antibody. In another aspect, the positive control and/or calibrator is a pentameric IgM antibody. For example, a pentameric IgG or IgM version of CR3018, CR3022 or any derivatives or variants thereof can be made for use in the methods described herein using routine techniques known in the art. More specifically, in one aspect, the positive control and/or calibrator is a pentameric IgG version of CR3018 or any derivative or variant thereof. In yet another aspect, the positive control and/or calibrator is a pentameric IgM version of CR3022 or any derivative or variant thereof.

[0484] Alternatively, the positive control can be mammalian serum or plasma that is reactive for β-coronavirus (e.g., SARS-CoV or SARS-CoV-2). An example of a negative control that can be used in the methods described herein is mammalian serum or plasma, such as human plasma or serum, which is not reactive for β-coronavirus, such as SARS-CoV or SARS-CoV-2 (e.g., is from a single subject or population of subjects that is naïve for, i.e., has not been exposed to, β-coronavirus (such as SARS-CoV or SARS-CoV-2), or in whom insufficient time has passed following β-coronavirus (such as SARS-CoV or SARS-CoV-2) exposure for any β-coronavirus (such as SARS-CoV or SARS-CoV-2) -reactive antibodies to have been raised). Optionally plasma as described herein is recalcified.

[0485] The control may be analyzed separately from, or concurrently with, the sample from the subject as described above. The results obtained from the subject sample can be compared to the results or information obtained from the control sample. Standard curves may be provided or developed with use of the calibrators and controls, with which assay results for the sample may

be compared. Such standard curves typically present levels of marker as a function of assay units (i.e., fluorescent signal intensity, if a fluorescent label is used).

[0486] It may also be desirable to include one or more calibrators for use in calibrating of any automated or semi-automated system for which the methods and kits described herein are adapted for use. The use of calibrators in such systems is well known in the art. For example, one or more calibrators can include mammalian serum or plasma that is reactive for β-coronavirus (e.g., SARS-CoV or SARS-CoV-2) due to the serum or plasma containing anti-SARS-CoV or anti-SARS-CoV-2 IgG and/or IgM antibody. For example, in one aspect, the calibrator may be human plasma that is reactive for β-coronavirus (such as SARS-CoV or SARS-CoV-2) due to the plasma containing anti-SARS-CoV or anti-SARS-CoV-2 IgM antibody. In another aspect, the calibrator may be human plasma that is reactive for a β-coronavirus (such as SARS-CoV or SARS-CoV-2) due to the plasma containing anti-SARS-CoV or anti-SARS-CoV-2 IgG antibody. The calibrator is optionally, part of a series of calibrators in which each of the calibrators differs from the other calibrators in the series by the concentration of an anti-β-coronavirus antibody (e.g., anti-β-coronavirus IgG and/or IgM antibody, such as an (such as an anti-SARS-CoV IgG and/or IgM antibody or an anti-SARS-CoV-2 IgG and/or IgM antibody)).

[0487] When one or more calibrators is used in connection with the methods of the present disclosure, the methods can have a signal-to-calibrator ratio of about 1.0, about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, about 2.0, about 2.1, about 2.2, about 2.3, about 2.4, about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, about 3.0, about 3.1, about 3.2, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, about 3.9, about 4.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.8, about 7.9, about 8.0, about 8.1, about 8.2, about 8.3, about 8.4, about 8.5, about 8.6, about 8.7, about 8.8, about 8.9, about 9.0, about 9.1, about 9.2, about 9.3, about 9.4, about 9.5, about 9.6, about 9.7, about 9.8, about 9.9, about 10.0, about 10.1, about 10.2, about 10.3, about 10.4, about 10.5, about 10.6, about 10.7, about 10.8, about 10.9, about 11.0, about 11.1, about 11.2, about 11.3, about 11.4, about 11.5, about 11.6, about 11.7, about 11.8, about 11.9, or about 12.0. In one aspect, the methods can have a signal-to-calibrator ratio of about 1.0

to about 12.0, about 1.0 to about 11.0, about 1.0 to about 10.0, about 1.0 to about 9.0, about 1.0 to about 8.0, about 1.0 to about 7.0, about 1.0 to about 6.0 or about 1.0 to about 5.0.

11. Kit

[0488] Provided herein is a kit, which may be used in the methods described herein for assaying or assessing a test sample for an anti-β-coronavirus antibody (such as an anti-SARS-CoV (IgG and/or IgM) antibody or an anti-SARS-CoV-2 (IgG and/or IgM) antibody). The kit comprises at least one component for assaying the test sample for an anti-β-coronavirus antibody (such as an anti-SARS-CoV (IgG and/or IgM) antibody or an anti-SARS-CoV-2 (IgG and/or IgM) antibody) instructions for assaying the test sample an anti-β-coronavirus antibody (such as an anti-SARS-CoV (IgG and/or IgM) antibody or an anti-SARS-CoV-2 (IgG and/or IgM) antibody). For example, the kit can comprise instructions for assaying the test sample for an anti-β-coronavirus antibody (such as an anti-SARS-CoV (IgG and/or IgM) antibody or an anti-SARS-CoV-2 (IgG and/or IgM) antibody) immunoassay, e.g., chemiluminescent microparticle immunoassay. Instructions included in kits can be affixed to packaging material, can be included as a package insert, or can be viewed or downloaded from a particular website that is recited as part of the kit packaging or inserted materials. While the instructions are typically written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this disclosure. Such media include, but are not limited to, electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. As used herein, the term "instructions" can include the address of an internet site that provides the instructions.

[0489] The at least one component for assaying the test sample for an anti-β-coronavirus antibody may include at least one composition comprising one or more isolated recombinant antigens or antigen fragments thereof that specifically bind to anti-β-coronavirus antibody (such as an anti-SARS-CoV (IgG and/or IgM) antibody or an anti-SARS-CoV-2 (IgG and/or IgM) antibody) as described previously herein.

[0490] Alternatively or additionally, the kit can comprise a calibrator or control, e.g., purified, and optionally frozen or lyophilized, as described previously herein, and/or at least one container (e.g., tube, microtiter plates or strips, which can be already coated with a β-coronavirus (such as SARS-CoV or SARS-CoV-2) recombinant antigen (such as one that binds an anti-SARS-CoV antibody or an anti-SARS-CoV-2 antibody) for conducting the assay, and/or a buffer, such as an

assay buffer or a wash buffer, either one of which can be provided as a concentrated solution, a substrate solution for the detectable label (e.g., an enzymatic label), or a stop solution. Preferably, the kit comprises all components, i.e., reagents, standards, buffers, diluents, etc., which are necessary to perform the assay. The instructions also can include instructions for generating a standard curve.

[0491] The kit may further comprise reference standards for quantifying anti-β-coronavirus antibody (such as an anti-SARS-CoV (IgG and/or IgM) antibody or an anti-SARS-CoV-2 (IgG and/or IgM) antibody). The reference standards may be employed to establish standard curves for interpolation and/or extrapolation of anti-β-coronavirus antibody (such as an anti-SARS-CoV (IgG and/or IgM) antibody or an anti-SARS-CoV-2 (IgG and/or IgM) antibody) concentration.

[0492] Any antibodies, which are provided in the kit, such as recombinant antibodies, can incorporate a detectable label, such as a fluorophore, radioactive moiety, enzyme, biotin/avidin label, chromophore, chemiluminescent label, or the like, or the kit can include reagents for labeling the antibodies or reagents for detecting the antibodies (e.g., detection antibodies) and/or for labeling the analytes (e.g., an anti-β-coronavirus antibody) or reagents for detecting the analyte (e.g., an anti-β-coronavirus antibody). The antigens (e.g., polypeptides), antibodies, calibrators, and/or controls can be provided in separate containers or pre-dispensed into an appropriate assay format, for example, into microtiter plates,

[0493] Optionally, the kit includes quality control components (for example, sensitivity panels, calibrators, and positive controls). Preparation of quality control reagents is well-known in the art and is described on insert sheets for a variety of immunodiagnostic products. Sensitivity panel members optionally are used to establish assay performance characteristics, and further optionally are useful indicators of the integrity of the immunoassay kit reagents, and the standardization of assays,

[0494] The kit can also optionally include other reagents required to conduct a diagnostic assay or facilitate quality control evaluations, such as buffers, salts, enzymes, enzyme co-factors, substrates, detection reagents, and the like. Other components, such as buffers and solutions for the isolation and/or treatment of a test sample (e.g., pretreatment reagents), also can be included in the kit. The kit can additionally include one or more other controls. One or more of the components of the kit can be lyophilized, in which case the kit can further comprise reagents suitable for the reconstitution of the lyophilized components.

[0495] The various components of the kit optionally are provided in suitable containers as necessary, e.g., a microtiter plate. The kit can further include containers for holding or storing a sample (e.g., a container or cartridge for a urine, whole blood, plasma, or serum sample). Where appropriate, the kit optionally also can contain reaction vessels, mixing vessels, and other components that facilitate the preparation of reagents or the test sample. The kit can also include one or more instrument for assisting with obtaining a test sample, such as a syringe, pipette, forceps, measured spoon, or the like.

[0496] If the detectable label is at least one acridinium compound, the kit can comprise at least one acridinium-9-carboxamide, at least one acridinium-9-carboxylate aryl ester, or any combination thereof. If the detectable label is at least one acridinium compound, the kit also can comprise a source of hydrogen peroxide, such as a buffer, solution, and/or at least one basic solution. If desired, the kit can contain a solid phase, such as a magnetic particle, bead, test tube, microtiter plate, cuvette, membrane, scaffolding molecule, film, filter paper, disc, or chip.

[0497] If desired, the kit can further comprise one or more components, alone or in further combination with instructions, for assaying the test sample for another analyte, which can be a biomarker, such as a biomarker of traumatic brain injury or disorder.

12. Adaptation of Kit and Method for use in Automated and Semi-Automated Systems

[0498] The method and kit (or components thereof) for detecting the presence of or determining the amount,level or concentration of β-coronavirus (such as SARS-CoV or SARS-CoV-2), such as, an anti-β-coronavirus antibody, in a test sample by an immunoassay as described herein, can be adapted for use in a variety of automated and semi-automated systems or platforms (including those wherein the solid phase comprises a microparticle) known in the art. The following adaptations of automated and/or semi-automated systems are included herein as merely exemplary. Specifically, the method and kit described herein can be adapted for use in automated and semi-automated systems or platforms such as those described, e.g., U.S. Patent No. 5,063,081, U.S. Patent Application Publication Nos. 2003/0170881, 2004/0018577, 2005/0054078, and 2006/0160164 and as commercially marketed *e.g.*, by Abbott Laboratories (Abbott Park, IL) as Abbott Point of Care (i-STAT® or i-STAT Alinity, Abbott Laboratories) as well as those described in U.S. Patent Nos. 5,089,424 and 5,006,309, and as commercially marketed, e.g., by Abbott Laboratories (Abbott Park, IL) as ARCHITECT® or the series of Abbott Alinity devices. Such systems include one or more devices and/or components that can

be used to detect one or more labels in the resulting complexes formed in the methods described previously herein.

[0499] Some of the differences between an automated or semi-automated system as compared to a non-automated system include the substrate to which the first specific binding partner (e.g., recombinant antigen or capture reagent) is attached, and the length and timing of the capture, detection, and/or any optional wash steps. Whereas a non-automated format may require a relatively longer incubation time with test sample and capture reagent (e.g., about 2 hours), an automated or semi-automated format (e.g., ARCHITECT® and any successor platform, Abbott Laboratories) may have a relatively shorter incubation time (e.g., approximately 18 minutes for ARCHITECT®). Similarly, whereas a non-automated format may incubate a detection antibody such as the conjugate reagent for a relatively longer incubation time (e.g., about 2 hours), an automated or semi-automated format (e.g., ARCHITECT® and any successor platform) may have a relatively shorter incubation time (e.g., approximately 4 minutes for the ARCHITECT® and any successor platform).

[0500] Other platforms available from Abbott Laboratories include, but are not limited to, AxSYM®, IMx® (see, *e.g.*, U.S. Patent No. 5,294,404, which is hereby incorporated by reference in its entirety), PRISM®, EIA (bead), and Quantum™ II, as well as other platforms. Additionally, the assays, kits, and kit components can be employed in other formats, for example, on electrochemical or other hand-held or point-of-care assay systems. As mentioned previously, the present disclosure is, for example, applicable to the commercial Abbott Point of Care (i-STAT®, Abbott Laboratories) electrochemical immunoassay system that performs sandwich immunoassays. Immunosensors and their methods of manufacture and operation in single-use test devices are described, for example in, U.S. Patent No. 5,063,081, U.S. Patent App. Publication Nos. 2003/0170881, 2004/0018577, 2005/0054078, and 2006/0160164, which are incorporated in their entireties by reference for their teachings regarding same.

[0501] In particular, with regard to the adaptation of an assay to the i-STAT® system, the following configuration is preferred. A microfabricated silicon chip is manufactured with a pair of gold amperometric working electrodes and a silver-silver chloride reference electrode. On one of the working electrodes, polystyrene beads (0.2 mm diameter) with immobilized capture antibody are adhered to a polymer coating of patterned polyvinyl alcohol over the electrode. This chip is assembled into an i-STAT® cartridge with a fluidics format suitable for

immunoassay. On a portion of the silicon chip, there is a specific binding partner for an anti-β-coronavirus antibody, such as at least one specific binding partner as described herein (e.g., recombinant antigen) or one or more anti-β-coronavirus (such as SARS-CoV or SARS-Co-V2) DVD-Igs (or a fragment thereof, a variant thereof, or a fragment of a variant thereof that can bind an anti-β-coronavirus antibody (such as SARS-CoV or SARS-CoV-2), either of which can be detectably labeled. Within the fluid pouch of the cartridge is an aqueous reagent that includes p-aminophenol phosphate.

[0502] In operation, a sample from a subject suspected of having or being exposed to a β-coronavirus (such as SARS-CoV or SARS-CoV-2) is added to the holding chamber of the test cartridge, and the cartridge is inserted into the i-STAT® reader. A pump element within the cartridge pushes the sample into a conduit containing the chip. The sample is brought into contact with the sensors allowing the enzyme conjugate to dissolve into the sample. The sample is oscillated across the sensors to promote formation of the sandwich of approximately 2-12 minutes. In the penultimate step of the assay, the sample is pushed into a waste chamber and wash fluid, containing a substrate for the alkaline phosphatase enzyme, is used to wash excess enzyme conjugate and sample off the sensor chip. In the final step of the assay, the alkaline phosphatase label reacts with p-aminophenol phosphate to cleave the phosphate group and permit the liberated p-aminophenol to be electrochemically oxidized at the working electrode. Based on the measured current, the reader is able to calculate the amount of anti-β-coronavirus antibody in the sample by means of an embedded algorithm and factory-determined calibration curve. Adaptation of a cartridge for multiplex use, such as used for i-STAT®, has been described in the patent literature, such as for example, U.S. Patent No. 6,438,498, the contents of which are herein incorporated by reference.

[0503] The methods, kits and systems as described herein necessarily encompass other reagents and methods for carrying out the immunoassay. For instance, encompassed are various buffers such as are known in the art and/or which can be readily prepared or optimized to be employed, e.g., for washing, as a conjugate diluent, and/or as a calibrator diluent. An exemplary conjugate diluent is ARCHITECT® conjugate diluent employed in certain kits (Abbott Laboratories, Abbott Park, IL) and containing 2-(N-morpholino)ethanesulfonic acid (MES), a salt, a protein blocker, an antimicrobial agent, and a detergent. An exemplary calibrator diluent is ARCHITECT® human calibrator diluent employed in certain kits (Abbott Laboratories,

Abbott Park, IL), which comprises a buffer containing MES, other salt, a protein blocker, and an antimicrobial agent. Additionally, as described in U.S. Patent No. 8,445,199, improved signal generation may be obtained, e.g., in an i-STAT® cartridge format, using a nucleic acid sequence linked to the signal antibody as a signal amplifier.

13. Analysis, Interpretation of Results and Algorithims

[0504] The results obtained using the methods of the present disclosure (e.g., detecting the presence of at least one anti-β-coronavirus antibody or determining the quantity, amount, level or concentration of at least one anti-β-coronavirus antibody in a biological sample) can be analyzed and interpreted individually or in combination with other any other results obtained prior to, during or after the results of the methods of the present disclosure are performed. The nature of the other results analyzed and interpreted with the results of the present disclosure are changeable. For example, in one aspect, if the methods of present disclosure are used to detect the presence of or determine the quantity, amount, level or concentration of at least one anti-β-coronavirus IgM antibody (such as an anti-SARS-CoV IgM antibody or anti-SARS-CoV-2 IgM antibody) in a biological sample, these results can be used alone or in combination with concurrently, previously, or later obtained results relating to detecting the presence of or determining the quantity, amount, level or concentration of at least one anti-β-coronavirus IgG antibody in a biological sample obtained from the same subject (e.g., such as using the methods of the present disclosure). The results relating to detecting the presence of or determining the quantity, amount, level or concentration of at least one anti-β-coronavirus IgG antibody in a biological sample obtained from the subject may have been obtained at the same time, or minutes, hours or days before or after the results relating to detecting the presence of or determining the quantity, amount, level or concentration of anti-β-coronavirus IgM antibody in the sample were obtained. Analyzing the combined results regarding the presence of or amount, level, or concentration of anti-β-coronavirus IgM and IgG antibody levels can guide treatment and/or monitoring decisions to be made by a clinician.

[0505] In yet another aspect, if the methods of present disclosure are used to detect the presence of or determine the quantity, amount, level or concentration of at least one anti-β-coronavirus IgM antibody (such as an anti-SARS-CoV IgM antibody or anti-SARS-CoV-2 IgM antibody) in a biological sample, these results can be used alone or in combination with concurrently, previously, or later obtained results relating to detecting the presence of or

determining the quantity, amount, level or concentration of at least one β-coronavirus antigen (such as SARS-CoV or SARS-CoV-2) in a biological sample obtained from the same subject (e.g., such as using the routine techniques known in the art). For example, SARS-CoV-2 antigen tests that can be used include the Sampinute COVID-19 Antigen MIA test available from Celltrion USA, Inc., the BD Veritor System for Rapid Detection of SARS-CoV-2 test available from Becton, Dickinson and Company, BinaxNOW COVID-19 Antigen Card available from Abbott Diagnostics Scarborough Inc., and the QuickVue SARS Antigen Test available from Quidel Corporation. The results relating to detecting the presence of or determining the quantity, amount, level or concentration of at least one β-coronavirus antigen (such as SARS-CoV or SARS-CoV-2) in a biological sample obtained from the subject may have been obtained at the same time, or minutes, hours or days before or after the results relating to detecting the presence of or determining the quantity, amount, level or concentration of anti-β-coronavirus IgM antibody (such as an anti-SARS-CoV IgM antibody or an anti-SARS-CoV-2 IgM antibody) in the sample were obtained. Analyzing the combined results regarding the presence of or amount, level, or concentration of anti-β-coronavirus IgM antibody and β-coronavirus antigen (such as SARS-CoV or SARS-CoV-2) levels can guide treatment and/or monitoring decisions to be made by a clinician.

[0506] In still yet another aspect, if the methods of present disclosure are used to detect the presence of or determine the quantity, amount, level or concentration of at least one anti-β-coronavirus IgG antibody (such as an anti-SARS-CoV IgG antibody or anti-SARS-CoV-2 IgG antibody) in a biological sample, these results can be used alone or in combination with concurrently, previously, or later obtained results relating to detecting the presence or determining the quantity, amount, level or concentration of at least one anti-β-coronavirus IgM antibody (such as an anti-SARS-CoV IgM antibody or an anti-SARS-CoV-2 IgM antibody) in a biological sample obtained from the same subject (e.g., such as using the methods of the present disclosure). The results relating to detecting the presence of or determining the quantity, amount, level or concentration of at least one anti-β-coronavirus IgM antibody (such as an anti-SARS-CoV IgM antibody or an anti-SARS-CoV-2 IgM antibody) in a biological sample obtained from the subject may have been obtained at the same time, or minutes, hours or days before or after the results relating to detecting the presence of or determining the quantity, amount, level or concentration of anti-β-coronavirus IgG antibody(such as an anti-SARS-CoV

IgG antibody or an anti-SARS-CoV-2 IgG antibody) in the sample were obtained. Analyzing the combined results regarding the presence of or amount, level, or concentration of anti-β-coronavirus IgG and IgM antibody (such as an anti-SARS-CoV IgG or IgM antibody or an anti-SARS-CoV-2 IgG or IgM antibody) levels can guide treatment and/or monitoring decisions to be made by a clinician.

[0507] In yet another aspect, if the methods of present disclosure are used to detect the presence of or determine the quantity, amount, level or concentration of at least one anti-β-coronavirus IgG antibody (such as an anti-SARS-CoV IgG antibody or anti-SARS-CoV-2 IgG antibody) in a biological sample, these results can be used alone or in combination with concurrently, previously, or later obtained results relating to detecting the presence of or determining the quantity, amount, level or concentration of at least one β-coronavirus antigen (such as SARS-CoV or SARS-CoV-2) in a biological sample obtained from the same subject (e.g., such as using the routine techniques known in the art). The results relating to detecting the presence of or determining the quantity, amount, level or concentration of at least one β-coronavirus antigen (such as SARS-CoV or SARS-CoV-2) in a biological sample obtained from the subject may have been obtained at the same time, or minutes, hours or days before or after the results relating to detecting the presence of or determining the quantity, amount, level or concentration of anti-β-coronavirus (such as SARS-CoV or SARS-CoV-2) IgG antibody in the sample were obtained. Analyzing the combined results regarding the presence of or amount, level, or concentration of anti-β-coronavirus (such as SARS-CoV or SARS-CoV-2) IgG antibody and β-coronavirus antigen (such as SARS-CoV or SARS-CoV-2) levels can guide treatment and/or monitoring decisions to be made by a clinician.

[0508] In still a further aspect, if the methods of present disclosure are used to detect the presence of or determine the quantity, amount, level or concentration of at least one anti-β-coronavirus IgG antibody (such as an anti-SARS-CoV IgG antibody or anti-SARS-CoV-2 IgG antibody) in a biological sample, these results can be used alone or in combination with concurrently, previously, or later obtained results relating to detecting the presence of or determining the quantity, amount, level or concentration of at least one anti-β-coronavirus IgM antibody (such as an anti-SARS-CoV IgG antibody or anti-SARS-CoV-2 IgM antibody) in a biological sample, and further can be used alone or in combination with concurrently, previously, or later obtained results relating to detecting the presence of or determining the quantity, amount,

level or concentration of at least one β-coronavirus antigen (such as SARS-CoV or SARS-CoV-2) in a biological sample obtained from the same subject (e.g., such as using the routine techniques known in the art). The results relating to detecting the presence of or determining the quantity, amount, level or concentration of at least one β-coronavirus antigen (such as SARS-CoV or SARS-CoV-2) in a biological sample obtained from the subject may have been obtained at the same time, or minutes, hours or days before or after the results relating to detecting the presence of or determining the quantity, amount, level or concentration of anti-β-coronavirus (such as SARS-CoV or SARS-CoV-2) IgG antibody in the sample were obtained, and may have been obtained at the same time, or minutes, hours or days before or after the results relating to detecting the presence of or determining the quantity, amount, level or concentration of anti-β-coronavirus (such as SARS-CoV or SARS-CoV-2) IgM antibody in the sample were obtained. Analyzing the combined results regarding the presence of or amount, level, or concentration of anti-β-coronavirus (such as SARS-CoV or SARS-CoV-2) IgG and/or IgM antibody, and/or β-coronavirus antigen (such as SARS-CoV or SARS-CoV-2) levels can guide treatment and/or monitoring decisions to be made by a clinician.

[0509] In some aspects, the methods of the present disclosure can be used to identify one or more subjects (e.g., candidate subjects) who have recovered from a β-coronavirus (such as SARS-CoV or SARS-CoV-2) and possess anti-β-coronavirus IgG antibodies, such as anti-SARS-CoV IgG antibodies or anti-SARS-CoV-2 IgG antibodies. Biological samples, such as serum or plasma obtained from whole blood collected from subjects who have tested positive for anti-SARS-CoV IgG antibodies or anti-SARS-CoV-2 IgG antibodies, can be used as convalescent serum or plasma to neutralize (e.g., said serum or plasma may contain one or more IgG neutralizing antibodies targeting one or more of S1, RBD or S2 of the spike protein) and/or treat subjects suffering from SARS-CoV or SARS-CoV-2 and/or used to develop other blood based therapies using routine techniques known in the art. In some aspects, candidate subjects can meet the following donor eligibility requirements: (a) evidence of SARS-CoV or SARS-CoV-2 documented by a laboratory test either by (i) a diagnostic test (e.g., nasopharyngeal swab) at the time of illiness; or (ii) a positive serologic test for SARS-CoV or SARS-CoV-2 IgG and/or SARS-CoV or SARS-CoV-2 IgM antibodies after recovery (if prior diagnostic testing was not performed at the time infection was suspected); (b) complete resolution of symptoms at least 14 days before donation; and (c) SARS-CoV or SARS-CoV-2 IgG neutralizing antibody titers, if

available (e.g., if IgG neutralizing antibody titers are available, IgG neutralizing antibody titers such as, for example, at least 1:160; a titer of 1:80 may be considered acceptable if an alternative matched unit is not available).

[0510] In other aspects, a subject can be identified as a candidate to provide a biological sample, such as serum or plasma, for use in convalescent therapy if the level of one or more anti-SARS-CoV IgG antibodies or anti-SARS-CoV-2 IgG antibodies and/or anti-SARS-CoV IgM antibodies or anti-SARS-CoV-2 IgM antibodies is from about 550 BAU/mL to about 650 BAU/mL. In other aspects, a subject can be identified as a candidate to provide a biological sample, such as serum or plasma, for use in convalescent therapy if the level of one or more anti-SARS-CoV IgG antibodies or anti-SARS-CoV-2 IgG antibodies and/or anti-SARS-CoV IgM antibodies or anti-SARS-CoV-2 IgM antibodies is from about 580 BAU/mL to about 640 BAU/mL. In still other aspects, a subject can be identified as a candidate to provide a biological sample, such as serum or plasma, for use in convalescent therapy if the level of one or more anti-SARS-CoV IgG antibodies or anti-SARS-CoV-2 IgG antibodies and/or anti-SARS-CoV IgM antibodies or anti-SARS-CoV-2 IgM antibodies is from about 600 BAU/mL to about 640 BAU/mL.

[0511] In still yet other aspects, a subject can be identified as a candidate to provide a biological sample, such as serum or plasma, for use in convalescent therapy if the level of one or more anti-SARS-CoV IgG antibodies or anti-SARS-CoV-2 IgG antibodies and/or anti-SARS-CoV IgM antibodies or anti-SARS-CoV-2 IgM antibodies is from at least about from at least about 100 BAU/mL to about 490 BAU/mL; at least about 110 BAU/mL to about 490 BAU/mL; at least about 200 BAU/mL to about 490 BAU/mL; at least from about 300 BAU mL to about 490 BAU/mL; at least about 400 BAU mL to about 490 BAU/mL; at least about 100 BAU/mL; at least about 110 BAU/mL; at least about 120 BAU/mL; at least about 130 BAU/mL; at least about 140 BAU/mL; at least about 150 BAU/mL; at least about 160 BAU/mL; at least about 170 BAU/mL; at least about 180 BAU/mL; at least about 190 BAU/mL; at least about 200 BAU/mL; at least about 210 BAU/mL; at least about 220 BAU/mL; at least about 230 BAU/mL; at least about 240 BAU/mL; at least about 250 BAU/mL; at least about 260 BAU/mL; at least about 270 BAU/mL; at least about 280 BAU/mL; at least about 290 BAU/mL; at least about 300 BAU/mL; at least about 300 BAU/mL; at least about 310 BAU/mL; at least about 320 BAU/mL; at least about 330 BAU/mL; at least about 340 BAU/mL; at least about 350 BAU/mL; at least about 360

BAU/mL; at least about 370 BAU/mL; at least about 380 BAU/mL; at least about 390 BAU/mL; at least about 400 BAU/mL; at least about 400 BAU/mL; at least about 410 BAU/mL; at least about 420 BAU/mL; at least about 430 BAU/mL; at least about 440 BAU/mL; at least about 450 BAU/mL; at least about 460 BAU/mL; at least about 470 BAU/mL; at least about 480 BAU/mL; or at least about 490 BAU/mL.

[0512] In still yet other aspects, a subject can be identified as a candidate to provide a biological sample, such as serum or plasma, for use in convalescent therapy if the level of one or more anti-SARS-CoV IgG antibodies or anti-SARS-CoV-2 IgG antibodies and/or anti-SARS-CoV IgM antibodies or anti-SARS-CoV-2 IgM antibodies is from at least about 500 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 500 BAU/mL; from at least about 510 BAU/mL; at least about 520 BAU/mL; at least about 530 BAU/mL; at least about 540 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL; at least about 560 BAU/mL; at least about 570 BAU/mL; at least about 580 BAU/mL; at least about 590 BAU/mL; at least about 600 BAU/mL; at least about 610 BAU/mL; at least about 620 BAU/mL; at least about 630 BAU/mL; at least about 639 BAU/mL; at least about 640 BAU/mL; or at least about 650 BAU/mL.

[0513] In still yet other aspects, a subject can be identified as a candidate to provide a biological sample, such as serum or plasma, for use in convalescent therapy if the level of one or more anti-SARS-CoV IgG antibodies or anti-SARS-CoV-2 IgG antibodies and/or anti-SARS-CoV IgM antibodies or anti-SARS-CoV-2 IgM antibodies is at least about 590 BAU/mL, at least about 600 BAU/mL, at least about 610 BAU/mL, at least about 620 BAU/mL, at least about 630 BAU/mL, at least about 631 BAU/mL, at least about 632 BAU/mL, at least about 633 BAU/mL, at least about 634 BAU/mL, at least about 635 BAU/mL, at least about 636 BAU/mL, at least about 637 BAU/mL, at least about 638 BAU/mL, at least about 639 BAU/mL, at least about 640 BAU/mL, at least about 641 BAU/mL, at least about 642 BAU/mL, at least about 643 BAU/mL, at least about 644 BAU/mL or at least about 645 BAU/mL.

[0514] In some aspects, an amount of anti-β-coronavirus antibody (e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) or anti-β-coronavirus neutralizing antibody (e.g., anti-SARS-CoV IgG neutralizing antibody or anti-SARS-CoV-2 IgG neutralizing antibody (e.g., anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof)) that is sufficient to impart immunity to a subject (e.g., human) thereby

allowing a subject to be identified as a candidate to provide a biological sample, such as serum or plasma, for use in convalescent therapy appears to be at least from about 4000 AU/mL to about 4500 AU/mL (unpublished studies done by Abbott Laboratories, Abbott Park, IL using the CE-marked Abbott anti-S SARS-CoV-2 IgG II Quantitative antibody assay (available for use on Abbott's ARCHITECT® and Alinity i™ platforms)). The measure AU/mL can be converted to the WHO International standard of binding antibody unit per mL (BAU/mL) using the following formula: BAU/mL=0.142 x AU/mL (e.g., (e.g., about 4000 AU/mL is about 568 BAU/mL (4000 AU/mL x 0.142 = 568 BAU/mL) and about 4500 AU/mL is about 639 BAU/mL (4500 AU/mL x 0.142 = 639 BAU/mL)).

[0515] In some aspects, the time course of viral and host immunity biomarkers during SARS-CoV-2 infection is described in FIG. 5. The incubation phase or initial period of infection is an asymptomatic period when a subject does not exhibit any symptoms of a SARS-CoV-2 infection. The incubation phase or initial period can last for a period of about 0 to about 14 days after infection. During the incubation phase or initial period, viral RNA and/or viral antigen become detectable. After the incubation phase or initial period of infection, the subject progresses to the active phase of infection. Initially, the subject enters into the early acute phase of infection. The early acute phase is the phase when the subject starts to experience the onset of one or more symptoms of a SARS-CoV-2 infection and begins to develop an immune response to the virus and begins to produce antibodies. The early acute phase may last for a period of about 0 to about 14 days after the onset of symptoms. During the early acute phase, viral RNA and/or viral antigen are detectable and anti-SARS-CoV-2 IgM antibodies become detectable. After the early acute phase, the subject progresses to the acute or active phase of infection. During the acute phase of infection, the symptoms of SARS-CoV-2 infection worsen (e.g., the subject may have to be hospitalized, may need oxygen support, etc.), the immune response of the subject continues to progress, viral RNA and/or viral antigen are detectable, anti-SARS-CoV-2 IgM antibodies are detectable and anti-SARS-CoV-2 IgG antibodies become detectable. The acute phase may last for for a period of about 0 to about 14 days after the onset of worsening symptoms of the virus. After the acute phase, the subject progresses into the late acute phase. The late acute phase is the stage when the subject starts to recover from the SARS-CoV-2 infection and one or more symptoms of infection begin to improve, lessen and/or disappear. The late acute phase may last for a period of about 1 day to about 28 days. During the late acute phase, viral RNA and viral

antigen levels start to decline and eventually are not detectable, anti-SARS-CoV-2 IgM antibodies are detectable and anti-SARS-CoV-2 IgG antibodies are detectable. The convalescence or recovery phase of infection begins after the late acute phase. During this period, the subject no longer exhibits any symptoms of SARS-CoV-2 infection and viral RNA and viral antigen are no longer detectable, anti-SARS-CoV-2 IgM antibodies begin to decline and eventually become non-detectable, and anti-SARS-CoV-2 IgG antibodies remain in the blood and provide long-term immunity for the subject.

[0516] The possible combination of viral and host biomarkers in SARS-CoV-2 infection and likely interpretation criteria are provided in below Table A. The separate assessment of anti-SARS-CoV-2 IgM antibody and anti-SARS-CoV-2 IgG antibody response may provide information as to whether the subject is in the early acute phase, the late acute phase or in convalence/recovery phase. This allows clinicians (e.g., laboratorians and physicians) to dissect the immune response and provide clear actionable answers (see, Table A). Combination of the anti-SARS-CoV-2 IgG antibody and anti-SARS-CoV-2 IgM antibody assay methods described herein may provide earlier detection, e.g., where the anti-SARS-CoV-2 IgG and IgM antibody assessments are combined in single assay, or where separate assays of the anti-SARS-CoV-2 IgG antibody and anti-SARS-CoV-2 IgM antibody are run on the same sample, e.g., at the same or different times. Similarly, assay of SARS-CoV-2 IgG antibody and SARS-CoV-2 IgM antibody can be done at multiple sampling times. However, using the methods described herein (e.g., methods to detect anti-SARS-CoV-2 IgG antibodies and anti-SARS-CoV-2 IgM antibodies) separately, or together, provides a continuum of data from infection all the way thorough to recovery and could prove of value when testing is carried out in asymptomatic subjects that are not tested or that test negative for viral RNA. The methods described herein (e.g., anti-SARS-CoV-2 IgG and IgM antibody assays) alone, or in combination, may not at all times allow the assessment (e.g., if the person has been infected only recently and still harbors the virus).

Table A

Item	Test Results			General Interpretation*
	PCR and/or Antigen	IgM	IgG	
1	+	-	-	Subject may be in the initial period of infection when antibodies are not yet produced or are under the limit of detection (incubation phase or initial period of infection)
2	+	+	-	Subject is in the active phase of infection and has started to develop an immune response with antibody production (early acute phase)
3	-	+	-	Subject may be in the early stage of infection. PCR result may be a false negative or IgM false positive (early acute phase)
4	+	+	+	Subject may be in the active phase of the infection; immune response has progressed (acute phase)
5	+	-	+	Subjection may be in the late stage of infection or has developed a recurrent infection (late acute phase)
6	-	+	+	Subject may be in the late or recovery phases of infection or PCR false negative (late acute phase or convalescence or recovery phase)
7	-	-	+	Subject may have recovered or has been infected in the past (convalescence or recovery phase).
8	-	-	-	Subject may have recovered (convalescence or recovery phase)

*Test results typically are considered along with other clinical data available to the treating clinician.

[0517] Thus, in some aspects, the present disclosure also relates to one or more algorithms that can be used in connection with the methods described herein to instruct and/or guide clinicians in making the appropriate treatment decisions for subjects suspected of having being exposed to and/or having SARS-CoV-2. For example, as depicted in Item 1 of Table A, a subject that tests PCR positive (and/or positive for a viral antigen) but is negative for both anti-SARS-CoV-2 IgM and anti-SARS-CoV-2 IgG antibodies (using the methods described herein) would be classified as being in the incubation phase or initial period of infection and advised to quarantine; any health care workers in contact with the subject would be advised to use appropriate personal protective equipment. In another aspect, as depicted in Item 3 of Table A, a

subject that tests PCR negative (or viral antigen negative), but positive for anti-SARS-CoV-2 IgM antibodies and negative for anti-SARS-CoV-2 IgG antibodies (using the methods described herein) may indicate a missed diagnosis (e.g., false negative PCR or false negative viral antigen result, a false negative IgG result, both a false negative PCR (or viral antigen) and IgG result, or a false positive IgM result), which may call for a retest on the PCR, or the anti-SARS-CoV-2 IgM and anti-SARS-CoV-2 IgG antibody assays, or management as if the subject is in the early acute phase of infection. Alternatively, as depicted in Item 7 of Table A, a subject that tests PCR negative (or viral antigen negative) and negative for anti-SARS-CoV-2 IgM antibodies (using the methods described herein) but positive for anti-SARS-CoV-2 IgG antibodies (using the methods described herein), indicates that the subject likely has been previously infected (e.g., has been past infected) with SARS-CoV-2 or is in the convalescence or recovery phase. Moreover, as depicted in Item 2, 4, and 6 of Table A, once any anti-SARS-CoV-2 IgM antibodies (using the methods described herein) are detected in a subject tested, this indicates to the treating clinician that the subject likely has entered the active phase of infection (such as the early acute phase, acute phase and/or late acute phase) and suggests that clinical action (e.g., treatment, subject isolation/quarantine, or return visit) be taken. The assessment of anti-SARS-CoV-2 IgG antibodies, as depicted in Item 2, 4, and 6 of Table A, adds further information on the subject's status. Namely, as depicted in Item 2 of Table A, a subject that tests PCR positive (or viral antigen positive), positive for anti-SARS-CoV-2 IgM antibodies (using the methods described herein) but negative for anti-SARS-CoV-2 IgG antibodies (using the methods described herein), indicates that the subject likely is in the active phase, namely, the early acute phase, of infection and is starting to develop an immune response with antibody production. As depicted in Item 4 of Table A, a subject that tests PCR positive (or viral antigen positive), positive for anti-SARS-CoV-2 IgM antibodies (using the methods described herein) and positive for anti-SARS-CoV-2 IgG antibodies (using the methods described herein), indicates that the subject is in the acute phase of infection and the subject's immune response with antibody production has progressed. As depicted in Item 6 of Table A, a subject that tests PCR negative (or viral antigen negative), positive for anti-SARS-CoV-2 IgM antibodies (using the methods described herein) and positive for anti-SARS-CoV-2 IgG antibodies (using the methods described herein), indicates that the subject may be in late acute phase or convalescence or recovery phase of infection, or that this may be a false negative PCR result or viral antigen result. Additionally, as depicted in Item 5 of

Table A, a subject that tests PCR positive (or viral antigen positive), negative for anti-SARS-CoV-2 IgM antibodies (using the methods described herein) and positive for anti-SARS-CoV-2 IgG antibodies (using the methods described herein), indicates that the subject may be in the late acute phase of infection, or perhaps has developed a recurrent infection. The presence of the anti-SARS-CoV-2 IgM and anti-SARS-CoV-2 IgG antibody assays also can be used in locations where PCR equipment is not readily available. The continuum of data from infection to recovery helps enable better subject assessment and management. Understanding the amounts of anti-SARS-CoV-2 IgM antibodies and anti-SARS-CoV-2 IgG antibodies in a subject at different infection stages may help inform of a subject's progress towards clearing the virus, or provide an indication of the cytokine storm initiation.

14. Systems for assessing whether a subject is likely to have immunity from infection by at least one type of SARS-CoV-2

[0518] In still further aspects, the present disclosure relates to a system for assessing whether a subject is likely to have immunity (e.g., has developed protective immunity) from infection by at least one type of SARS-CoV-2 in a biological sample. The system, which can be automated or semi-automated, comprises: (1) at least one type of first specific binding partner comprising at least one type of β-coronavirus (SARS-CoV or SARS-CoV-2) isolated polypeptide or variant thereof selected from a C-terminal domain nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain nucleocapsid protein and a receptor binding domain (RBD) of a spike protein from at least one type of β-coronavirus (SARS-CoV or SARS-CoV-2) that specifically binds to at least one type of anti-β-coronavirus antibody (anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) and at least one type of second specific binding partner comprising at least one detectable label; (2) at least one device that detects the at least one label from a complex formed between the at least one type of first specific binding partner and the at least one type of second specific binding partner, wherein the amount of signal from the label indicates the presence or amount of at least one type of anti-SARS-CoV-2 antibody in the biological sample; and (3) a means for assigning a differentiative rating which indicates or reflects whether the subject is likely immune (e.g., or has likely developed protective immunity) from infection by at least one type of SARS-CoV-2. The determination of whether a subject is likely immune (e.g., or has likely developed protective immunity) is based on detecting the presence of or determining the amount, level and/or concentration of at least one type of anti-

SARS-CoV-2 antibody in the biological sample using the methods described in Section 2 (e.g., employing the at least one type of first specific binding partner and at least one type of second specific binding partner) alone, or in combination with other information obtained from the biological sample as the result of other laboratory tests, such as, for example, that the subject is positive or negative for at least one type of SARS-CoV-2 viral RNA and/or antigen.

[0519] Any device or platform capable of detecting at least one label from the complex formed between at least one first type of specific binding partner and at least one type of second specific binding partner can be used. Such devices or platforms are well-known in the art. Exemplary devices or platforms are described, for example, in U.S. Patent No. 5,063,081, U.S. Patent Application Publication Nos. 2003/0170881, 2004/0018577, 2005/0054078, and 2006/0160164 and as commercially marketed *e.g.*, by Abbott Laboratories (Abbott Park, IL) as Abbott Point of Care (i-STAT® or i-STAT Alinity, Abbott Laboratories) as well as those described in U.S. Patent Nos. 5,089,424 and 5,006,309, and as commercially marketed, e.g., by Abbott Laboratories (Abbott Park, IL) as ARCHITECT® or the series of Abbott Alinity devices.

[0520] Additionally, the system comprises a means for assigning a differentiative rating indicating whether the subject is likely immune from infection by at least one type of SARS-CoV-2. An example of a differentiative rating is the assigning a color and/or a number rating indicating whether the subject is likely immune from infection by at least one type of SARS-CoV-2. Methods for assigning such a differentiative rating are well known in the art and can involve one or more software programs. In some aspects, the one or more software programs are incorporated into the device or platform for detecting the detectable label. In other aspects, the one or more software programs are incorporated into a device that does not detect a detectable label (e.g., such as a computer, etc.). In other aspects, the one or more software programs are incorporated into the device or platform for detecting the detectable label as well as on a device that does not detect the detectable label.

[0521] The differentiative rating (e.g., color and/or a number rating) or assessing immunity can be developed using routine techniques known in the art. An example of a color and/or number differentiative rating for determining whether a subject likely has immunity (e.g., or has likely developed protective immunity) from infection by at least one type of SARS-CoV-2 is provided below in Table D.

[0522] Table D

Differentiative rating - Number/Color Rating	SARS-CoV-2 Viral RNA/Antigen	Anti-SARS-CoV-2 IgM antibody	Anti-SARS-CoV-2 IgG antibody	Likelihood that a subject is immune from infection by at least one type of SARS-CoV-2
Level 1/Red	Negative	Negative	Negative	Subject likely is not immune from infection by at least one type of SARS-CoV-2
Level 2/Orange	Positive	Negative or Positive	Negative	Subject likely is not immune from infection by at least one type of SARS-CoV-2 may develop symptoms of at least one type of SARS-CoV-2 and/or may be contagious
Level 3/Yellow	Positive	Positive	Positive	Subject may have developed symptoms of at least one type of SARS-CoV-2 and/or is developing immunity to infection.
Level 4/Green	Negative	Negative or Positive	Positive	Subject likely has immunity from infection from at least one type of SARS-CoV-2

[0523] In still other aspects, the differentiative rating (e.g., color and/or number rating) can be displayed on a mobile or smart device through the use of one or more mobile or smart device applications as well known in the art. In some aspects, in a semi-automated system, the differentiative rating (e.g., the color and/or number rating) can be uploaded and/or verified by a QR or bar code.

[0524] Some of the differences between an automated or semi-automated system as compared to a non-automated system include the substrate to which the at least one type of first specific binding partner is attached, and the length and timing of the capture, detection, and/or any optional wash steps. Whereas a non-automated format may require a relatively longer incubation time with test sample and capture reagent (e.g., about 2 hours), an automated or semi-automated format (e.g., ARCHITECT® and any successor platform, Abbott Laboratories) may have a relatively shorter incubation time (e.g., approximately 18 minutes for ARCHITECT®). Similarly, whereas a non-automated format may incubate at least one type of second specific binding reagent such as the conjugate reagent for a relatively longer incubation time (e.g., about 2 hours), an automated or semi-automated format (e.g., ARCHITECT® and any successor platform) may have a relatively shorter incubation time (e.g., approximately 4 minutes for the ARCHITECT® and any successor platform).

[0525] Other devices or platforms available from Abbott Laboratories include, but are not limited to, AxSYM®, IMx® (see, *e.g.*, U.S. Patent No. 5,294,404, which is hereby incorporated by reference in its entirety), PRISM®, EIA (bead), and Quantum™ II, as well as other platforms. Additionally, device can be employed in other formats, for example, on electrochemical or other hand-held or point-of-care assay systems. As mentioned previously, the present disclosure is, for example, applicable to the commercial Abbott Point of Care (i-STAT®, Abbott Laboratories) electrochemical immunoassay system that performs sandwich immunoassays. Immunosensors and their methods of manufacture and operation in single-use test devices are described, for example in, U.S. Patent No. 5,063,081, U.S. Patent App. Publication Nos. 2003/0170881, 2004/0018577, 2005/0054078, and 2006/0160164, which are incorporated in their entireties by reference for their teachings regarding same.

[0526] In particular, with regard to the adaptation of an assay to the i-STAT® system, the following configuration is preferred. A microfabricated silicon chip is manufactured with a pair of gold amperometric working electrodes and a silver-silver chloride reference electrode. On one of the working electrodes, polystyrene beads (0.2 mm diameter) with immobilized capture antibody are adhered to a polymer coating of patterned polyvinyl alcohol over the electrode. This chip is assembled into an i-STAT® cartridge with a fluidics format suitable for immunoassay. On a portion of the silicon chip, there is a specific binding partner for an anti-β-coronavirus antibody, such as at least one specific binding partner as described in Section 3 (e.g.,

recombinant antigen) or one or more anti-β-coronavirus (such as SARS-CoV or SARS-Co-V2) DVD-Igs (or a fragment thereof, a variant thereof, or a fragment of a variant thereof that can bind an anti-β-coronavirus antibody (such as SARS-CoV or SARS-CoV-2), either of which can be detectably labeled. Within the fluid pouch of the cartridge is an aqueous reagent that includes p-aminophenol phosphate.

[0527] In operation, a biological sample is added to the holding chamber of the test cartridge, and the cartridge is inserted into the i-STAT® reader. A pump element within the cartridge pushes the sample into a conduit containing the chip. The sample is brought into contact with the sensors allowing the enzyme conjugate to dissolve into the sample. The sample is oscillated across the sensors to promote formation of the sandwich of approximately 2-12 minutes. In the penultimate step of the assay, the sample is pushed into a waste chamber and wash fluid, containing a substrate for the alkaline phosphatase enzyme, is used to wash excess enzyme conjugate and sample off the sensor chip. In the final step, the alkaline phosphatase label reacts with p-aminophenol phosphate to cleave the phosphate group and permit the liberated p-aminophenol to be electrochemically oxidized at the working electrode. Based on the measured current, the reader is able to calculate the amount of anti-β-coronavirus antibody in the sample by means of an embedded algorithm and factory-determined calibration curve. Adaptation of a cartridge for multiplex use, such as used for i-STAT®, has been described in the patent literature, such as for example, U.S. Patent No. 6,438,498, the contents of which are herein incorporated by reference.

[0528] The system as described herein necessarily encompass other reagents and methods for detecting the presence or amount of at least one anti-β-coronavirus antibody e.g., anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody) in a sample. For instance, encompassed are various buffers such as are known in the art and/or which can be readily prepared or optimized to be employed, e.g., for washing, as a conjugate diluent, and/or as a calibrator diluent. An exemplary conjugate diluent is ARCHITECT® conjugate diluent employed in certain kits (Abbott Laboratories, Abbott Park, IL) and containing 2-(N-morpholino)ethanesulfonic acid (MES), a salt, a protein blocker, an antimicrobial agent, and a detergent. An exemplary calibrator diluent is ARCHITECT® human calibrator diluent employed in certain kits (Abbott Laboratories, Abbott Park, IL), which comprises a buffer containing MES, other salt, a protein blocker, and an antimicrobial agent. Additionally, as described in U.S. Patent No. 8,445,199,

improved signal generation may be obtained, e.g., in an i-STAT® cartridge format, using a nucleic acid sequence linked to the signal antibody as a signal amplifier.

[0529] The present disclosure has multiple aspects, illustrated by the following non-limited examples.

15. Examples

[0530] It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the present disclosure described herein are readily applicable and appreciable, and may be made using suitable equivalents without departing from the scope of the present disclosure or the aspects and embodiments disclosed herein. Having now described the present disclosure in detail, the same will be more clearly understood by reference to the following examples, which are merely intended only to illustrate some aspects and embodiments of the disclosure, and should not be viewed as limiting to the scope of the disclosure. The disclosures of all journal references, U.S. patents, and publications referred to herein are hereby incorporated by reference in their entireties.

[0531] **Example 1: Nucleocapsid protein**

[0532] Recombinant proteins comprising either the C-terminal domain (CTD) of SARS-CoV-2 ("CTD peptide") or the epitope for the monoclonal antibody CR3018 (described in van den Brink et al., *J. Virol.* 79(3):1635-1644 (February 2005) and U.S. Patent No. 7,696,330) fused at the N-terminal end of the CTD ("epitope-grafted CTD" fusion protein or peptide) were expressed in *E. coli* cultured cells. For the isolation of CTD peptide of SARS-CoV-2, the thawed cell paste is lysed with lysozyme and benzonase. The lysate is centrifuged and protein solution is loaded onto a Nickel-affinity capture column providing for capture of the CTD peptide. The clarified nickel pool eluted from the column is then subjected to SP polishing followed by sterile filtration and aliquoting. Samples of CTD peptide antigen are taken and purity and integrity of purified protein was confirmed.

[0533] **Example 2: Preparation of CTD Nucleocapsid Coated Microparticles**

[0534] Paramagnetic (styrene) microparticles were reacted with carbodiimide (EDAC) in 2-(N-morpholino) ethanesulfonic acid in a centrifuge tube and then supernatant was aspirated out. The microparticles were then washed and reacted with either the CTD peptide or epitope-grafted fusion protein or peptide of Example 1. The coated microparticles were separated from the

supernatant, washed, and then stored in buffer with surfactant, preservative, and an antifoam agent, until used in an assay.

[0535] Example 3: Detection of Anti-SARS-CoV-2 IgG Antibodies in Human Plasma Samples

[0536] 161 EDTA plasma samples from 28 SARS-CoV-2-positive subjects (nasopharyngeal swabs assessed by a PCR-based assay for SARS-CoV-2 virus) were obtained. Days since symptom onset was assessed for all subjects. 2 subjects were transplant recipients with compromised immune systems. 76 subjects presented with signs and symptoms similar to COVID-19 but tested negative by SARS-CoV-2 PCR.

[0537] Antibody production was assessed for each subject using an indirect assay on the ARCHITECT® automated analyzer (Abbott Laboratories, Abbott Park, IL). The assay format utilized either the CTD peptide-or epitope-grafted fusion peptide-coated microparticles of Example 2 diluted into an ARCHITECT® buffer solution, incubated with patient serum or plasma. After an 18 minute incubation, the magnetic microparticles were washed and reacted with a conjugate diluent comprising acridinylated anti-human IgG (mouse, monoclonal) IgG (e.g., a comparable antibody to that used in other commercially-available ARCHITECT/Alinity viral assay kits (Abbott Laboratories, Abbott Park, IL), although any other commercially-available anti-species IgG (e.g., anti-human-IgG IgG) antibody can be used) to detect the anti-SARS-CoV-2 IgG bound to the solid phase via immunocomplex. After another wash, pre-trigger (hydrogen peroxide) and trigger (sodium hydroxide) were added to generate a signal (S). Additionally, the assay employed either reactive human serum or plasma, or monoclonal antibody CR3018 as the positive control and/or calibrator, and either non-reactive human serum or plasma, or human IgG, as the negative control. The signal (S) was expressed in relative light units (RLU). The signal (S) and signal to calibrator (C) ratio for the subjects tested is shown in below Tables 1-7.

[0538] Table 1 shows a study in of earlier times following infection in which 7 subjects who were SARS-CoV-2 PCR-positive were found to be non-reactive in the IgG assay; however, two of these subjects were transplant recipients that presumably had a compromised immune responsive due to immunosuppressive drug treatment (subjects 1 and 13), 2 subjects appeared to be building an immune response (subjects 7 and 28), and 2 subjects appeared to be in the pre-

seroconversion window (subjects 24 and 26). "#VALUE!" means the value could not be calculated due to unavailability of sample.

[0539] As shown in Tables 2-4, 13 SARS-CoV-2 PCR-positive subjects who were tested at later times following infection were found to be reactive in the IgG assay. Tables 5-7 also show PCR-positive subjects who were tested at later times following infection, with some data also included for earlier times following infection as well. Tables 5-7 show that 8 subjects (subjects 2, 4, 6, 8, 17, 21, 25 and 27) who were SARS-CoV-2 positive (as tested by PCR), were in the seroconversion window. As demonstrated by the data provided in the tables below, the IgG assay identified subjects that were positive for SARS-CoV-2 at least as early as 7 days after the onset of symptoms. When compared against known positives and negative samples for SARS-CoV-2 at more than 7 days after onset of symptoms, the IgG assay showed clinical agreement of percent positives greater than 90%, and percent negatives greater than 99.5%. Additional tests of the IgG assay disclosed 100% specificity for SARS-CoV-2 at 14 days or later after the onset of symptoms, with specificity of 99.63% (1070 specimens; 100% specificity observed for 73 patients with other respiratory illness). In still another study involving samples from 1492 donors tested using the above assay, the assay correctly identified subjects not having SARS-CoV-2 IgG antibodies with about 99.7% specificity.

Table 1

	Subject	Sample Code	Patient Code	Collection Date	Date of Symptom Onset	Days collection-symptom onset	Days (integer value)	Past Medical History	ARCHITECT SARS-CoV-2 IgG RLU	Calibrator RLU	ARCHITECT SARS-CoV-2 IgG S/C	Result Based on qRT-PCR
Set 1	Subject 1	1614	COV1-1	3/25/20 13:55	3/5/20	20.57	21	73 y/o with renal transplant, on tacrolimus and Cellcept	872	62706.2	0.01	Positive
		1609	COV1-2	3/26/20 5:09	3/5/20	21.21	21	73 y/o with renal transplant, on tacrolimus and Cellcept	866	62706.2	0.01	Positive
	Subject 7	1606	COV7-1	3/23/20 12:00 AM	3/16/20	7.00	7		339	62706.2	0.01	Positive
		1613	COV7-2	3/25/20 14:35	3/16/20	9.61	10		7,247	62706.2	0.12	Positive
		1608	COV7-3	3/26/20 5:37	3/16/20	10.23	10		22,039	62706.2	0.35	Positive
	Subject 13	1587	COV13-1				4	lung transplant recipient	318	62706.2	0.01	Positive
		1588	COV13-2				5	lung transplant recipient	336	62706.2	0.01	Positive
	Subject 23	C75	L	3/31/20 17:57	3/30/20		0		1,266	64363.0	0.02	Negative
		C76	L	3/31/20 23:25	3/30/20		0		1,155	64363.0	0.02	Negative
		C77	L	4/1/20 6:21	3/30/20		1		No Sample	64363.0	#VALUE!	Negative
Set 2	Subject 24	C80	M	3/31/20 19:31	3/30/20		0		2,563	64363.0	0.04	Positive
		C81	M	4/1/20 3:15	3/30/20		1		3,956	64363.0	0.06	Positive
	Subject 26	C84	O	3/30/20 16:00	3/23/20		7		413	64363.0	0.01	Positive
		C85	O	4/1/20 6:16	3/23/20		8		435	64363.0	0.01	Positive
	Subject 28	C93	Q	4/1/20 18:30	3/29/20		2		20,086	64363.0	0.31	Positive

Table 2

	Subject	Sample Code	Patient Code	Collection Date	Date of Symptom Onset	Days collection-symptom onset	Days (integer value)	Past Medical History	ARCHITECT SARS-CoV-2 IgG RLU	Calibrator RLU	ARCHITECT SARS-CoV-2 IgG S/C	Result Based on qRT-PCR
	Subject 3	1615	COV3-1	3/25/20 3:49	3/12/20	13.16	13		218,219	62706.2	3.48	Positive
		1616	COV3-2	3/27/20 0:00	3/12/20	15.00	15		271,479	62706.2	4.33	Positive
	Subject 5	1628	COV5-1	3/22/20 21:24	3/6/20	16.89	17		76,921	62706.2	1.23	Positive
		1632	COV5-2	3/24/20 3:24	3/6/20	18.14	18		184,172	62706.2	2.94	Positive
		1633	COV5-3	3/25/20 4:30	3/6/20	19.19	19		264,462	62706.2	4.22	Positive
		1634	COV5-4	3/25/20 16:19	3/6/20	19.68	20		304,095	62706.2	4.85	Positive
		1630	COV5-5	3/26/20 3:03	3/6/20	20.13	20		327,702	62706.2	5.23	Positive
		1631	COV5-6	3/26/20 16:06	3/6/20	20.67	21		347,148	62706.2	5.54	Positive
		1629	COV5-7	3/27/20 4:23	3/6/20	21.18	21		356,401	62706.2	5.68	Positive
Set 1	Subject 9	1597	COV9-1	3/23/20 4:22 PM	2/25/20	27.68	28		378,957	62706.2	6.04	Positive
		1599	COV9-2	3/23/20 4:22 PM	2/25/20	27.68	28		381,967	62706.2	6.08	Positive
		1598	COV9-3	3/23/20 8:58 PM	2/25/20	27.87	28		375,321	62706.2	5.99	Positive
		1600	COV9-4	3/23/20 8:58 PM	2/25/20	27.87	28		366,797	62706.2	5.85	Positive
		1601	COV9-5	3/24/20 2:33 PM	2/25/20	28.11	28		372,164	62706.2	5.94	Positive
		1602	COV9-6	3/24/20 9:27 AM	2/25/20	28.39	28		376,925	62706.2	6.01	Positive
		1627	COV9-7	3/26/20 17:08	2/25/20	30.71	31		381,969	62706.2	6.08	Positive
		1626	COV9-8	3/27/20 3:28	2/25/20	31.14	31		384,371	62706.2	6.13	Positive
	Subject 10	1607	COV10-1	3/22/20 12:00 AM	3/13/20	9.00	9		131,668	62706.2	2.10	Positive
	Subject 11	1579	COV11-1				7		84,720	62706.2	1.35	Positive
		1580	COV11-2				8		111,306	62706.2	1.75	Positive
		1581	COV11-3				8		113,177	62706.2	1.80	Positive
	Subject 12	1582	COV12-1				14		365,720	62706.2	5.83	Positive
		1583	COV12-2				14		364,581	62706.2	5.81	Positive
		1584	COV12-3				14		374,307	62706.2	5.97	Positive
		1585	COV12-4				15		365,390	62706.2	5.83	Positive
		1586	COV12-5				15		370,677	62706.2	5.91	Positive

Table 3

	Subject	Sample Code	Patient Code	Collection Date	Date of Symptom Onset	Days collection-symptom onset	Days (integer value)	Past Medical History	ARCHITECT SARS-CoV-2 IgG RLU	Calibrator RLU	ARCHITECT SARS-CoV-2 IgG S/C	Result Based on qRT-PCR
Set 2	Subject 14	34	A	3/28/2020	3/20/20		8		284,887	64363.0	4.58	Positive
		35	A	3/29/2020	3/20/20		9		307,979	64363.0	4.78	Positive
		36	A	3/30/2020	3/20/20		10		306,890	64363.0	4.77	Positive
		C88	A	3/31/20 2:26	3/19/20		12		312,350	64363.0	4.85	Positive
	Subject 15	13	B	3/27/2020	2/25/20		32		370,631	64363.0	5.76	Positive
		14	B	3/28/2020	2/25/20		33		373,498	64363.0	5.8	Positive
		15	B	3/28/2020	2/25/20		33		369,896	64363.0	5.75	Positive
		16	B	3/28/2020	2/25/20		33		367,101	64363.0	5.7	Positive
		17	B	3/28/2020	2/25/20		33		356,623	64363.0	5.54	Positive
		18	B	3/29/2020	2/25/20		34		368,968	64363.0	5.73	Positive
		19	B	3/30/2020	2/25/20		35		368,374	64363.0	5.72	Positive
		C64	B	3/30/20 11:50	2/25/20		35		371,018	64363.0	5.76	Positive
		C66	B	3/30/20 17:01	2/25/20		35		368,257	64363.0	5.72	Positive
		C65	B	3/30/20 21:03	2/25/20		35		376,485	64363.0	5.85	Positive
		C67	B	3/31/20 4:43	2/25/20		36		373,329	64363.0	5.8	Positive
		C68	B	3/31/20 14:23	2/25/20		36		371,235	64363.0	5.77	Positive
		C69	B	4/1/20 2:13	2/25/20		36		376,493	64363.0	5.85	Positive
		C96	B	4/1/20 12:04	2/25/20		36		368,124	64363.0	5.72	Positive
		C95	B	4/1/20 16:24	2/25/20		36		360,484	64363.0	5.60	Positive
	Subject 16	9	D	3/27/2020	3/16/20		11		93,601	64363.0	1.45	Positive
		10	D	3/28/2020	3/16/20		12		155,061	64363.0	2.41	Positive
		11	D	3/29/2020	3/16/20		13		190,624	64363.0	2.96	Positive
		12	D	3/30/2020	3/16/20		14		207,217	64363.0	3.22	Positive
		C73	D	3/31/20 1:41	3/16/20		15		226,338	64363.0	3.52	Positive
		C74	D	4/1/20 2:46	3/16/20		15		258,338	64363.0	4.01	Positive
	Subject 18	28	G	3/27/2020	3/16/20		11		231,588	64363.0	3.60	Positive
		29	G	3/28/2020	3/16/20		12		273,772	64363.0	4.25	Positive
		30	G	3/29/2020	3/16/20		13		311,576	64363.0	4.84	Positive
		31	G	3/30/2020	3/16/20		14		334,787	64363.0	5.20	Positive
		C78	G	3/31/20 3:13	3/16/20		15		347,546	64363.0	5.40	Positive
		C79	G	4/1/20 4:20	3/16/20		15		356,538	64363.0	5.54	Positive

Table 4

	Subject	Sample Code	Patient Code	Collection Date	Date of Symptom Onset	Days collection-symptom onset	Days (integer value)	Past Medical History	ARCHITECT SARS-CoV-2 IgG RLU	Calibrator RLU	ARCHITECT SARS-CoV-2 IgG S/C	Result Based on qRT-PCR
Set 2	Subject 19	2	H	3/28/2020	3/6/20		22		354,865	64363.0	5.51	Positive
		3	H	3/29/2020	3/6/20		23		360,899	64363.0	5.61	Positive
		4	H	3/29/2020	3/6/20		23		361,162	64363.0	5.61	Positive
		5	H	3/30/2020	3/6/20		24		360,448	64363.0	5.60	Positive
		C70	H	3/30/20 15:46	3/6/20		24		356,698	64363.0	5.54	Positive
		C71	H	3/31/20 4:05	3/6/20		25		353,627	64363.0	5.49	Positive
		C72	H	3/31/20 16:02	3/6/20		25		353,700	64363.0	5.50	Positive
		C94	H	4/1/20 3:23	3/6/20		25		351,681	64363.0	5.46	Positive
		C92	H	4/1/20 17:36	3/6/20		25		354,465	64363.0	5.51	Positive
	Subject 20	20	I	3/27/2020	3/13/20		14		218,053	64363.0	3.39	Positive
		21	I	3/27/2020	3/13/20		14		238,597	64363.0	3.63	Positive
		22	I	3/28/2020	3/13/20		15		248,413	64363.0	3.86	Positive
		23	I	3/28/2020	3/13/20		15		250,331	64363.0	3.89	Positive
		24	I	3/28/2020	3/13/20		15		268,968	64363.0	4.18	Positive
		25	I	3/28/2020	3/13/20		15		268,506	64363.0	4.17	Positive
		26	I	3/29/2020	3/13/20		16		288,363	64363.0	4.48	Positive
		27	I	3/30/2020	3/13/20		17		301,952	64363.0	4.69	Positive
		C47	I	3/30/20 9:53	3/13/20		17		303,845	64363.0	4.72	Positive
		C48	I	3/30/20 16:46	3/13/20		17		314,073	64363.0	4.88	Positive
		C49	I	3/30/20 22:07	3/13/20		17		311,594	64363.0	4.84	Positive
		C50	I	3/31/20 4:00	3/13/20		18		308,674	64363.0	4.80	Positive
		C51	I	3/31/20 10:27	3/13/20		18		322,521	64363.0	5.01	Positive
		C52	I	3/31/20 15:13	3/13/20		18		317,910	64363.0	4.94	Positive
		C53	I	3/31/20 22:00	3/13/20		18		322,957	64363.0	5.02	Positive
		C54	I	4/1/20 4:42	3/13/20		18		330,369	64363.0	5.13	Positive
		C90	I	4/1/20 9:40	3/13/20		18		326,209	64363.0	5.07	Positive
		C91	I	4/1/20 15:40	3/13/20		18		325,488	64363.0	5.06	Positive
	Subject 22	C55	K	3/27/20 15:03	3/21/20		6		304,162	64363.0	4.73	Positive
		C56	K	3/28/20 4:21	3/21/20		7		322,540	64363.0	5.01	Positive
		C57	K	3/28/20 21:38	3/21/20		7		344,780	64363.0	5.36	Positive
		C58	K	3/29/20 3:45	3/21/20		8		338,834	64363.0	5.26	Positive
		C59	K	3/29/20 18:01	3/21/20		8		352259	64363.0	5.47	Positive
		C60	K	3/30/20 3:50	3/21/20		9		361049	64363.0	5.61	Positive
		C61	K	3/31/20 4:22	3/21/20		10		364706	64363.0	5.67	Positive
		C62	K	3/31/20 16:14	3/21/20		10		376015	64363.0	5.84	Positive
		C63	K	4/1/20 2:57	3/21/20		10		370968	64363.0	5.76	Positive
		C97	K	4/1/20 15:16	3/21/20		10		371582	64363.0	5.77	Positive

Table 5

Subject	Sample Code	Patient Code	Collection Date	Date of Symptom Onset	Days collection-symptom onset	Days (integer value)	Past Medical History	ARCHITECT SARS-CoV-2 IgG RLU	Calibrator RLU	ARCHITECT SARS-CoV-2 IgG S/C	Result Based on qRT-PCR
Subject 2	1603	COV2-1	3/21/20 12:00 AM	3/12/20	9.00	9		24,441	62706.2	0.39	Positive
	1605	COV2-2	3/24/20 12:00 AM	3/12/20	12.00	12		174,344	62706.2	2.78	Positive
Subject 4	1594	COV4-1	3/22/20 12:00 AM	3/20/20	2.00	2		2,274	62706.2	0.04	Positive
	1590	COV4-2	3/22/20 5:30 AM	3/20/20	2.23	2		507	62706.2	0.01	Positive
	1591	COV4-3	3/22/20 4:57 PM	3/20/20	2.71	3		761	62706.2	0.01	Positive
	1595	COV4-4	3/23/20 2:20 AM	3/20/20	3.10	3		7,980	62706.2	0.13	Positive
	1593	COV4-5	3/24/20 12:00 AM	3/20/20	4.00	4		1,350	62706.2	0.02	Positive
	1617	COV4-6	3/25/20 3:50	3/20/20	5.16	5		49,336	62706.2	0.79	Positive
	1618	COV4-7	3/25/20 23:35	3/20/20	5.98	6		127,526	62706.2	2.03	Positive
	1619	COV4-8	3/26/20 3:18	3/20/20	6.14	6		143,257	62706.2	2.28	Positive
	1611	COV4-9	3/26/20 16:45	3/20/20	6.70	7		198,919	62706.2	3.17	Positive
	1612	COV4-10	3/27/20 0:41	3/20/20	7.03	7		239,821	62706.2	3.82	Positive
	1610	COV4-11	3/27/20 3:59	3/20/20	7.17	7		243,255	62706.2	3.88	Positive
Subject 6	1624	COV6-1	3/23/20 5:26	3/16/20	7.23	7		910	62706.2	0.01	Positive
	1620	COV6-2	3/24/20 5:47	3/16/20	8.24	8		3,325	62706.2	0.05	Positive
	1622	COV6-3	3/25/20 2:16	3/16/20	9.09	9		14,386	62706.2	0.23	Positive
	1621	COV6-4	3/26/20 2:00	3/16/20	10.08	10		69,135	62706.2	1.10	Positive
	1623	COV6-5	3/26/20 16:30	3/16/20	10.69	11		129,857	62706.2	2.07	Positive
	1625	COV6-6	3/27/20 2:00	3/16/20	11.08	11		163,347	62706.2	2.60	Positive

Table 6

Subject	Sample Code	Patient Code	Collection Date	Date of Symptom Onset	Days collection-symptom onset	Days (integer value)	Past Medical History	ARCHITECT SARS-CoV-2 IgG RLU	Calibrator RLU	ARCHITECT SARS-CoV-2 IgG S/C	Result Based on qRT-PCR
Subject 8	1645	COV8-1	3/22/20 18:29	3/13/20	9.77	10		743	62706.2	0.01	Positive
	1639	COV8-2	3/23/20 9:16	3/13/20	10.39	10		1,604	62706.2	0.03	Positive
	1635	COV8-3	3/23/20 18:03	3/13/20	10.75	11		2,633	62706.2	0.04	Positive
	1644	COV8-4	3/24/20 3:25	3/13/20	11.14	11		6,245	62706.2	0.10	Positive
	1640	COV8-5	3/24/20 15:57	3/13/20	11.66	12		15,765	62706.2	0.25	Positive
	1642	COV8-6	3/25/20 3:00	3/13/20	12.13	12		32,949	62706.2	0.53	Positive
	1636	COV8-7	3/25/20 16:59	3/13/20	12.71	13		72,122	62706.2	1.15	Positive
	1643	COV8-8	3/26/20 3:30	3/13/20	13.15	13		105,781	62706.2	1.69	Positive
	1637	COV8-9	3/26/20 12:16	3/13/20	13.51	14		146,231	62706.2	2.33	Positive
	1641	COV8-10	3/26/20 15:21	3/13/20	13.64	14		157,859	62706.2	2.52	Positive
	1638	COV8-11	3/27/20 4:19	3/13/20	14.18	14		198,945	62706.2	3.17	Positive
Subject 17	37	F	3/27/2020	3/5/20		22		2,399	64363.0	0.04	Positive
	38	F	3/28/2020	3/5/20		23		4,758	64363.0	0.07	Positive
	39	F	3/29/2020	3/5/20		24		19,355	64363.0	0.30	Positive
	40	F	3/30/2020	3/5/20		25		68,721	64363.0	1.07	Positive
	C87	F	3/31/20 6:11	3/5/20		26		148,187	64363.0	2.30	Positive
Subject 21	1	J	3/27/20 0:00	3/25/20		2.0	Flier? Gunky sample	345,419	64363.0	5.37	Positive
	32	J	3/27/20 0:00	3/25/20		2.0		1,210	64363.0	0.02	Positive
	33	J	3/29/20 0:00	3/25/20		4.0		3,777	64363.0	0.06	Positive
	C44	J	3/30/20 18:50	3/25/20		5.0		19,703	64363.0	0.31	Positive
	C45	J	3/31/20 3:29	3/25/20		6.0		34,065	64363.0	0.53	Positive
	C46	J	4/1/20 3:21	3/25/20		6.0		115,858	64363.0	1.80	Positive
	C89	J	4/1/20 5:33	3/25/20		6.0		118,783	64363.0	1.85	Positive
	518	J	4/2/2020	3/25/20		7.0	Mislabeled as negative	203,581	64363.0	3.16	Positive

Table 7

Subject	Sample Code	Patient Code	Collection Date	Date of Symptom Onset	Days coleiction-symptom onset	Days (integer value)	Past Medical History	ARCHITECT SARS-CoV-2 IgG RLU	Calibrator RLU	ARCHITECT SARS-CoV-2 IgG S/C	Result Based on qRT-PCR
Subject 25	6	N	3/28/2020	3/19/20		9		24,105		0.37	Positive
	7	N	3/29/2020	3/19/20		10		62,692		0.97	Positive
	8	N	3/30/2020	3/19/20		11		108,748		1.69	Positive
	C82	N	3/31/20 5:40	3/19/20		12		176,567		2.74	Positive
	C83	N	4/1/20 4:50	3/19/20		12		243,378		3.78	Positive
Subject 27	41	P	3/27/2020	3/26/20		1		733		0.01	Positive
	42	P	3/28/2020	3/26/20		2		728		0.01	Positive
	43	P	3/29/2020	3/26/20		3		2,529		0.04	Positive
	C86	P	3/31/20 6:37	3/26/20		5		82,978		1.29	Positive

[0540] Additionally, no cross-reactivity was observed with the assay described herein and a population of patients with non-CoV-2 respiratory illnesses. The above assay was evaluated for potential cross-reactivity from subjects with other medical conditions. A total of 182 specimens from 36 different categories were tested. One hundred eight-one (181) specimens were negative and 1 specimen was positive by the SARS-CoV-2 IgG assay. The 1 specimen that tested positive was cytomegalovirus (CMV) IgG (1 out 5 tested positive; 4 out of 5 tested negative). The other categories of specimens that tested negative were: adenovirus, antinuclear antibody (ANA), autoimmune hepatitis, CMV Immunoglobulin Class M (IgM), double-stranded deoxyribonucleic acid (dsDNA) antibody, Epstein-Barr Virus (EBV) IgG, EBV IgM, *E. coli* antibody, human anti-mouse antibodies (HAMA), hemodialysis patients, Hepatitis A virus (HAV), Hepatitis B Core (HBc) IgM, Hepatitis B virus, Hepatitis C Virus, Hepatitis D virus, herpex simplex virus (HSV), heterophilic antibody positive, human immunodeficiency virus, human T-lymphotropic virus (HTLV) Type I, HTLV Type 2, Influenza A, Influenza B, Influenza (type unknown), Influenza vaccine, lupus, monoclonal hyper IgG, picornavirus, polyclonal hyper IgG, pregnant females, pregnant females (multiparous), respiratory syncytial virus, Rheumatoid factor (RF), Rubella IgG, Toxoplasmosis IgG, and Varicella Zoster virus.

[0541] Additionally, specimens from subjects who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits employing such monoclonal antibodies. Further, heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Subjects routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Moreover, rheumatoid factor in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. As mentioned, the assay described herein did not exhibit any HAMA, heterophilic antibody or rheumatoid factor cross-reactivity.

[0542] **Example 4: Spike RBD protein**

[0543] Recombinant proteins comprising the receptor binding domain (RBD) of spike of of SARS-CoV-2 ("RBD peptide") were expressed in *E. coli* cultured cells. All recombinant proteins were affinity purified from the cell culture supernatant by affinity capture followed by

sterile filtration. In some aspects, the purification can involve separating any monomeric RBD peptide from any dimeric RBD peptide from the supernatant using routine techniques known in the art, such as for example, using immobilized metal affinity chromatography (IMAC). Purity and integrity of purified protein was confirmed. These methods were done as described, e.g., in Example 1.

[0544] Example 5: Preparation of RBD Spike Coated Microparticles

[0545] Paramagnetic (styrene) microparticles were reacted with carbodiimide (EDAC) in 2-(N-morpholino) ethanesulfonic acid in a centrifuge tube and then supernatant was aspirated out. The microparticles were then washed and reacted with the RBD peptide of Example 4. The coated microparticles were separated from the supernatant, washed, and then stored in buffer with surfactant, preservative, and an antifoam agent, until used in an assay.

[0546] Example 6: Detection of Anti-SARS-CoV-2 IgM Antibodies in Human Plasma Samples

[0547] Plasma samples from SARS-CoV-2-positive subjects (e.g., nasopharyngeal swabs assessed by a PCR-based assay for SARS-CoV-2 virus) were obtained. Days since symptom onset was assessed for the subjects. Antibody production was assessed for each subject using an indirect assay on the ARCHITECT® automated analyzer (Abbott Laboratories, Abbott Park, IL). The assay format utilized the spike RBD peptide-coated microparticles of Example 5 diluted into an ARCHITECT® buffer solution, incubated with the patient serum or plasma. After an 18 minute incubation, the magnetic microparticles were washed and reacted with a conjugate diluent comprising an acridinium-labeled anti-human IgM (e.g., a comparable antibody to that used in other commercially-available ARCHITECT/Alinity viral assay kits (Abbott Laboratories, Abbott Park, IL), although any other commercially-available anti-species IgM (e.g., anti-human-IgM IgG) antibody can be used) to detect the anti-SARS-CoV-2 IgM bound to the solid phase via immunocomplex. After another wash, pre-trigger (hydrogen peroxide) and trigger (sodium hydroxide) were added to generate a signal (S). Additionally, the assay employed either reactive human serum or plasma, or monoclonal antibody CR3022 as the positive control and/or calibrator, and either non-reactive human serum or (recalcified) plasma, or human IgM, as the negative control. The signal (S) was expressed in relative light units (RLU). The signal (S) and signal to calibrator (C) ratio for the subjects was tested.Based on these studies, it was confirmed that the spike IgM assay was able to detect IgM-reactive antibodies in SARS-CoV-2 PCR-

positive subjects, e.g., who were tested at various times following infection. Specifically, in one study involving samples from 812 donors tested using the above assay, the assay correctly identified subjects not having SARS-CoV-2 IgM antibodies with about 99.6% specificity. In yet another study involving 698 samples tested from 0 to 21 days post onset of SARS-CoV-2, the assay exhibited a sensitivity (e.g., correctly identified subjects as having SARS-CoV-2) of between about 66% to about 98% beginning at day 7 and continuing past day 21 after onset, and a sensitivity of between about 42% to about 60% from day 0 to day 6. In yet another study involving serial bleed samples from 22 different donors tested at days post-onset ranging from 8-31 days, the above assay and the assay described in Example 3 were used to identify subject samples with anti-SARS-CoV-2 IgM antibodies as early as 8 days post-onset, and anti-SARS-CoV-2 IgG antibodies as early as 10 days post onset. The number of samples testing positive for anti-SARS-CoV-2 IgM antibodies and anti-SARS-CoV-2 IgG antibodies peaked at day 15and then declined, with detection of positive samples continuing 31 days post-onset.

[0548] Example 7: Detection of Anti-SARS-CoV-2 IgG Antibody Levels in Human Plasma Samples

[0549] Plasma or serum samples from SARS-CoV-2-positive subjects (e.g., nasopharyngeal swabs assessed by a PCR-based assay for SARS-CoV-2 virus) were obtained and tested. Days since symptom onset was available for the subjects.

[0550] Antibody production was assessed and quantified for each subject using a two-step immunoassay on the ARCHITECT® automated analyzer (Abbott Laboratories, Abbott Park, IL). The assay format utilized the microparticles coated with spike RBD peptide as in Example 5 (produced from an established Chinese Hamster Ovary cell line versus transiently expressed from cell pools) diluted into an ARCHITECT® buffer solution, incubated with the patient plasma. Patient plasma or serum was diluted (e.g., about 30-fold) in order to expand the linear range of the assay. After an 18 minute incubation, the magnetic microparticles were washed and reacted with a conjugate solution comprising an acridinium-labeled anti-human IgG IgG (e.g., a comparable antibody to that used in other commercially-available ARCHITECT/Alinity viral assay kits (Abbott Laboratories, Abbott Park, IL), although any other commercially-available anti-human-IgG IgG antibody can be used) to detect the anti-SARS-CoV-2 IgG bound to the solid phase via immunocomplex. After another wash, pre-trigger (hydrogen peroxide) and trigger (sodium hydroxide) were added to generate a signal (S). Additionally, the assay employed either

reactive human serum or plasma, or monoclonal antibody CR3022 as the positive control and/or calibrator, and either non-reactive human serum or (recalcified) plasma, or human IgG, as the negative control. The signal (S) was expressed in relative light units (RLU). The signal (S) for the subjects was measured and the quantity of anti-SARS-CoV-2 IgG in the samples determined in terms of Arbitrary Units (AU)/mL, which are capable being standardized to an internationally-recognized standard. Based on these studies, it was confirmed that the spike IgG assay was able to determine the amount, level and/or concentration of anti-Spike RBD IgG-reactive antibodies in SARS-CoV-2 PCR-positive subjects, e.g., who were tested at various times following infection.

[0551] In one study, the above assay was used to correctly identify 10 out 10 SARS-CoV-2 positive plasma samples as containing anti-SARS-CoV-2 IgG antibodies. Additionally, the assay was compared against other commercially available assays (some of which use an index to determine the quantity of anti-SARS-CoV-2 IgG antibody in a sample) and was found to detect all members of a commercially available test panel, whereas other commercially-available tests missed one or more samples.

[0552] In an exemplary study, about 1000 pre-SARS-CoV-2 samples were tested using the above assay, to establish a cutoff of about 50 AU/mL with ≥99.50% specificity. In yet another study, 552 SARS-CoV-2 positive samples were tested to determine the linear range of the assay. Using the above assay, the linear range from 50 AU/mL up to between about 35,000 AU/mL to about 50,000 AU/mL. In some aspects, the linear range of the assay was determined to be from 50 AU/mL: up to about 35,000 AU/mL, up to about 40,000 AU/mL, up to about 45,000 AU/mL, or up to about 50,000 AU/mL.

[0553] In another study, the above assay was found not to require manual dilution of the biological sample, such as, for example, when monitoring one or more subjects for a response to a SARS-CoV-2 vaccine as shown in FIG. 8. Specifically, in FIG. 8, dose 1 was measured at about 3 weeks post-immunization and dose 2 was measured about 2 weeks after subjects received a booster (e.g., second vaccination with a SARS-CoV-2 vaccine). Specifically, the above assay shows quantification of 98.8% of anti-SARS-CoV-2 IgG antibodies on an ARCHITECT instrument within the extended measuring interval (EMI) with automated dilution and 90.6% within the upper limit measuring interval (ULMI) with no dilution. Specifically, as shown in FIG. 13 and described in this Example 7, in some aspects, the ULMI can be from about

15,500 to about 40,000 AU/mL and/or the EMI can be from about 40,000 to about 80,000 AU/mL. In yet other aspects, the ULMI can be from about 15,500 to about 25,000 AU/mL and/or the EMI can be from about 25,000 to about 50,000 AU/mL. Additionally, tests of the assay on ARCHITECT show that the primary measuring interval (up to 40,000 AU/mL) coupled with the instrument's 1:2 auto dilution (providing the user up to 80,000 AU/mL) encompassed ~ 99% of the samples tested to date, confirming that the assay has sufficiently broad range to encompass vaccine response with no (or at most minimal) need for manual dilution.

[0554] In still further aspects, in yet another study (unpublished data), the above assay can be performed without dilution at an ULMI from about 7.1 to about 5680 BAU/mL.

[0555] Example 8: Predicting Outcome in SARS-CoV-2 Subjects

[0556] Specimens: Specimens were collected between March 21, 2020 and July 20, 2020 from hospitalized patients at Montefiore Medical Center (Bronx, NY) or Washington University Medical Center (St. Louis, MO). All specimens were collected in speckled red top serum tubes (BD, Franklin Lakes, NJ) or EDTA plasma tubes (BD) and frozen at -80°C within 5 days of collection. Residual specimens were deidentified for serological testing. Age, sex, comorbid conditions, immune status, symptom onset date, mortality, and collection date were obtained from electronic medical records.

[0557] Testing: All patient specimens were analyzed on the Abbott ARCHITECT® i2000 instrument using the methods of Examples 3 and 6. A minimum of 100 µL of residual specimen was used for each assay. While both assays are intended for qualitative determination of antibodies to SARS-CoV-2, they report a semi-quantitative signal using chemiluminescent microparticles to detect IgG and IgM binding to the SARS-CoV-2 nucleocapsid protein and spike protein, respectively. Assay results are reported as an index value of the ratio of specimen to calibrator absorbance (S/C or S/CO). An index value of ≥1.4 S/C indicates IgG seropositivity and ≥1.0 S/C indicates IgM positivity. The results are shown below in Tables 8 and FIG. 6.

Table 8

	Number of Patients		Percentage A		Percentage B	
Parameter	Died	Survived	Died (out of Total Died)	Survived (out of Total Survived)	Died (out of Total per Parameter)	Survived (out of Total per Parameter)
SARS-CoV-2 IgG - seroconverted first	3	6	25	13.04	33	67
SARS-CoV-2 IgM - seroconverted first	4	15	33.33	32.61	21	79
SARS-CoV-2 IgG and IgM seroconverted on the same day	5	25	41.67	54.35	17	83
Total	12	46	100	100	100	100

[0558] Table 8 shows in the "Percentage A" columns that subjects who converted to anti-SARS-CoV-2 IgG before IgM were two times more likely to have an unfavorable outcome (i.e., die) (25% vs. 13%). By contrast, subjects who converted to anti-SARS-CoV-2 IgM before IgG had about the same likelihood of death as survival. Subjects who converted to anti-SARS-CoV-2 IgM at the same time as seroconverting to IgG had a slightly better chance of surviving than dying. This trend is even more apparent by comparing on a per parameter basis ("Percentage B" columns of Table 8), where it can been seen that seroconversion to IgG and IgM on the same day appears to favor a favorable outcome (i.e., survival), whereas dysregulation of seroconversion, particularly seroconversion to IgG earlier than IgM, increases the possibility of an unfavorable outcome. Additionally, FIG. 6 shows that subjects having anti-SARS-CoV-2 IgM levels equal or greater than about 10 S/CO, specifically, about 10 to about 15 S/CO at more than 10 days (e.g., from about 11 to about 25 days) after symptom onset also had an unfavorable outcome (e.g., died).

[0559] Example 9: Correlation between Results Obtained from Quantitative SARS-CoV-2 IgG Assay and IgG Neutralizing Antibodies

[0560] Eighty-six (86) serum or plasma samples from convalescent SARS-CoV-2 patients were obtained and tested using the SARS-CoV-2 IgG assay described below.

[0561] Antibody production was assessed and quantified for each subject using a two-step immunoassay on the ARCHITECT® automated analyzer (Abbott Laboratories, Abbott Park, IL). The assay format utilized the microparticles coated with spike RBD peptide as in Example 5 (produced from an established Chinese Hamster Ovary cell line versus transiently expressed from cell pools) diluted into an ARCHITECT® buffer solution, incubated with the patient plasma. Patient plasma or serum was diluted (e.g., about 2-fold up to about 30-fold) in order to expand the linear range of the assay. After an 18 minute incubation, the magnetic microparticles were washed and reacted with a conjugate solution comprising an acridinium-labeled anti-human IgG IgG (e.g., a comparable antibody to that used in other commercially-available ARCHITECT/Alinity viral assay kits (Abbott Laboratories, Abbott Park, IL), although any other commercially-available anti-human-IgG IgG antibody can be used) to detect the anti-SARS-CoV-2 IgG bound to the solid phase via immunocomplex. After another wash, pre-trigger (hydrogen peroxide) and trigger (sodium hydroxide) were added to generate a signal (S). Additionally, the assay employed either reactive human serum or plasma, or monoclonal antibody CR3022 as the positive control and/or calibrator, and either non-reactive human serum or (recalcified) plasma, or human IgG, as the negative control. The signal (S) was expressed in relative light units (RLU). The signal (S) for the subjects was measured and the quantity of anti-SARS-CoV-2 IgG in the samples determined in terms of Arbitrary Units (AU)/mL, which are capable being standardized to an internationally-recognized standard.

[0562] A plaque-reduction neutralization test (PRNTs) from the Broad Institute (Eli and Edythe L. Broad Institute of MIT and Harvard) was also performed on the serum or plasma samples from the above convalescent SARS-CoV-2 patients. Plaque-reduction neutralization tests are used to quantify the titer of IgG neutralizing antibodies for a virus.

[0563] Table 9A below shows the results of a positive percent agreement study performed with the SARS-CoV-2 IgG Assay (also referred to as the SARS-CoV-2 IgG II Quant Assay) on the ARCHITECT® instrument using the 86 samples demonstrated to be positive ($\geq$ 1:20) using the PRNT test.

[0564] Table 9A

	Neutralization Test	
SARS-CoV-2 IgG II Quant	Positive	Negative
Positive	86	0

Positive % agreement = 100.0% (86/86); 95% CI = 95.72, 100.00

[0565] Logistic regression analyses were performed using the 86 samples above to estimate the SARS-CoV-2 IgG concentration corresponding to 95% probability of being at or above the PRNT dilutions listed in Table 9B below:

[0566] Table 9B

PRNT Dilution	SARS-CoV-2 IgG AU/mL
1:80	1050
1:160	3550
1:250	4160
1:640	6950

[0567] An example of the probability profile using the PRNT ID_{50} at 1:250 dilution as a representative high titer is shown in Table 9C and in Figure 7.

[0568] Table 9C

SARS-CoV-2 IgG AU/mL	Probability of High Titer	95% CI*
2150	0.8	0.65, 0.90
3200	0.9	0.73, 0.97
4160	0.95	0.78, 0.99
6350	0.99	0.87, 1.00

[0569] *In the above table, under 95% CI, the first number is the low end of the 95% CI and the second number is the high end of the CI.

[0570] The results as demonstrated in Tables 9A-9C demonstrate: (1) high qualitative agreement between the PRNT assay and the SARS-CoV-2 IgG assay; (2) rising nAb titers are

associated with increasing values in the SARS-CoV-2 assay; and (3) the probability profile for the SARS-CoV-2 IgG assay corresponds to high titer levels in the PRNT assay.

[0571] Example 10: Use of Assay Formats in Connection with Subjects Receiving One or More SARS-CoV-2 Vaccinations

[0572] A total of 1090 healthcare workers who received the Pfizer-BioNTech vaccination (BNT162b2) at a medical center in Southern California were evaluated using the assay format described in Examples 2, 5 and 7 to quantify circulating levels of SARS-CoV-2 anti-nucleocapsid (N) protein IgG and anti-spike (S) protein IgG at 3 time points: (1) before or up to 3 days after dose 1; (2) within 7 to 21 days after dose 1; and (3) within 7 to 21 days after dose 2. More specifically, the CE-marked Abbott anti-S SARS-CoV-2 IgG II Quantitative antibody assay (available for use on Abbott's ARCHITECT® and Alinity i™ platforms) was employed for assessing anti-S IgG. An ACE2 binding inhibition assay (Abbott Laboratories internally developed prototype assay) that correlates well with the SARS-CoV-2 plaque reduction neutralization test methodology and exhibits a high correlation with the Abbott anti-S SARS-CoV-2 IgG II Quantitative antibody assay threshold (r^2=0.95) was also used.

[0573] All participants provided survey data on medical history, including prior SARS-CoV-2 exposures and infection, in addition to data on symptoms experienced after each dose of vaccine. Prior SARS-CoV-2 infection status and timing were determined in relation to date of the first vaccine dose received, based on concordance of data on SARS-CoV-2 diagnosis documented in the electronic health record, presence of anti-N IgG antibodies at baseline pre-vaccination testing, and the self-reported survey information collected. Antibody level and symptom responses between those with and without a prior SARS-CoV-2 diagnosis were compared. In addition to between-group comparisons at each time point, the time point shifted levels of antibody response for those with a prior SARS-CoV-2 diagnosis (at baseline and following dose 1) compared to those without a prior SARS-CoV-2 diagnosis (following dose 1 and dose 2) were examined.

[0574] A total of N=1090 vaccine recipients provided at least one blood sample for antibody testing. Of this total sample, N=980 (78 with prior SARS-CoV-2 infection) provided baseline (pre-vaccine) samples, N=525 (35 with prior infection) provided samples after dose 1, and 239 (11 with prior infection) provided samples after dose 2. A total of 217 individuals (10 with prior

infection) provided blood samples at all 3 time points. For both anti-N IgG (representing response to prior infection) and anti-S IgG (representing response to either prior infection or vaccine), SARS-CoV-2 recovered persons had expectedly higher antibody levels at all time points (FIG. 10 and Tables 10A-10B, with sensitivity analysis at all 3 time points shown in Tables 10C-10D). Specifically, anti-S IgG levels were only slightly lower in SARS-CoV-2 recovered persons at baseline, when compared to infection naïve persons who had received a single vaccine dose. Moreover, anti-S IgG levels were not significantly different between SARS-CoV-2 recovered persons following a single dose and infection naïve persons who had received 2 doses.

[0575] Table 10A – Comparison of antibody levels between participants with and without prior SARS-CoV-2 infection at matched time points. Values are shown as median and interquartile range [IQR] with comparisons performed with two-sided Wilcoxon tests. Sample sizes at baseline, after Dose 1, and after Dose 2 included: N=903, N=490, and N=228 for infection naïve persons; and N=78, N=25, and N=11 for persons with prior infection.

	No Prior Infection	Prior Infection	P value
Log IgG(S-RBD)			
Baseline	0.6 [0.2, 1.2]	6.0 [4.6, 6.9]	<0.001
After Dose 1	7.0 [6.3, 7.6]	10.0 [9.2, 10.4]	<0.001
After Dose 2	9.9 [9.4, 10.3]	10.6 [10.3, 10.7]	<0.001
Log IgG(N)			
Baseline	-3.9 [-4.6, -3.2]	0.6 [-0.5, 1.2]	<0.001
After Dose 1	-2.6 [-3.9, -1.7]	0.6 [-0.1, 1.2]	<0.001
After Dose 2	-2.7 [-3.7, -1.3]	1.0 [0.2, 1.1]	<0.001
Log IgM(S)			
Baseline	-3.2 [-3.5, -2.7]	-0.3 [-1.4, 0.8]	<0.001
After Dose 1	0.1 [-0.8, 0.8]	0.1 [-0.4, 1.0]	0.43
After Dose 2	0.7 [-0.1, 1.3]	-0.1 [-0.6, 1.4]	0.59
ACE2 binding, %			
After Dose 1	42.5 [26.1, 58.0]	99.6 [97.4, 100.0]	<0.001
After Dose 2	98.6 [96.9, 99.2]	100.0 [99.0, 100.0]	<0.001

[0576] Table 10B - Comparison of antibody levels between participants with and without prior SARS-CoV-2 infection at shifted time points. Values are shown as median and interquartile range [IQR] with comparisons performed with two-sided Wilcoxon tests. Sample sizes at baseline, after Dose 1, and After Dose 2 included: After Dose 1, and After Dose 2 included: N=903, N=490, and N=228 for infection naïve persons; and N=78, N=25, and N=11 for persons with prior infection.

	No Prior Infection	Prior Infection	P value
	After Dose 1	*At Baseline*	
Log IgG(S-RBD)	7.0 [6.3, 7.6]	6.0 [4.6, 6.9]	<0.001
Log IgG(N)	-2.8 [-3.9, -1.7]	0.6 [-0.5, 1.2]	<0.001
Log IgM(S)	0.1 [-0.8, 0.8]	-0.3 [-1.4, 0.8]	0.09
	After Dose 2	*After Dose 1*	
Log IgG(S-RBD)	9.9 [9.4, 10.3]	10.0 [9.2, 10.4]	0.82
Log IgG(N)	-2.7 [-3.6, -1.3]	0.6 [-0.1, 1.2]	<0.001
Log IgM(S)	0.7 [-0.1, 1.3]	0.1 [-0.4, 1.0]	0.050
ACE2 binding, %	98.8 [96.9, 99.2]	99.8 [97.4, 100.0]	<0.001

[0577] Table 10C – Sensitivity analysis comparising antibody levels between participants with and without prior SARS-CoV-2 infection at matched time points. Values are shown as median and interquartile range [IQR] with comparisons performed with two-sided Wilcoxon tests.The sample with data available at all time points included N=207 infection naïve persons and N=10 with prior infection.

	No Prior Infection	Prior Infection	P value
Log IgG(S-RBD)			
Baseline	0.7 [0.2, 1.2]	5.9 [2.7, 7.2]	<0.001
After Dose 1	7.0 [6.2, 7.6]	10.2 [8.4, 10.5]	<0.001
After Dose 2	9.9 [9.4, 10.3]	10.6 [10.3, 10.8]	0.001
Log IgG(N)			
Baseline	-3.9 [-4.6, -3.0]	0.7 [0.1, 1.0]	<0.001
After Dose 1	-2.7 [-3.9, -1.6]	0.8 [-0.1, 1.2]	<0.001
After Dose 2	-2.7 [-3.9, -1.3]	0.9 [0.0, 1.1]	<0.001
Log IgM(S)			
Baseline	-3.2 [-3.5, -2.7]	-1.2 [-1.9, 0.5]	<0.001
After Dose 1	0.0 [-0.7, 0.7]	0.1 [-0.2, 1.0]	0.49
After Dose 2	0.7 [-0.1, 1.3]	-0.2 [-0.7, 1.1]	0.24
ACE2 binding, %			
After Dose 1	42.1 [26.3, 58.8]	99.9 [77.3, 100.0]	<0.001
After Dose 2	98.6 [96.9, 99.2]	100.0 [99.0, 100.0]	0.001

[0578] Table 10D – Sensitivity analysis comparing antibody levels between participants with and without prior SARS-CoV-2 infection at shifted time points. Values are shown as median and interquartile range [IQR], with comparisons performed using two-sided Wilcoxon tests. The sample with data available at all time points included N=207 infection naïve persons and N=10

with prior infection.

	No Prior Infection	Prior Infection	P value
	After Dose 1	*At Baseline*	
Log IgG(S-RBD)	7.0 [6.2, 7.6]	5.9 [2.7, 7.2]	0.050
Log IgG(N)	-2.7 [-3.9, -1.8]	0.7 [0.1, 1.0]	0.001
Log IgM(S)	0.0 [-0.7, 0.7]	-1.2 [-1.9, 0.5]	0.08
	After Dose 2	*After Dose 1*	
Log IgG(S-RBD)	9.9 [9.4, 10.3]	10.2 [9.4, 10.5]	0.58
Log IgG(N)	-2.7 [-3.9, -1.3]	0.8 [-0.1, 1.2]	<0.001
Log IgM(S)	0.7 [-0.1, 1.3]	0.1 [-0.2, 1.0]	0.31
ACE2 binding, %	98.6 [96.9, 99.2]	99.9 [77.3, 100.0]	0.033

[0579] In parallel with analyses of antibody response, it was observed that overall frequency and severity of post-vaccine symptoms were more prominent for SARS-CoV-2 recovered after dose 1 compared to infection naïve persons after dose 2. Additionally, as shown in FIG. 9, anti-S IgG levels were not significantly different between previously infected persons following a single dose and infection naïve persons who had received 2 doses. Additionally, it was found that ACE2 binding inhibition was significantly higher among previously infected individuals than infection naïve participants after a single vaccine dose with no between-group difference seen after the second dose. In time shifted analyses, there was no difference in ACE2 binding between participants with prior SARS-CoV-2 infection after a single dose compared with infection naïve participants following 2 doses as shown in FIG. 11. Finally, as shown in FIG. 12, previously infected individual experienced significant post-vaccine symptoms (i.e., reactogenicity) more frequently than infection naïve persons following dose 1. However, there was no between-group difference in significant symptoms following dose 2.

[0580] The present disclosure has multiple aspects, illustrated by the following non-limiting examples.

[0581] It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope, which is defined solely by the appended claims and their equivalents.

[0582] Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation those relating to the chemical structures, substituents, derivatives, intermediates, syntheses,

compositions, formulations, or methods of use, may be made without departing from the spirit and scope thereof.

[0583] For reasons of completeness, various aspects are set out in the following numbered clauses:

[0584] Clause 1. A method for detecting a presence or determining an amount of at least one anti-β-coronavirus antibody in a subject, the method comprising the steps of:

[0585] a) contacting at least one biological sample from the subject, either simultaneously or sequentially, in any order, with

[0586] at least one first specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof, selected from a C-terminal domain of a nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain of a nucleocapsid protein and a receptor binding domain (RBD) of a spike protein, wherein the polypeptide specifically binds to at least one β-coronavirus antibody in the sample, and

[0587] at least one second specific binding partner comprising a detectable label,

[0588] thereby producing one or more first complexes comprising the first specific binding partner-anti-β-coronavirus antibody-second specific binding partner; and

[0589] b) assessing a signal from the one or more first complexes, wherein the amount of detectable signal from the detectable label indicates the presence or amount of anti-β-coronavirus antibody in the sample.

[0590] Clause 2. The method of clause 1, wherein the anti-β-coronavirus antibody is an anti-SARS-CoV antibody or an anti-SARS-CoV-2 antibody.

[0591] Clause 3. The method of any of clauses 1 or 2, wherein the anti-β-coronavirus antibody is an anti-SARS-CoV-2 antibody.

[0592] Clause 4. The method of any of clauses 1-3, wherein the biological sample is whole blood, serum, plasma, saliva, a nasal mucus specimen, an oropharyngeal specimen, anal swab specimen, or a nasopharyngeal specimen.

[0593] Clause 5. The method of any of clauses 1-4, wherein the isolated polypeptide is a fusion polypeptide.

[0594] Clause 6. The method of any of clauses 1-5, wherein the at least one isolated polypeptide has a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48,

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651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000, 1001, 1002, 1003, 1004, 1005, 1006, 1007, 1008, 1009, 1010, 1011, 1012, 1013, 1014, 1015, 1016, 1017, 1018, 1019, 1020, 1021, 1022, 1023, 1024, 1025, 1026, 1027, 1028, 1029, 1030, 1031, 1032, 1033, 1034, 1035, 1036, 1037, 1038, 1039, 1040, 1041, 1042, 1043, 1044, 1045, 1046, 1047, 1048, 1049, 1050, 1051, 1052, 1053, 1054, 1055, 1056, 1057, 1058, 1059, 1060, 1061, 1062, 1063, 1064, 1065, 1066, 1067, 1068, 1069, 1070, 1071, 1072, 1073, 1074, 1075, 1076, 1077, 1078, 1079, 1080, 1081, 1082, 1083, 1084, 1085, 1086, 1087, 1088, 1089, 1090, 1091, 1092, 1093, 1094, 1095, 1096, 1097, 1098, 1099, 1100, 1101, 1102, 1103, 1104, 1105, 1106, 1107, 1108, 1109, 1110, 1111, 1112, 1113, 1114, 1115, 1116, 1117, 1118, 1119, 1120, 1121, 1122, 1123, 1124, 1125, 1126, 1127, 1128, 1129, 1130, 1131, 1132, 1133, 1134, 1135, 1136, 1137, 1138, 1139, 1140, 1141, 1142, 1143, 1144, 1145, 1146, 1147, 1148, 1149, 1150, 1151, 1152, 1153, 1154, 1155, 1156, 1157, 1158, 1159, 1160, 1161, 1162, 1163, 1164, 1165, 1166, 1167, 1168, 1169, 1170, 1171, 1172, 1173, 1174, 1175, 1176, 1177, 1178, 1179, 1180, 1181, 1182, 1183, 1184, 1185, 1186, 1187, 1188, 1189,

1190, 1191, 1192, 1193, 1194, 1195, 1196, 1197, 1198, 1199, 1200, 1201, 1202, 1203, 1204, 1205, 1206, 1207, 1208, 1209, 1210, 1211, 1212, 1213, 1214, 1215, 1216, 1217, 1218, 1219, 1220, 1221, 1222, 1223, 1224, 1225, 1226, 1227, 1228, 1229, 1230, 1231, 1232, 1233, 1234, 1235, 1236, 1237, 1238, 1239, 1240, 1241, 1242, 1243, 1244, 1245, 1246, 1247, 1248, 1249, 1250, 1251, 1252, 1253, 1254, 1255, 1256, 1257, 1258, 1259, 1260, 1261, 1262, 1263, 1264, 1265, 1266, 1267, 1268, 1269, 1270, 1272, 1272, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1286, 1287, 1288, 1289, 1290, 1291, 1292, 1293, 1294, 1295, 1296, 1297, 1298, 1299, 1300, 1301, 1302, 1303, 1304, 1305, 1306, 1307, 1308, 1309, 1310, 1311, 1312, 1313, 1314, 1315, 1316, 1317, 1318, 1319, 1320, 1321, 1322, 1323, 1324, 1325, 1326, 1327, 1328, 1329, 1330, 1331, 1332, 1333, 1334, 1335, 1336, 1337, 1338, 1339, 1340, 1341, 1342, 1343, 1344, 1345, 1346, 1347, 1348, 1349, 1350, 1351, 1352, 1353, 1354, 1355, 1356, 1357, 1358, 1359, 1360, 1361, 1362, 1363, 1364, 1365, 1366, 1367, 1368, 1369, 1370, 1371, 1372, 1373, 1374, 1375, 1376, 1377, 1378, 1379, 1380, 1381, 1382, 1383, 1384, 1385, 1386, 1387, 1388, 1389, 1390, 1391, 1392, 1393, 1394, 1395, 1396, 1397, 1398, 1399, 1400, 1401, 1402, 1403, 1404, 1405, 1406, 1407, 1408, 1409, 1410, 1411, 1412, 1413, 1414, 1415, 1416, 1417, 1418, 1419, 1420, 1421, 1422, 1423, 1424, 1425, 1426, 1427, 1428, 1429, 1430, 1431, 1432, 1433, 1434, 1435, 1436, 1437, 1438, 1439, 1440, 1441, 1442, 1443, 1444, 1445, 1446, 1447, 1448, 1449, 1450, 1451, 1452, 1453, 1454, 1455, 1456, 1457, 1458, 1459, 1460, 1461, 1462, 1463, 1464, 1465, 1466, 1467, 1468, 1469, 1470, 1471, 1472, 1473, 1474, 1475, 1476, 1477, 1478, 1479, 1480, 1481, 1482, 1483, 1484, 1485, 1486, 1487, 1488, 1489, 1490, 1491, 1492, 1493, 1494, 1495, 1496, 1497, 1498, 1499 or 1500 amino acids.

[0595] Clause 7. The method of any of clauses 1-6, wherein the isolated polypeptide is a C-terminal domain of a nucleocapsid protein, said polypeptide having a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185,

186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404 or 405 amino acids.

[0596] Clause 8. The method of any of clauses 1-7, wherein the isolated polypeptide is a C-terminal domain of a nucleocapsid protein, said polypeptide having a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249 or 250 amino acids.

[0597] Clause 9. The method of any of clauses 1-8, wherein the isolated polypeptide is a a receptor binding domain (RBD) of a spike protein, said polypeptide having a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84,

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1211, 1212, 1213, 1214, 1215, 1216, 1217, 1218, 1219, 1220, 1221, 1222, 1223, 1224, 1225, 1226, 1227, 1228, 1229, 1230, 1231, 1232, 1233, 1234, 1235, 1236, 1237, 1238, 1239, 1240, 1241, 1242, 1243, 1244, 1245, 1246, 1247, 1248, 1249, 1250, 1251, 1252, 1253, 1254, 1255, 1256, 1257, 1258, 1259, 1260, 1261, 1262, 1263, 1264, 1265, 1266, 1267, 1268, 1269, 1270, 1272, 1272, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1286, 1287, 1288, 1289, 1290, 1291, 1292, 1293, 1294, 1295, 1296, 1297, 1298, 1299, 1300, 1301, 1302, 1303, 1304, 1305, 1306, 1307, 1308, 1309, 1310, 1311, 1312, 1313, 1314, 1315, 1316, 1317, 1318, 1319, 1320, 1321, 1322, 1323, 1324, 1325, 1326, 1327, 1328, 1329, 1330, 1331, 1332, 1333, 1334, 1335, 1336, 1337, 1338, 1339, 1340, 1341, 1342, 1343, 1344, 1345, 1346, 1347, 1348, 1349, 1350, 1351, 1352, 1353, 1354, 1355, 1356, 1357, 1358, 1359, 1360, 1361, 1362, 1363, 1364, 1365, 1366, 1367, 1368, 1369, 1370, 1371, 1372, 1373, 1374, 1375, 1376, 1377, 1378, 1379, 1380, 1381, 1382, 1383, 1384, 1385, 1386, 1387, 1388, 1389, 1390, 1391, 1392, 1393, 1394, 1395, 1396, 1397, 1398, 1399, 1400, 1401, 1402, 1403, 1404, 1405, 1406, 1407, 1408, 1409, 1410, 1411, 1412, 1413, 1414, 1415, 1416, 1417, 1418, 1419, 1420, 1421, 1422, 1423, 1424, 1425, 1426, 1427, 1428, 1429, 1430, 1431, 1432, 1433, 1434, 1435, 1436, 1437, 1438, 1439, 1440, 1441, 1442, 1443, 1444, 1445, 1446, 1447, 1448, 1449, 1450, 1451, 1452, 1453, 1454, 1455, 1456, 1457, 1458, 1459, 1460, 1461, 1462, 1463, 1464, 1465, 1466, 1467, 1468, 1469, 1470, 1471, 1472, 1473, 1474, 1475, 1476, 1477, 1478, 1479, 1480, 1481, 1482, 1483, 1484, 1485, 1486, 1487, 1488, 1489, 1490, 1491, 1492, 1493, 1494, 1495, 1496, 1497, 1498, 1499 or 1500 amino acids.

[0598] Clause 10. The method of any of clauses 1-9, wherein the at least one anti-β-coronavirus antibody is an IgG antibody, an IgM antibody, or an IgG antibody and an IgM antibody.

[0599] Clause 11. The method of any of clauses 1-10, wherein the at least one first specific binding partner comprises at least one isolated polypeptide from a C-terminal domain of a nucleocapsid protein from a β-coronavirus, wherein said specific binding partner specifically binds to (a) an IgG antibody; (b) an IgM antibody; or (b) both an IgG and IgM antibody.

[0600] Clause 12. The method of any of clauses 1-11, wherein the at least one first specific binding partner comprises at least one isolated polypeptide from a receptor binding domain (RBD) of a spike protein from a β-coronavirus, wherein said specific binding partner

specifically binds to (a) an IgG antibody; and (b) an IgM antibody; or (b) both an IgG and IgM antibody.

[0601] Clause 13. The method of any of clauses 1-12, wherein the at least one second specific binding partner is an antibody.

[0602] Clause 14. The method any of clauses 1-13, wherein the method further comprises a pre-treatment step done at the same time as, or prior to contacting the at least one first specific binding partner, the at least one second specific binding partner, or the at least one first specific binding partner and the at least one second specific binding partner, with the biological sample, and wherein the pretreatment step optionally comprises treatment with anti-human IgG, anti-human IgM, or anti-human IgG and anti-human IgM.

[0603] Clause 15. The method of any of clauses 1-14, wherein the at least one first specific binding partner is immobilized on a solid support.

[0604] Clause 16. The method of any of clauses 1-16, wherein the method is performed in less than about 20 minutes, optionally, less than about 5 minutes, less than about 10 minutes or less than about 15 minutes.

[0605] Clause 17. The method of any of clauses 1-16, wherein the method further comprises use with at least one calibrator reagent, at least one control reagent, or at least one calibrator reagent and at least one control reagent.

[0606] Clause 18. The method of any of clauses 1-17, wherein the method is selected from the group consisting of an immunoassay, or a clinical chemistry assay.

[0607] Clause 19. The method of any of clauses 1-18, wherein the method is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0608] Clause 20. The method of any of clauses 1-19, wherein the at least one second specific binding partner is an anti-human-IgG IgG antibody, an anti-human-IgM IgG antibody, or an anti-human-IgG IgG and an anti-human-IgM IgG antibody.

[0609] Clause 21. The method of any of clauses 1-20, wherein the method is adapted for use in an automated system or a semi-automated system.

[0610] Clause 22. The method of any of clauses 1-21, wherein the method further comprises (a) monitoring the subject for anti-β-coronavirus IgG and/or IgM antibodies; (b) treating the subject for a β-coronavirus; (c) monitoring the subject for anti-β-coronavirus IgG and/or IgM

antibodies and treating the subject for a β-coronavirus; or (d) treating the subject for a β-coronavirus and monitoring the subject for anti-β-coronavirus IgG and/or IgM antibodies.

[0611] Clause 23. A kit for performing the method of clause 1, wherein the kit comprises:

[0612] a. at least one specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof selected from a C-terminal domain nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain nucleocapsid protein and a receptor binding domain (RBD) of a spike protein from a β-coronavirus; and

[0613] b. at least one second specific binding partner comprising at least one detectable label.

[0614] Clause 24. The kit of clause 23, wherein the kit further comprises, or is configured to be used with, at least one calibrator reagent, at least one control reagent, or at least one calibrator reagent and at least one control reagent.

[0615] Clause 25. The kit of any of clauses 23 or 24, wherein the kit further comprises at least one solid support.

[0616] Clause 26. The kit of any of clauses 23-25, wherein the kit further comprises, or is configured to be used with, at least one pretreatment reagent.

[0617] Clause 27. The kit of any of clauses 23-26, wherein the isolated polypeptide is a fusion polypeptide.

[0618] Clause 28. The kit of any of clauses 23-27, wherein the at least one isolated polypeptide has a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251,

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1339, 1340, 1341, 1342, 1343, 1344, 1345, 1346, 1347, 1348, 1349, 1350, 1351, 1352, 1353, 1354, 1355, 1356, 1357, 1358, 1359, 1360, 1361, 1362, 1363, 1364, 1365, 1366, 1367, 1368, 1369, 1370, 1371, 1372, 1373, 1374, 1375, 1376, 1377, 1378, 1379, 1380, 1381, 1382, 1383, 1384, 1385, 1386, 1387, 1388, 1389, 1390, 1391, 1392, 1393, 1394, 1395, 1396, 1397, 1398, 1399, 1400, 1401, 1402, 1403, 1404, 1405, 1406, 1407, 1408, 1409, 1410, 1411, 1412, 1413, 1414, 1415, 1416, 1417, 1418, 1419, 1420, 1421, 1422, 1423, 1424, 1425, 1426, 1427, 1428, 1429, 1430, 1431, 1432, 1433, 1434, 1435, 1436, 1437, 1438, 1439, 1440, 1441, 1442, 1443, 1444, 1445, 1446, 1447, 1448, 1449, 1450, 1451, 1452, 1453, 1454, 1455, 1456, 1457, 1458, 1459, 1460, 1461, 1462, 1463, 1464, 1465, 1466, 1467, 1468, 1469, 1470, 1471, 1472, 1473, 1474, 1475, 1476, 1477, 1478, 1479, 1480, 1481, 1482, 1483, 1484, 1485, 1486, 1487, 1488, 1489, 1490, 1491, 1492, 1493, 1494, 1495, 1496, 1497, 1498, 1499 or 1500 amino acids.

[0619] Clause 29. The kit of any of clauses 23-28, wherein the isolated polypeptide is a C-terminal domain nucleocapsid protein, said polypeptide having a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376,

377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404 or 405 amino acids.

[0620] Clause 30. The kit of any of clauses 23-29, wherein the isolated polypeptide is a C-terminal domain of a nucleocapsid protein, said polypeptide having a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249 or 250 amino acids.

[0621] Clause 31. The kit of any of clauses 23-30, wherein the isolated polypeptide is a receptor binding domain (RBD) of a spike protein, said polypeptide having a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 272, 273, 274, 275, 276, 277, 278,

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1361, 1362, 1363, 1364, 1365, 1366, 1367, 1368, 1369, 1370, 1371, 1372, 1373, 1374, 1375, 1376, 1377, 1378, 1379, 1380, 1381, 1382, 1383, 1384, 1385, 1386, 1387, 1388, 1389, 1390, 1391, 1392, 1393, 1394, 1395, 1396, 1397, 1398, 1399, 1400, 1401, 1402, 1403, 1404, 1405, 1406, 1407, 1408, 1409, 1410, 1411, 1412, 1413, 1414, 1415, 1416, 1417, 1418, 1419, 1420, 1421, 1422, 1423, 1424, 1425, 1426, 1427, 1428, 1429, 1430, 1431, 1432, 1433, 1434, 1435, 1436, 1437, 1438, 1439, 1440, 1441, 1442, 1443, 1444, 1445, 1446, 1447, 1448, 1449, 1450, 1451, 1452, 1453, 1454, 1455, 1456, 1457, 1458, 1459, 1460, 1461, 1462, 1463, 1464, 1465, 1466, 1467, 1468, 1469, 1470, 1471, 1472, 1473, 1474, 1475, 1476, 1477, 1478, 1479, 1480, 1481, 1482, 1483, 1484, 1485, 1486, 1487, 1488, 1489, 1490, 1491, 1492, 1493, 1494, 1495, 1496, 1497, 1498, 1499 or 1500 amino acids.

[0622] Clause 32. The kit of any of clauses 23-31, wherein the at least one second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM (e.g., anti-human-IgM IgG) antibody, or an anti-species IgG (e.g., anti-human-IgG IgG) and an anti-species IgM (e.g., anti-human-IgM IgG) antibody.

[0623] Clause 33. The kit of any of clauses 22-32, wherein the kit is adapted for use with an automated or semi-automated system.

[0624] Clause 34. A system for detecting an anti-β-coronavirus antibody in a biological sample obtained from a subject comprising:

[0625] at least one first specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof selected from a C-terminal domain nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain nucleocapsid protein and a receptor binding domain (RBD) of a spike protein from a β-coronavirus that specifically binds to at least one β-coronavirus antibody and at least one second specific binding partner comprising at least one detectable label; and

[0626] at least one device for detecting the at least one label from the complex.

[0627] Clause 35. The system of clause 34, wherein the device for detecting the label from the complex is automated or semi-automated.

[0628] Clause 36. The system of any of clauses 34-35, wherein the anti-β-coronavirus antibody is anti-SARS-CoV antibody or anti-SARS-CoV-2 antibody.

[0629] Clause 37. The system of any of clauses 34-36, wherein the anti-β-coronavirus antibody is an anti-SARS-CoV IgG antibody, an anti-SARS-CoV IgM antibody, an anti-SARS-CoV-2 IgG antibody, an anti-SARS-CoV-2 IgM antibody, or any combination thereof.

[0630] Clause 38. The system of any of clauses 34-37, wherein the at least one second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM (e.g., anti-human-IgM IgG) antibody, or an anti-species IgG (e.g., anti-human-IgG IgG) and anti-species IgM (e.g., anti-human-IgM IgG) antibody.

[0631] Clause 39. The method of any of clauses 1-22, wherein the method further employs use of a calibrator or a control comprising a sequence of monoclonal antibody CR3018 or CR3022.

[0632] Clause 40. The method of any of clauses 1-22 or 39, wherein the at least one first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

[0633] Clause 41. The method of any of clauses 1-22 or 39, wherein the at least one first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

[0634] Clause 42. The kit of any of clauses 22-33, wherein the kit further comprises at least one calibrator or control comprising a sequence of monoclonal antibody CR3018 or CR3022.

[0635] Clause 43. The kit of any of clauses 22-33 or 42, wherein the at least one first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

[0636] Clause 44. The kit of any of clauses 22-33 or 42, wherein the at least one first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

[0637] Clause 45. The system of clauses 34-38, wherein the at least one first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

[0638] Clause 46. The system of clauses 34-38, wherein the at least one first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

[0639] Clause 47. In an improvement of a method of detecting an anti-β-coronavirus antibody in a biological sample, wherein the method comprises detecting a complex comprising a first specific binding partner, said sample anti-β-coronavirus antibody, and a second specific binding partner comprising at least one detectable label, wherein the improvement comprises using a first specific binding partner comprising at least one isolated polypeptide from a C-terminal domain of a β-coronavirus nucleocapsid protein or a variant thereof.

[0640] Clause 48. In the improvement of clause 47, wherein the first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

[0641] Clause 49. In an improvement of a method of detecting an anti-β-coronavirus antibody in a biological sample, wherein the method comprises detecting a complex comprising a first specific binding partner, said sample anti-β-coronavirus antibody, and a second specific

binding partner comprising at least one detectable label, wherein the improvement comprises using a first specific binding partner comprising at least one isolated polypeptide from a receptor binding domain (RBD) of a β-coronavirus spike protein or a variant thereof.

[0642] Clause 50. In the improvement of clause 49, wherein the first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

[0643] Clause 51. A method for detecting a presence or determining an amount of at least one anti-β-coronavirus antibody in a subject, the method comprising the steps of:

[0644] a) contacting at least one biological sample from the subject, either simultaneously or sequentially, in any order, with

[0645] at least one first specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof, selected from a C-terminal domain of a nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain of a nucleocapsid protein and a receptor binding domain (RBD) of a spike protein, wherein the polypeptide specifically binds to at least one β-coronavirus antibody in the sample, and

[0646] at least one second specific binding partner comprising a detectable label,

[0647] thereby producing one or more first complexes comprising the first specific binding partner-anti-β-coronavirus antibody-second specific binding partner; and

[0648] b) assessing a signal from the one or more first complexes, wherein the amount of detectable signal from the detectable label indicates the presence or amount of anti-β-coronavirus antibody in the sample.

[0649] Clause 52. The method of clause 51, wherein the anti-β-coronavirus antibody is an anti-SARS-CoV-2 antibody.

[0650] Clause 53. The method of any of clauses 51 or 52, wherein the biological sample is whole blood, serum, plasma, saliva, a nasal mucus specimen, an anal swab specimen, an oropharyngeal specimen, or a nasopharyngeal specimen.

[0651] Clause 54. The method of any of clauses 51-53, wherein the at least one anti-β-coronavirus antibody is an anti-β-coronavirus IgG antibody, an anti-β-coronavirus IgM antibody, or an anti-β-coronavirus IgG antibody and an anti-β-coronavirus IgM antibody.

[0652] Clause 55. The method of any of clauses 51-54, wherein the at least one first specific binding partner comprises at least one isolated polypeptide from a C-terminal domain of a

nucleocapsid protein from a β-coronavirus, wherein the method is carried out so that said specific binding partner specifically binds to (a) an anti-β-coronavirus IgG antibody; (b) an anti-β-coronavirus IgM antibody; or (b) both an anti-β-coronavirus IgG and an anti-β-coronavirus IgM antibody.

[0653] Clause 56. The method of any of clauses 51-54, wherein the at least one first specific binding partner comprises at least one isolated polypeptide from a receptor binding domain (RBD) of a spike protein from a β-coronavirus, wherein the method is carried out so that said specific binding partner specifically binds to (a) an anti-β-coronavirus IgG antibody; (b) an anti-β-coronavirus IgM antibody; or (b) both an anti-β-coronavirus IgG antibody and anti-β-coronavirus IgM antibody.

[0654] Clause 57. The method of any of clauses 51-56, wherein the at least one second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM (e.g., anti-human-IgM IgG) antibody, or an (e.g., anti-human-IgG IgG) antibody and an anti-species IgM (e.g., anti-human-IgM IgG) antibody.

[0655] Clause 58. The method of any of clauses 51-55, wherein the at least one first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that

[0656] (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

[0657] Clause 59. The method of any of clauses 51-54 or 56, wherein the at least one first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

[0658] Clause 60. The method of clauses 58 or 59, wherein the method is carried out so that the at least one first specific binding partner binds to (a) an anti-SARS-CoV-2 IgG antibody; (b) an anti-SARS CoV-2 IgM antibody; or (c) both an anti-β-coronavirus IgG antibody and anti-β-coronavirus IgM antibody.

[0659] Clause 61. The method of any of clauses 51-60, wherein the method comprises detecting:

[0660] (a) at least one anti-SARS-CoV-2 IgG antibody in at least one biological sample obtained from a subject wherein the at least one first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2 and specifically binds to at least one anti-SARS-CoV-2 IgG antibody, and/or

[0661] (b) at least one anti-SARS-CoV-2 IgM antibody in at least one biological sample obtained in a subject wherein the at least one first specific binding partner wherein the at least one first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17 and specifically binds to at least one anti-SARS-CoV-2 IgM antibody; and

[0662] wherein the at least one anti-SARS-CoV-2 IgG antibody and/or at least one anti-SARS-CoV-2 IgG antibody are detected in a single biological sample obtained from the subject or in multiple biological samples obtained from the subject; and

[0663] further wherein, when at least one anti-SARS-CoV-2 IgG antibody and at least one anti-SARS-CoV-2 IgG antibody are detected simultaneously or sequentially, in any order, in a single biological sample obtained from the subject or in multiple biological samples obtained from the subject.

[0664] Clause 62. The method of any of clauses 51-61, wherein the method further comprises detecting SARS-CoV-2 from at least one biological sample obtained from the subject, said at least one biological sample being a single biological sample or multiple biological samples.

[0665] Clause 63. The method of clauses 61 or 62, wherein the SARS-CoV-2 is detected by its viral RNA using polymerase chain reaction, or by its viral antigen.

[0666] Clause 64. The method of any of clause 61-63, wherein said multiple biological samples are obtained at the same or different times.

[0667] Clause 65. The method of any of clauses 61-64, wherein the method further comprises detecting whether the subject is in:

[0668] (a) an initial period of infection without any antibodies being produced, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen, negative for anti-SARS-CoV-2 IgM antibodies, and negative for anti-SARS-CoV-2 IgG antibodies; and/or

[0669] (b) an early acute phase of infection, and is developing an immune response to the virus and producing antibodies, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen, positive for an anti-SARS-CoV-2 IgM antibodies, and negative for anti-SARS-CoV-2 IgG antibodies; and/or

[0670] (c) an early acute phase of infection or had a false negative viral RNA result or viral antigen result, or a false positive anti-SARS-CoV-2 IgM antibody result, when the at least one biological sample obtained from the subject is negative for viral RNA, positive for anti-SARS-CoV-2 IgM antibodies, and negative for anti-SARS-CoV-2 IgG antibodies; and/or

[0671] (d) an acute phase of infection, and is progressing in an immune response, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen, positive for anti-β-coronavirus IgM antibodies, and positive for anti-SARS-CoV-2 IgG antibodies; and/or

[0672] (e) a late acute phase of infection, or has developed a recurrent infection with SARS-CoV-2, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen, negative for anti-SARS-CoV-2 IgM antibodies, and positive for anti-SARS-CoV-2 IgG antibodies; and/or

[0673] (f) a late acute phase of infection or recovery phase, or had a false negative viral RNA result, when the at least one biological sample obtained from the subject is negative for viral RNA or viral antigen, positive for anti-β-coronavirus IgM antibodies, and positive for anti-SARS-CoV-2 IgG antibodies; and/or

[0674] (g) recovery phase when the at least one biological sample obtained from the subject is negative for viral RNA or viral antigen, negative for anti-SARS-CoV-2 IgM antibodies and positive for anti-SARS-CoV-2 IgG antibodies; and/or

[0675] (h) recovery phase when the at least one biological sample obtained from the subject is negative for viral RNA or viral antigen and negative for anti-SARS-CoV-2 IgM antibodies and anti-SARS-CoV-2 IgG antibodies.

[0676] Clause 66. The method any of clauses 51-65, wherein the method further comprises a pre-treatment step done at the same time as, or prior to, contacting the at least one first specific binding partner, the at least one second specific binding partner, or the at least one first specific binding partner and the at least one second specific binding partner, with the biological sample, and wherein the pretreatment step optionally comprises treatment with anti-human IgG, anti-human IgM, or anti-human IgG and anti-human IgM.

[0677] Clause 67. The method of any of clauses 51-66, wherein the at least one first specific binding partner is immobilized on a solid support.

[0678] Clause 68. The method of any of clauses 51-67, wherein the method is performed in less than about 20 minutes, optionally, less than about 5 minutes, less than about 10 minutes or less than about 15 minutes.

[0679] Clause 69. The method of any of clauses 51-68, wherein the method further comprises use with at least one calibrator reagent, at least one control reagent, or at least one calibrator reagent and at least one control reagent.

[0680] Clause 70. The method of any of clauses 51-69, wherein the method further employs use of at least one calibrator or a control comprising a sequence of monoclonal antibody CR3018 or CR3022.

[0681] Clause 71. The method of any of clauses 51-70, wherein the method is selected from the group consisting of an immunoassay, or a clinical chemistry assay.

[0682] Clause 72. The method of any of clauses 51-71, wherein the method is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0683] Clause 73. The method of any of clauses 51-72, wherein the method is adapted for use in an automated system or a semi-automated system.

[0684] Clause 74. The method of any of clauses 51-73, wherein the method further comprises (a) monitoring the subject for anti-β-coronavirus, optionally, SARS-CoV-2, IgG and/or IgM antibodies; (b) treating the subject for a β-coronavirus, optionally, SARS-CoV-2; (c) monitoring the subject for anti-β-coronavirus, optionally, SARS-CoV-2, IgG and/or IgM antibodies and treating the subject for a β-coronavirus, optionally, SARS-CoV-2; or (d) treating

the subject for a β-coronavirus, optionally, SARS-CoV-2 and monitoring the subject for anti-β-coronavirus, optionally, SARS-CoV-2, IgG and/or IgM antibodies.

[0685] Clause 75. A kit for performing the method of clause 51, wherein the kit comprises:

[0686] a. at least one specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof selected from a C-terminal domain nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain nucleocapsid protein and a receptor binding domain (RBD) of a spike protein from a β-coronavirus; and

[0687] b. at least one second specific binding partner comprising at least one detectable label.

[0688] Clause 76. The kit of clause 75, wherein the kit further comprises, or is configured to be used with, at least one calibrator reagent, at least one control reagent, or at least one calibrator reagent and at least one control reagent.

[0689] Clause 77. The kit of any of clauses 75 or 76, wherein the kit further comprises at least one solid support.

[0690] Clause 78. The kit of any of clauses 75-77, wherein the kit further comprises, or is configured to be used with, at least one pretreatment reagent.

[0691] Clause 79. The kit of any of clauses 75-78, wherein the isolated polypeptide is a fusion polypeptide.

[0692] Clause 80. The kit of any of clauses 75-79, wherein the at least one second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM (e.g., anti-human-IgM IgG) antibody, or an anti-species IgG (e.g., anti-human-IgG IgG) and an anti-species IgM (e.g., anti-human-IgM IgG) antibody.

[0693] Clause 81. The kit of any of clauses 75-80, wherein the kit further comprises at least one calibrator or control comprising a sequence of monoclonal antibody CR3018 or CR3022.

[0694] Clause 82. The kit of any of clauses 75-81, wherein the at least one first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that

[0695] (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids

211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

[0696] Clause 83. The kit of any of clauses 75-81, wherein the at least one first specific binding partner is a receptor binding domain (RBD) of a spike protein that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

[0697] Clause 84. The kit of any of clauses 75-83, wherein the at least one first specific binding partner binds to (a) an anti-β-coronavirus IgG antibody, (b) an anti-β-coronavirus IgM antibody, or (c) combinations of (a) and (b).

[0698] Clause 85. The kit of any of clauses 75-84, wherein the kit is adapted for use with an automated or semi-automated system.

[0699] Clause 86. A system for detecting an anti-β-coronavirus antibody in a biological sample obtained from a subject comprising:

[0700] at least one first specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof selected from a C-terminal domain nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain nucleocapsid protein and a receptor binding domain (RBD) of a spike protein from a β-coronavirus that specifically binds to at least one β-coronavirus antibody and at least one second specific binding partner comprising at least one detectable label; and

[0701] at least one device for detecting the at least one label from the complex.

[0702] Clause 87. The system of clause 86, wherein the device for detecting the label from the complex is automated or semi-automated.

[0703] Clause 88. The system of any of clauses 86-87, wherein the anti-β-coronavirus antibody is an anti-SARS-CoV-2 antibody.

[0704] Clause 89. The system of any of clause 88, wherein the anti-SARS-CoV-2 antibody is an anti-SARS-CoV-2 IgG antibody, an anti-SARS-CoV-2 IgM antibody, or any combination thereof.

[0705] Clause 90. The system of any of clauses 86-89, wherein the at least one second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM (e.g., anti-human-IgM IgG) antibody, or an anti-species IgG (e.g., anti-human-IgG IgG) and anti-species IgM (e.g., anti-human-IgM IgG) antibody.

[0706] Clause 91. The system of clauses 86-90, wherein the at least one first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

[0707] Clause 92. The system of clauses 86-90, wherein the at least one first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

[0708] Clause 93. The system of clause 86-92, wherein the at least one first specific binding partner binds to (a) an anti-β-coronavirus IgG antibody, (b) an anti-β-coronavirus IgM antibody, or (c) combinations of (a) and (b).

[0709] Clause 94. In an improvement of a method of detecting an anti-β-coronavirus antibody in a biological sample, wherein the method comprises detecting a complex comprising a first specific binding partner, said sample anti-β-coronavirus antibody, and a second specific binding partner comprising at least one detectable label, wherein the improvement comprises using a first specific binding partner comprising at least one isolated polypeptide from a C-terminal domain of a β-coronavirus nucleocapsid protein or a variant thereof.

[0710] Clause 95. In an improvement of a method of detecting an anti-β-coronavirus antibody in a biological sample, wherein the method comprises detecting a complex comprising a first specific binding partner, said sample anti-β-coronavirus antibody, and a second specific binding partner comprising at least one detectable label, wherein the improvement comprises using a first specific binding partner comprising at least one isolated polypeptide from a receptor binding domain (RBD) of a β-coronavirus spike protein or a variant thereof.

[0711] Clause 96. In the improvement of clause 94, wherein the first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an

initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

[0712] Clause 97. In the improvement of clause 95, wherein the first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

[0713] Clause 98. In the improvement of clauses 96 or 97, wherein the at least one first specific binding partner binds to (a) an anti-β-coronavirus IgG antibody, (b) an anti-β-coronavirus IgM antibody, or (c) combinations of (a) and (b).

Clause 99. The method of any of clauses 51-74, wherein the method further comprises identifying a subject having one or more anti-β-coronavirus IgG and/or anti-β-coronavirus IgM antibodies as a candidate subject to provide a biological sample for use in convalescent therapy against a β-coronavirus.

[0714] Clause 100. The method of clause 99, wherein the biological sample is a plasma sample.

[0715] Clause 101. The method of any of clauses 51-74, wherein the method involves determining an amount or quantity of at least one anti-β-coronavirus antibody in a subject based on the amount of detectable signal assessed.

[0716] Clause 102. The method of clause 101, wherein at least one anti-β-coronavirus antibody is an anti-SARS-CoV-2 IgG antibody.

[0717] Clause 103. The method of clause 102, wherein the at least one first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17 and specifically binds to at least one anti-SARS-CoV-2 IgG antibody.

[0718] Clause 104. The method of clause 103, wherein the at least one second specific binding partner is anti-human-IgG IgG antibody.

[0719] Clause 105. The system of any of clauses 86-93, wherein the system further comprises determining an amount of at least one anti-β-coronavirus antibody based on the amount of label detected from the at least one complex.

[0720] Clause 106. The system of clause 105, wherein at least one anti-β-coronavirus antibody is an anti-SARS-CoV-2 IgG antibody.

[0721] Clause 107. The system of clause 106, wherein the at least one first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17 and specifically binds to at least one anti-SARS-CoV-2 IgG antibody.

[0722] Clause 108. The system of clause 107, wherein the at least one second specific binding partner is an anti-human-IgG IgG antibody.

[0723] Clause 109. In an improvement of a method or system for determining an amount of an anti-SARS-CoV-2 IgG antibody in a subject based on the amount of detectable signal assessed, wherein the improvement comprises: (a) a linear assay range of up to 50,000 AU/mL; (b) determining an amount in arbitrary units (e.g., AU/mL) rather than an index; and/or (c) improved detection of positive samples as compared to other assays.

[0724] Clause 110. A method of predicting outcome in a subject that is or was infected with SARS-CoV-2, the method comprising the steps of:

a) detecting an anti-SARS-CoV-2 IgG antibody in at least one biological sample obtained from the subject within a first ten days after onset of symptoms of SARS-CoV-2 infection;

b) detecting an anti-SARS-CoV-2 IgM antibody in the at least one biological sample obtained from the subject within the first ten days after onset of symptoms of SARS-CoV-2 infection;

c) determining which of the anti-SARS-CoV-2 IgG or anti-SARS-CoV-2 IgM antibody detected in a) and b) first appears in the subject;

d) predicting that the subject is more likely to have an unfavorable outcome if anti-SARS-CoV-2 IgG antibody first appears in the subject prior to the appearance of anti-SARS-CoV-2 IgM antibody; and

e) predicting that the subject is not more likely to have an unfavorable outcome if anti-SARS-CoV-2 IgG antibody first appears in the subject at the same time or after the appearance of anti-SARS-CoV-2 IgM antibody.

[0725] Clause 111. A method of predicting outcome in a subject that is or was infected with SARS-CoV-2, the method comprising the steps of:

a) detecting an anti-SARS-CoV-2 IgG antibody in at least one biological sample obtained from the subject within a first ten days after onset of symptoms of SARS-CoV-2 infection;

b) detecting an anti-SARS-CoV-2 IgM antibody in the at least one biological sample obtained from the subject within the first ten days after onset of symptoms of SARS-CoV-2 infection;

c) determining which of the anti-SARS-CoV-2 IgG or anti-SARS-CoV-2 IgM antibody detected in a) and b) first appears in the subject;

d) predicting that the subject is more likely to have an unfavorable outcome if anti-SARS-CoV-2 IgG antibody first appears in the subject prior to the appearance of anti-SARS-CoV-2 IgM antibody; and

e) predicting that the subject is more likely to have an favorable outcome if anti-SARS-CoV-2 IgG antibody first appears in the subject at the same time or after the appearance of anti-SARS-CoV-2 IgM antibody.

[0726] Clause 112. The method of clause 110 or 111, wherein the subject is hospitalized for symptoms of SARS-CoV-2.

[0727] Clause 113. The method of any of clauses 110-112, wherein (i) the anti-SARS-CoV-2 IgG antibody and the one anti-SARS-CoV-2 IgM antibody are detected in the same biological sample; or (ii) the anti-SARS-CoV-2 IgG antibody and the anti-SARS-CoV-2 IgM antibody are detected in different biological samples.

[0728] Clause 114. The method of any of clauses 110-113, wherein the anti-SARS-2 IgG antibody and the anti-SARS-CoV-2 IgM antibody are (i) detected on the same day within the first ten days after onset of symptoms; (ii) detected on different days within the first ten days after the onset of symptoms.

[0729] Clause 115. The method any of clauses 110-114, wherein the anti-SARS-CoV-2 IgG antibody is detected in at least one biological sample obtained from the subject within the first day after the onset of symptoms, within the second day after the onset of symptoms, within the third day after the onset of symptoms, within the fourth day after the onset of symptoms, within the fifth day after the onset of symptoms, within the sixth day after the onset of symptoms, within the seventh date after the onset of symptoms, within the eighth day after the onset of symptoms or within the ninth day after the onset of symptoms.

[0730] Clause 116. The method of any of clauses 110-114, wherein the anti-SARS-CoV-2 IgM antibody is detected in at least one biological sample obtained from the subject within the

first day after the onset of symptoms, within the second day after the onset of symptoms, within the third day after the onset of symptoms, within the fourth day of infection, within the fifth day after the onset of symptoms, within the sixth day after the onset of symptoms, within the seventh date after the onset of symptoms, within the eighth day after the onset of symptoms or within the ninth day after the onset of symptoms.

[0731] Clause 117. The method of any of clauses 110-116, wherein the unfavorable outcome is death.

[0732] Clause 118. A method of predicting outcome in a subject that is or was infected with SARS-CoV-2, the method comprising the steps of:

a) obtaining a signal-to-calibrator ratio (S/CO) from an assay of at least one anti-SARS-CoV-2 IgM antibody in at least one biological sample obtained from the subject at least ten days after onset of symptoms of SARS-CoV-2; and

b) predicting that the subject is more likely than not to have an unfavorable outcome if the S/CO ratio of anti-SARS-CoV-2 IgM antibody determined in the biological sample is equal to or greater than about 10 S/CO.

[0733] Clause 119. The method of clause 118, wherein the subject is hospitalized for symptoms of SARS-CoV-2.

[0734] Clause 120. The method of clause 118 or clause 119, further comprising that the subject is more likely than not to have an unfavorable outcome if the level of anti-SARS-CoV-2 IgM antibody determined in the biological sample is between about 10 S/CO to about 15 S/CO.

[0735] Clause 121. The method of clauses 110-120, wherein the method is selected from the group consisting of an immunoassay, or a clinical chemistry assay.

[0736] Clause 122. The method of any of clauses 110-121, wherein the method is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0737] Clause 123. A method for detecting a presence or determining an amount of at least one anti-SARS-CoV-2 IgG neutralizing antibody in a subject, the method comprising the steps of:

[0738] a) contacting at least one biological sample from the subject, either simultaneously or sequentially, in any order, with

[0739] at least one first specific binding partner comprising at least one SARS-CoV-2 isolated polypeptide or variant thereof, wherein said polypeptide comprises at least one receptor binding

domain (RBD) of a spike protein, and further wherein the polypeptide specifically binds to at least one anti-SARS-CoV-2 IgG neutralizing antibody in the sample, and

[0740] at least one second specific binding partner comprising a detectable label,

[0741] thereby producing one or more first complexes comprising the first specific binding partner-anti-SARS-CoV-2 IgG neutralizing antibody-second specific binding partner; and

[0742] b) assessing a signal from the one or more first complexes, wherein the amount of detectable signal from the detectable label indicates the presence or amount of anti-SARS-CoV-2 IgG neutralizing antibody.

[0743] Clause 124. The method of clause 123, wherein the biological sample is whole blood, serum, plasma, saliva, a nasal mucus specimen, an anal swab specimen, an oropharyngeal specimen, or a nasopharyngeal specimen.

[0744] Clause 125. The method of clauses 123 or clause 124, wherein the at least one second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM (e.g., anti-human-IgM IgG) antibody, or an anti-species IgG (e.g., anti-human-IgG IgG) and an anti-species IgM (e.g., anti-human-IgM IgG) antibody.

[0745] Clause 126. The method of any of clauses 123, wherein the at least one first specific binding partner has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

[0746] Clause 127. The method of any of clauses 123-126, wherein the method further comprises detecting the anti-SARS-CoV-2 IgG neutralizing antibody from at least one biological sample obtained from the subject, said at least one biological sample being a single biological sample or multiple biological samples.

[0747] Clause 128. The method of any of clauses 123-127, wherein the at least one first specific binding partner is immobilized on a solid support.

[0748] Clause 129. The method of any of clauses 123-128, wherein the method is performed in less than about 20 minutes, optionally, less than about 5 minutes, less than about 10 minutes or less than about 15 minutes.

[0749] Clause 130. The method of any of clauses 123-129, wherein the method further comprises use with at least one calibrator reagent, at least one control reagent, or at least one calibrator reagent and at least one control reagent.

[0750] Clause 131. The method of any of clauses 123-130, wherein the method further employs use of at least one calibrator or a control comprising a sequence of monoclonal antibody CR3018 or CR3022.

[0751] Clause 132. The method of any of clauses 123-131, wherein the method is selected from the group consisting of an immunoassay, or a clinical chemistry assay.

[0752] Clause 133. The method of any of clauses 123-132, wherein the method is performed using single molecule detection, a lateral flow assay or a point-of-care assay.

[0753] Clause 134. The method of any of clauses 123-133, wherein the method is adapted for use in an automated system or a semi-automated system.

[0754] Clause 135. The method of clause 52, wherein the method further comprises detecting at least one anti-SARS-CoV-2 IgG neutralizing antibody.

[0755] Clause 136. The method of clause 52, wherein the method demonstrates high qualitative agreement with a plaque reduction neutralization assay (e.g., of Broad Institute).

[0756] Clause 137. The method of clause 52, wherein increasing amounts of anti-SARS-CoV-2 IgG antibodies as detected by the method are associated with increasing amounts (e.g., rising titers) of anti-SARS-CoV-2 IgG neutralizing antibodies.

[0757] Clause 138. The method of claim 52, wherein probability profile for the method corresponds to high titer levels in the plaque reduction neutralization assay (e.g., of Broad Institute) such that there is a high probability of the levels of anti-SARS-CoV-2 IgG antibodies determined by the method being at or above the levels of anti-SARS-CoV-2 IgG neutralizing antibodies determined in the plaque reduction neutralization assay (e.g., of Broad Institute).

[0758] Clause 139. The method of clause 52, wherein the method comprises obtaining the at least one biological sample from the subject at a critical time of from about fourteen (14) days to about thirty-five (35) days after onset of symptoms of SARS-CoV-2.

[0759] Clause 140. The method of clause 134, wherein the method further comprises determining that the subject: (i) more likely than not will develop or experience at least one of a cytokine storm, acute respiratory distress syndrome (ARDS), or a combination of a cytokine storm and ARDS if anti-SARS-CoV-2 IgM antibody, anti-SARS-CoV-2 IgG antibody, or anti-SARS-CoV-2 IgM antibody and anti-SARS-CoV-2 IgG antibody are not detected in the biological sample within the critical time; or (ii) more likely than not will not develop or experience at least one of a cytokine storm, ARDS, or a combination of a cytokine storm and

ARDS if anti-SARS-CoV-2 IgM antibody, anti-SARS-CoV-2 IgG antibody, or anti-SARS-CoV-2 IgM antibody and anti-SARS-CoV-2 IgG antibody are not detected in the biological sample within the critical time.

[0760] Clause 141. The method of any of clauses 58 or 61, wherein the variant of the nucleocapsid protein comprises: (i) an amino acid substitution replacing serine with phenylalanine at position 235 within amino acids 210 to 419 of SEQ ID NO:2; or (ii) SEQ ID NO:24.

[0761] Clause 142. The kit of clause 82, wherein the variant of the nucleocapsid protein comprises (i) an amino acid substitution replacing serine with phenylalanine at position 235 within amino acids 210 to 419 of SEQ ID NO:2; or (ii) SEQ ID NO:24.

[0762] Clause 143. The system of clause 91, wherein the variant of the nucleocapsid protein comprises: (i) an amino acid substitution replacing serine with phenylalanine at position 235 within amino acids 210 to 419 of SEQ ID NO:2; or (ii) SEQ ID NO:24.

[0763] Clause 144. The improvement of clause 96, wherein the variant of the nucleocapsid protein comprises: (i) an amino acid substitution at position S235F within amino acids 210 to 419 of SEQ ID NO:2; or (ii) SEQ ID NO:24.

[0764] Clause 145. The method of any of clauses 59, 61, 103, 126, wherein the variant of the RBD of a spike protein comprises one or more amino substitutions within amino acids 319 to 542 of SEQ ID NOS:15 or 25 replacing lysine with asparagine at position 417, replacing glutamic acid with lysine at position 484, and/or replacing asparagine with tyrosine at position 501.

[0765] Clause 146. The system of any of clauses 92 or 107, wherein the variant of the RBD protein comprises one or more amino substitutions within amino acids 319 to 542 of SEQ ID NOS:15 or 25 replacing lysine with asparagine at position 417, replacing glutamic acid with lysine at position 484, and/or replacing asparagine with tyrosine at position 501.

[0766] Clause 147. The improvement of clause 97, wherein the variant of the RBD protein comprises one or more amino substitutions within amino acids 319 to 542 of SEQ ID NOS:15 or 25 replacing lysine with asparagine at position 417, replacing glutamic acid with lysine at position 484, and/or replacing asparagine with tyrosine at position 501.

[0767] Clause 148. The method of any of clauses 52 to 74, 118 to 141, or 145, wherein the anti-SARS-CoV-2 antibody detected or determined specifically binds to a variant:

[0768] a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

[0769] b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

[0770] c) from a SARS-CoV-2 virus comprising any combination of a) and b).

[0771] Clause 149. The method of any of clauses 62 to 74, wherein the SARS-CoV-2 detected comprises a variant:

[0772] a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

[0773] b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at

amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

[0774] c) from a SARS-CoV-2 virus comprising any combination of a) and b).

[0775] Clause 150. The kit of any of clauses 75 to 85 or 142, wherein the anti-β-coronavirus antibody detected or determined is an anti-SARS-CoV-2 antibody that specifically binds to a variant:

[0776] a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

[0777] b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

[0778] c) from a SARS-CoV-2 virus comprising any combination of a) and b).

[0779] Clause 151. The system of any of clauses 86 to 93, 143 or 146, wherein the anti-β-coronavirus antibody detected is an anti-SARS-CoV-2 antibody that specifically binds to a variant:

[0780] a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

[0781] b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

[0782] c) from a SARS-CoV-2 virus comprising any combination of a) and b).

[0783] Clause 152. The improvement of any of clauses 94 to 98, 144 or 147, wherein the anti-β-coronavirus antibody detected is an anti-SARS-CoV-2 antibody that specifically binds to a variant:

[0784] a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

[0785] b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine

with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

[0786] c) from a SARS-CoV-2 virus comprising any combination of a) and b).

[0787] Clause 153. A method for detecting a presence or determining an amount of at least one type of anti-SARS-CoV-2 antibody in a subject, the method comprising the steps of:

[0788] a) contacting at least one biological sample from the subject, either simultaneously or sequentially, in any order, with at least one type of first specific binding partner comprising at least one type of β-coronavirus isolated polypeptide or variant thereof, selected from a C-terminal domain of a nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain of a nucleocapsid protein and a receptor binding domain (RBD) of a spike protein, wherein the polypeptide specifically binds to at least one type of anti-SARS-CoV-2 antibody in the sample, and

[0789] at least one type of second specific binding partner comprising a detectable label, thereby producing one or more types of first complexes comprising the first specific binding partner-anti-SARS-CoV-2 antibody-second specific binding partner; and

b) assessing a signal from the one or more types of first complexes, wherein the amount of detectable signal from the detectable label indicates the presence or amount of at least one type of anti-β-coronavirus antibody in the sample.

Clause 154. The method of clause 153, wherein the at least one type of anti-SARS-CoV-2 antibody specifically binds to a variant:

a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of

(1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

c) from a SARS-CoV-2 virus comprising any combination of a) and b).

Clause 155. The method of clauses 153 or 154, wherein the biological sample is whole blood, serum, plasma, saliva, a nasal mucus specimen, an anal swab specimen, an oropharyngeal specimen, or a nasopharyngeal specimen.

Clause 156. The method of any of clauses 153-155, wherein the at least one type of anti-SARS-CoV-2 antibody is an IgG antibody, an IgM antibody, or IgG antibody and an IgM antibody.

Clause 157. The method of any of clauses 153-156, wherein the at least one type of first specific binding partner comprises at least one isolated polypeptide from a C-terminal domain of a nucleocapsid protein from a β-coronavirus, wherein the method is carried out so that said specific binding partner specifically binds to (a) an anti-β-coronavirus IgG antibody; (b) an anti-β-coronavirus IgM antibody; or (b) both an anti-β-coronavirus IgG and an anti-β-coronavirus IgM antibody.

Clause 158. The method of any of clauses 153-156, wherein the at least one type of first specific binding partner comprises at least one isolated polypeptide from a receptor binding domain (RBD) of a spike protein from a β-coronavirus, wherein the method is carried out so that said specific binding partner specifically binds to (a) an anti-β-coronavirus IgG antibody; (b) an anti-β-coronavirus IgM antibody; or (b) both an anti-β-coronavirus IgG antibody and anti-β-coronavirus IgM antibody.

Clause 159. The method of any of clauses 153-158, wherein the at least one type of second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM (e.g., anti-human-IgM IgG) antibody, or an anti-species IgG (e.g., anti-human-IgG IgG) and an anti-species IgM (e.g., anti-human-IgM IgG) antibody.

Clause 160. The method of any of clauses 153-159, wherein the at least one type of first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

Clause 161. The method of any of clauses 153-156 or 158, wherein the at least one type of first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

Clause 162. The method of clause 160 or clause 161, wherein the method is carried out so that the at least one type of first specific binding partner binds to (a) an anti-SARS-CoV-2 IgG antibody; (b) an anti-SARS CoV-2 IgM antibody; or (c) both an anti-SARS-CoV-2 IgG antibody and anti-SARS-CoV-2 IgM antibody.

Clause 163. The method of any of clauses 153-162, wherein the method comprises detecting:

(a) at least one anti-SARS-CoV-2 IgG antibody in at least one biological sample obtained from a subject wherein the at least one type of first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2 and specifically

binds to at least one anti-SARS-CoV-2 IgG antibody; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2, and/or

(b) at least one anti-SARS-CoV-2 IgM antibody in at least one biological sample obtained in a subject wherein the at least one first specific binding partner wherein the at least one first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17 and specifically binds to at least one anti-SARS-CoV-2 IgM antibody; and

wherein the at least one anti-SARS-CoV-2 IgG antibody and/or at least one anti-SARS-CoV-2 IgM antibody are detected in a single biological sample obtained from the subject or in multiple biological samples obtained from the subject; and

further wherein, when the at least one anti-SARS-CoV-2 IgG antibody and at least one anti-SARS-CoV-2 IgM antibody are detected simultaneously or sequentially, in any order, in a single biological sample obtained from the subject or in multiple biological samples obtained from the subject.

Clause 164. The method of any of clauses 156-163, wherein the method quantifies up to (a) about 91% or (b) about 99% of anti-SARS-CoV-2 IgG antibodies.

Clause 165. The method of any of clauses 153-164, wherein the method further comprises detecting SARS-CoV-2 from at least one biological sample obtained from the subject, said at least one biological sample being a single biological sample or multiple biological samples.

Clause 166. The method of clause 165, wherein the SARS-CoV-2 detected comprises a variant:

a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

c) from a SARS-CoV-2 virus comprising any combination of a) and b).

Clause 167. The method of any of clauses 164-166, wherein the SARS-CoV-2 is detected by its viral RNA using polymerase chain reaction, or by its viral antigen.

Clause 168. The method of any of clauses 164-166, wherein said multiple biological samples are obtained at the same or different times.

Clause 169. The method of any of clauses 164-166, wherein the method further comprises detecting whether the subject is in:

(a) an initial period of infection without any antibodies being produced, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen, negative for anti-SARS-CoV-2 IgM antibodies, and negative for anti-SARS-CoV-2 IgG antibodies; and/or

(b) an early acute phase of infection, and is developing an immune response to the virus and producing antibodies, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen, positive for an anti-SARS-CoV-2 IgM antibodies, and negative for anti-SARS-CoV-2 IgG antibodies; and/or

(c) an early acute phase of infection or had a false negative viral RNA result or viral antigen result, or a false positive anti-SARS-CoV-2 IgM antibody result, when the at least one biological sample obtained from the subject is negative for viral RNA, positive for anti-SARS-CoV-2 IgM antibodies, and negative for anti-SARS-CoV-2 IgG antibodies; and/or

(d) an acute phase of infection, and is progressing in an immune response, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen,

positive for anti-β-coronavirus IgM antibodies, and positive for anti-SARS-CoV-2 IgG antibodies; and/or

(e) a late acute phase of infection, or has developed a recurrent infection with SARS-CoV-2, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen, negative for anti-SARS-CoV-2 IgM antibodies, and positive for anti-SARS-CoV-2 IgG antibodies;

(f) a late acute phase of infection or recovery phase, or had a false negative viral RNA result, when the at least one biological sample obtained from the subject is negative for viral RNA or viral antigen, positive for anti-β-coronavirus IgM antibodies, and positive for anti-SARS-CoV-2 IgG antibodies;

(g) recovery phase when the at least one biological sample obtained from the subject is negative for viral RNA or viral antigen, negative for anti-SARS-CoV-2 IgM antibodies and positive for anti-SARS-CoV-2 IgG antibodies; and/or

(h) recovery phase when the at least one biological sample obtained from the subject is negative for viral RNA or viral antigen and negative for anti-SARS-CoV-2 IgM antibodies and anti-SARS-CoV-2 IgG antibodies.

Clause 170. The method any of clauses 153-169, wherein the method further comprises a pre-treatment step done at the same time as, or prior to, contacting the at least one type of first specific binding partner, the at least one type of second specific binding partner, or the at least one type of first specific binding partner and the at least one type of second specific binding partner, with the biological sample, and wherein the pretreatment step optionally comprises treatment with anti-human IgG, anti-human IgM, or anti-human IgG and anti-human IgM.

Clause 171. The method of any of clauses 153-170, wherein the at least one type of first specific binding partner is immobilized on a solid support.

Clause 172. The method of any of clauses 153-171, wherein the method is performed in from about 5 to about 20 minutes, less than about 20 minutes, and optionally is performed in less than about 5 minutes, less than about 10 minutes or less than about 15 minutes.

Clause 173. The method of any of clauses 153-172, wherein the method further comprises use with at least one calibrator reagent, at least one control reagent, or at least one calibrator reagent and at least one control reagent.

Clause 174. The method of any of clauses 153-173, wherein the method further employs use of at least one calibrator reagent or a control reagent comprising a sequence of monoclonal antibody CR3018 or CR3022.

Clause 175. The method of any of clauses 153-174, wherein the method is selected from the group consisting of an immunoassay, or a clinical chemistry assay.

Clause 176. The method of any of clauses 153-175, wherein the method is performed using single molecule detection, lateral flow assay, or a point-of-care assay.

Clause 177. The method of any of clauses 153-176, wherein the method further comprises identifying a subject having one or more anti-SARS-CoV-2 IgG and/or anti-SARS-CoV-2 IgM antibodies as a candidate subject to provide a biological sample for use in convalescent therapy againstSARS-CoV-2.

Clause 178. The method of clause 177, wherein the subject is identified as a candidate to provide a biological sample for use in convalescent therapy if the level of one or more SARS-CoV-2 IgG and/or SARS-CoV-2 IgM antibodies is from at least about 100 BAU/mL to about 490 BAU/mL; at least about 110 BAU/mL to about 490 BAU/mL; at least about 200 BAU/mL to about 490 BAU/mL; at least from about 300 BAU mL to about 490 BAU/mL; at least about 400 BAU mL to about 490 BAU/mL; at least about 100 BAU/mL; at least about 110 BAU/mL; at least about 120 BAU/mL; at least about 130 BAU/mL; at least about 140 BAU/mL; at least about 150 BAU/mL; at least about 160 BAU/mL; at least about 170 BAU/mL; at least about 180 BAU/mL; at least about 190 BAU/mL; at least about 200 BAU/mL; at least about 210 BAU/mL; at least about 220 BAU/mL; at least about 230 BAU/mL; at least about 240 BAU/mL; at least about 250 BAU/mL; at least about 260 BAU/mL; at least about 270 BAU/mL; at least about 280 BAU/mL; at least about 290 BAU/mL; at least about 300 BAU/mL; at least about 300 BAU/mL; at least about 310 BAU/mL; at least about 320 BAU/mL; at least about 330 BAU/mL; at least about 340 BAU/mL; at least about 350 BAU/mL; at least about 360 BAU/mL; at least about 370 BAU/mL; at least about 380 BAU/mL; at least about 390 BAU/mL; at least about 400 BAU/mL; at least about 400 BAU/mL; at least about 410 BAU/mL; at least about 420 BAU/mL; at least about 430 BAU/mL; at least about 440 BAU/mL; at least about 450 BAU/mL; at least about 460 BAU/mL; at least about 470 BAU/mL; at least about 480 BAU/mL; or at least about 490 BAU/mL.

Clause 179. The method of clause 178, wherein the subject is identified as a candidate to provide a biological sample for use in convalescent therapy if the level of one or more SARS-CoV-2 IgG and/or anti-β-coronavirus IgM antibodies is from at least about 500 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 500 BAU/mL; from at least about 510 BAU/mL; at least about 520 BAU/mL; at least about 530 BAU/mL; at least about 540 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL; at least about 560 BAU/mL; at least about 570 BAU/mL; at least about 580 BAU/mL; at least about 590 BAU/mL; at least about 600 BAU/mL; at least about 610 BAU/mL; at least about 620 BAU/mL; at least about 630 BAU/mL; at least about 639 BAU/mL; at least about 640 BAU/mL; or at least about 650 BAU/mL.

Clause 180. The method of any of clauses 153-179, wherein the method:

(a) further comprises detecting at least one type of anti-SARS-CoV-2 IgG neutralizing antibody;

(b) demonstrates high qualitative agreement with a plaque reduction neutralization assay;

(c) detects increasing amounts of anti-SARS-CoV-2 IgG antibodies as detected by the method that are associated with increasing amounts of anti-SARS-CoV-2 IgG neutralizing antibodies;

(d) has a probability profile that corresponds to high titer levels in the plaque reduction neutralization assay such that there is a high probability of the levels of anti-SARS-CoV-2 IgG antibodies determined by the method being at or above the levels of anti-SARS-CoV-2 IgG neutralizing antibodies determined in the plaque reduction neutralization assay; or

(e) demonstrates high qualitative agreement with an ACE2 binding inhibition assay.

Clause 181. The method of clause 180, wherein a level of anti-SARS-CoV-2 IgG antibodies of at least about 4160 AU/mL (about 590 BAU/mL) when used as a cut-off or threshold correspond to about a 0.95 probability of obtaining a plaque reduction neutralization assay ID50 at 1:250 dilution.

Clause 182. The method of clause 180, wherein the anti-SARS-CoV-2 neutralizing antibody is an anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof.

Clause 183. The method of any of clauses 153-182, wherein the method comprises obtaining the at least one biological sample from the subject at a critical time of from about fourteen (14) days to about thirty-five (35) days after onset of symptoms of SARS-CoV-2.

Clause 184. The method of clause 183, wherein the method further comprises determining that the subject: (i) more likely than not will develop or experience at least one of a cytokine storm, acute respiratory distress syndrome (ARDS), or a combination of a cytokine storm and ARDS if anti-SARS-CoV-2 IgM antibody, anti-SARS-CoV-2 IgG antibody, or anti-SARS-CoV-2 IgM antibody and anti-SARS-CoV-2 IgG antibody are not detected in the biological sample within the critical time; or (ii) more likely than not will not develop or experience at least one of a cytokine storm, ARDS, or a combination of a cytokine storm and ARDS if anti-SARS-CoV-2 IgM antibody, anti-SARS-CoV-2 IgG antibody, or anti-SARS-CoV-2 IgM antibody and anti-SARS-CoV-2 IgG antibody are not detected in the biological sample within the critical time.

Clause 185. The method of any of clauses 153-184, wherein the subject: (1) is naïve and was not previously vaccinated against SARS-CoV-2; (2) is naïve and was previously vaccinated against SARS-CoV-2; (3) is currently infected with SARS-CoV-2 and was not previously vaccinated against SARS-CoV-2; (4) is currently infected with SARS-CoV-2 and was previously vaccinated against SARS-CoV-2; (5) was previously infected with SARS-CoV-2, recovered, and was not previously vaccinated against SARS-CoV-2; or (6) was previously infected with SARS-CoV-2, recovered, and then was vaccinated against SARS-CoV-2.

Clause 186. The method of clause 185, wherein the method is performed to: (a) determine whether the subject can be administered a current vaccine for SARS-CoV-2; or (b) monitor the subject following the current vaccine or previous vaccination, based on detecting the presence of at least one type of anti-SARS-CoV-2 antibody in the sample.

Clause 187. The method of clause 186, performed regardless of variation in timing and/or severity of prior infection with SARS-CoV-2.

Clause 188. The method of any of clause 186 or 187, wherein the method comprises determining that the current vaccine for SARS-CoV-2:

(a) can be administered to the subject when no anti-SARS-CoV-2 antibodies, no anti-SARS-CoV-2 IgG neutralizing antibodies, or an amount of anti-SARS-CoV-2 IgG antibody or

anti-SARS-CoV-2 IgG neutralizing antibody that is insufficient to impart immunity, are detected in the biological sample; and/or

(b) should not be administered to the subject if an amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 IgG neutralizing antibody that is sufficient to impart immunity is detected in the biological sample.

Clause 189. The method of clause 188, wherein the amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 IgG neutralizing antibody sufficient to impart immunity is from at least about 100 BAU/mL to about 490 BAU/mL; at least about 110 BAU/mL to about 490 BAU/mL; at least about 200 BAU/mL to about 490 BAU/mL; at least from about 300 BAU mL to about 490 BAU/mL; at least about 400 BAU mL to about 490 BAU/mL; at least about 100 BAU/mL; at least about 110 BAU/mL; at least about 120 BAU/mL; at least about 130 BAU/mL; at least about 140 BAU/mL; at least about 150 BAU/mL; at least about 160 BAU/mL; at least about 170 BAU/mL; at least about 180 BAU/mL; at least about 190 BAU/mL; at least about 200 BAU/mL; at least about 210 BAU/mL; at least about 220 BAU/mL; at least about 230 BAU/mL; at least about 240 BAU/mL; at least about 250 BAU/mL; at least about 260 BAU/mL; at least about 270 BAU/mL; at least about 280 BAU/mL; at least about 290 BAU/mL; at least about 300 BAU/mL; at least about 300 BAU/mL; at least about 310 BAU/mL; at least about 320 BAU/mL; at least about 330 BAU/mL; at least about 340 BAU/mL; at least about 350 BAU/mL; at least about 360 BAU/mL; at least about 370 BAU/mL; at least about 380 BAU/mL; at least about 390 BAU/mL; at least about 400 BAU/mL; at least about 400 BAU/mL; at least about 410 BAU/mL; at least about 420 BAU/mL; at least about 430 BAU/mL; at least about 440 BAU/mL; at least about 450 BAU/mL; at least about 460 BAU/mL; at least about 470 BAU/mL; at least about 480 BAU/mL; or at least about 490 BAU/mL.

Clause 190. The method of clause 188, wherein the amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 IgG neutralizing antibody sufficient to impart immunity is: (a) from at least about 550 BAU/mL to about 650 BAU/mL; (b) at least about 550 BAU/mL; (c) at least about 560 BAU/mL; (d) at least about 570 BAU/mL; (e) at least about 580 BAU/mL; (f) at least about 590 BAU/mL; (g) at least about 600 BAU/mL; (h) at least about 610 BAU/mL; (i) at least about 620 BAU/mL; (j) at least about 630 BAU/mL; (k) at least about 639 BAU/mL; (l) at least about 640 BAU/mL; or (m) at least about 650 BAU/mL.

Clause 191. The method of clause 186, wherein the method comprises obtaining the biological sample within a time frame after the subject has received either the current vaccine or previous vaccination for SARS-CoV-2 of at least one day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, at least 15 days, at least 16 days, at least 17 days, at least 18 days, at least 19 days, at least 20 days, at least 21 days, at least 22 days, at least 23 days, at least 24 days, at least 25 days, at least 26 days, at least 27 days, at least 28 days, at least 29 days, at least 30 days, at least 31 days, at least 32 days, at least 33 days, at least 34 days, at least 35 days, at least 36 days, at least 37 days, at least 38 days, at least 39 days, at least 40 days, at least 41 days, at least 42 days, at least 43 days, at least 44 days, at least 45 days, at least 46 days, at least 47 days, at least 48 days, at least 49 days, or at least 50 days.

Clause 192. The method of clause 186, wherein the biological sample is obtained within about 7 to about 21 days after the subject has received either the current vaccine or previous vaccination.

Clause 193. The method of clause 191 or clause 192, wherein the method comprises determining that at least one further vaccine for SARS-CoV-2:

(a) can be administered to the subject after the current vaccine if no anti-SARS-CoV-2 antibodies, no anti-SARS-CoV-2 IgG neutralizing antibodies, and/or an amount of anti-SARS-CoV-2 IgG antibody or anti-SARS-CoV-2 IgG neutralizing antibody that is insufficient to impart immunity, is detected in the biological sample obtained from the subject; or

(b) should not be administered to the subject after the current vaccine if an amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 IgG neutralizing antibody that is sufficient to impart immunity is detected in the biological sample obtained from the subject.

Clause 194. The method of clause 193, wherein the amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 IgG neutralizing antibody sufficient to impart immunity is from at least about 100 BAU/mL to about 490 BAU/mL; at least about 110 BAU/mL to about 490 BAU/mL; at least about 200 BAU/mL to about 490 BAU/mL; at least from about 300 BAU mL to about 490 BAU/mL; at least about 400 BAU mL to about 490 BAU/mL; at least about 100 BAU/mL; at least about 110 BAU/mL; at least about 120 BAU/mL; at least about 130 BAU/mL;

at least about 140 BAU/mL; at least about 150 BAU/mL; at least about 160 BAU/mL; at least about 170 BAU/mL; at least about 180 BAU/mL; at least about 190 BAU/mL; at least about 200 BAU/mL; at least about 210 BAU/mL; at least about 220 BAU/mL; at least about 230 BAU/mL; at least about 240 BAU/mL; at least about 250 BAU/mL; at least about 260 BAU/mL; at least about 270 BAU/mL; at least about 280 BAU/mL; at least about 290 BAU/mL; at least about 300 BAU/mL; at least about 300 BAU/mL; at least about 310 BAU/mL; at least about 320 BAU/mL; at least about 330 BAU/mL; at least about 340 BAU/mL; at least about 350 BAU/mL; at least about 360 BAU/mL; at least about 370 BAU/mL; at least about 380 BAU/mL; at least about 390 BAU/mL; at least about 400 BAU/mL; at least about 400 BAU/mL; at least about 410 BAU/mL; at least about 420 BAU/mL; at least about 430 BAU/mL; at least about 440 BAU/mL; at least about 450 BAU/mL; at least about 460 BAU/mL; at least about 470 BAU/mL; at least about 480 BAU/mL; or at least about 490 BAU/mL.

Clause 195. The method of clause 193, wherein the amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 IgG neutralizing antibody sufficient to impart immunity is from at least about 500 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 500 BAU/mL; from at least about 510 BAU/mL; at least about 520 BAU/mL; at least about 530 BAU/mL; at least about 540 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL; at least about 560 BAU/mL; at least about 570 BAU/mL; at least about 580 BAU/mL; at least about 590 BAU/mL; at least about 600 BAU/mL; at least about 610 BAU/mL; at least about 620 BAU/mL; at least about 630 BAU/mL; at least about 639 BAU/mL; at least about 640 BAU/mL; or at least about 650 BAU/mL.

Clause 196. The method of any of clauses 180, 182, 188, 189, 190, 193, 194, or 195, wherein the anti-SARS-CoV-2 neutralizing antibody is an anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof.

Clause 197. The method of any of clauses 153-196, wherein the method further comprises (a) monitoring the subject for at least one type of anti- SARS-CoV-2 antibody, optionally, IgG and/or IgM antibodies; (b) treating the subject for SARS-CoV-2; (c) monitoring the subject for at least one type of anti- SARS-CoV-2, optionally, IgG and/or IgM antibodies and treating the subject for SARS-CoV-2; or (d) treating the subject for a SARS-CoV-2 and monitoring the subject for at least one type of anti- SARS-CoV-2 antibody, optionally, IgG and/or IgM antibodies.

Clause 198. The method of any of clauses 153-197, wherein the volume of the biological sample used in the method is from about 0.30 µL to about 0.40 µL.

Clause 199. The method of any of clauses 153-198, wherein the method comprises (a) diluting the biological sample prior to or during the method and further wherein the diluting comprises mixing about 10 µL of the biological sample with about 290 µL of at least one buffer to form a biological sampling mixture; and (b) removing about 10 µL from the biological sampling mixture for use in the method.

Clause 200. The method of any of clauses 153-160, or 162-197, wherein the method quantifies about 99% of anti-SARS-CoV-2 IgG antibodies within an extended measuring interval (EMI) with automated dilution and about 91% of anti-SARS-CoV-2 IgG antibodies within an upper limit measuring interval (ULMI) with no dilution.

Clause 201. The method of clause 200, wherein the method can be performed without dilution and the ULMI is from about 15,500 to about 40,000 and/or the EMI is from about 40,000 to about 80,000.

Clause 202. The method of clause 200, wherein the method can be performed without dilution and the ULMI is from about 15,500 to about 25,000 AU/mL and/or the EMI is from about 25,000 to about 50,000 AU/mL.

Clause 203. The method of any of clauses 153-202, wherein the method is (a) semi-quantitative; (b) quantitative; or (c) qualitative.

Clause 204. The method of the above clause 203, wherein when the method is (a) semi-quantitative, it is not standardized against an internally recognized standard; (b) quantitative, it is standardized against an internationally recognized standard; or (c) qualitative, a single-to-calibrator (S/CO) is obtained.

Clause 205. The method of any of clauses 153-204, wherein the method is adapted for use in an automated system or a semi-automated system.

Clause 206. A kit for performing the method of clause 153, wherein the kit comprises:

a. at least one type of specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof selected from a C-terminal domain nucleocapsid protein, a

receptor binding domain (RBD) of a spike protein, or a C-terminal domain nucleocapsid protein and a receptor binding domain (RBD) of a spike protein from a β-coronavirus; and

b. at least one type of second specific binding partner comprising at least one detectable label.

Clause 207. The kit of clause 206, wherein the at least one type of anti-SARS-CoV-2 antibody detected or determined specifically binds to a variant:

a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

c) from a SARS-CoV-2 virus comprising any combination of a) and b).

Clause 208. The kit of clause 206 or clause 207, wherein the kit further comprises, or is configured to be used with, at least one calibrator reagent, at least one control reagent, or at least one calibrator reagent and at least one control reagent.

Clause 209. The kit of any of clauses 206-208, wherein the kit further comprises at least one solid support.

Clause 210. The kit of any of clauses 206-209, wherein the kit further comprises, or is configured to be used with, at least one pretreatment reagent.

Clause 211. The kit of any of clauses 206-210, wherein the isolated polypeptide is a fusion polypeptide.

Clause 212. The kit of any of clauses 206-211, wherein the at least one type of second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM (e.g., anti-human-IgM IgG) antibody, or an anti-species IgG (e.g., anti-human-IgG IgG) antibody and an anti-species IgM (e.g., anti-human-IgM IgG) antibody.

Clause 213. The kit of any of clauses 206-212, wherein the kit further comprises at least one calibrator reagent or control reagent comprising a sequence of monoclonal antibody CR3018 or CR3022.

Clause 214. The kit of any of clauses 206-213, wherein the at least one type of first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

Clause 215. The kit of any of clauses 206-214, wherein the at least one type of first specific binding partner is a receptor binding domain (RBD) of a spike protein that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

Clause 216. The kit of any of clauses 206-215, wherein the at least one type of first specific binding partner binds to (a) an anti-SARS-CoV-2 IgG antibody, (b) an anti-SARS-CoV-2 IgM antibody, or (c) combinations of (a) and (b).

Clause 217. The kit of any of clauses 206-216, wherein the kit is adapted for use with an automated or semi-automated system.

Clause 218. A system for detecting a presence or determining an amount of anti-SARS-CoV-2 antibody in a biological sample obtained from a subject comprising:

at least one type of first specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof selected from a C-terminal domain nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain nucleocapsid protein and a receptor binding domain (RBD) of a spike protein from a β-coronavirus that specifically

binds to at least one type of anti-SARS-CoV-2 antibody and at least one type of second specific binding partner comprising at least one detectable label; and

at least one device for detecting the at least one label from the complex, wherein the amount of signal from the label indicates the presence or amount of anti- SARS-CoV-2antibody in the sample.

Clause 219. The system of clause 218, wherein the at least one type of anti-SARS-CoV-2 antibody detected specifically binds to a variant:

a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

c) from a SARS-CoV-2 virus comprising any combination of a) and b).

Clause 220. The system of clause 218 or clause 219, wherein the device for detecting the label from the complex is automated or semi-automated.

Clause 221. The system of any of clauses 218-220, wherein the at least one type of anti-SARS-CoV-2 antibody is an IgG antibody, an IgM antibody, or any combination thereof.

Clause 222. The system of any of clauses 218-221, wherein the at least one type of second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an

anti-species IgM (e.g., anti-human-IgM IgG) antibody, or an anti-species IgG (e.g., anti-human-IgG IgG) antibody, and anti-species IgM (e.g., anti-human-IgM IgG) antibody.

Clause 223. The system of clauses 218-222, wherein the at least one type of first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

Clause 224. The system of any of clauses 218-223, wherein the at least one type of first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

Clause 225. The system of any of clauses 218-224, wherein the at least one type of first specific binding partner binds to (a) an anti-SARS-CoV-2 IgG antibody, (b) an anti-SARS-CoV-2 IgM antibody, or (c) combinations of (a) and (b).

Clause 226. In an improvement of a method of detecting at least one type of anti-SARS-CoV-2 antibody in a biological sample, wherein the method comprises detecting a complex comprising a first type of specific binding partner, said sample anti-SARS-CoV-2 antibody, and a second type of specific binding partner comprising at least one detectable label, wherein the improvement comprises using at least one type of first specific binding partner comprising at least one isolated polypeptide from a C-terminal domain of a β-coronavirus nucleocapsid protein or a variant thereof.

Clause 227. In the improvement of clause 226, wherein the at least one type of first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID

NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

Clause 228. In an improvement of a method of detecting at least one type of anti-SARS-CoV-2 antibody in a biological sample, wherein the method comprises detecting a complex comprising a first type of specific binding partner, said sample anti- SARS-CoV-2 antibody, and a second type of specific binding partner comprising at least one detectable label, wherein the improvement comprises using a first specific binding partner comprising at least one isolated polypeptide from a receptor binding domain (RBD) of a β-coronavirus spike protein or a variant thereof.

Clause 229. In the improvement of clause 228, wherein the at least one type of first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

Clause 230. In the improvement of clause 228 or clause 229, wherein the at least one first type of specific binding partner binds to (a) an anti-SARS-CoV-2 IgG antibody, (b) an anti-SARS-CoV-2 IgM antibody, or (c) combinations of (a) and (b).

Clause 231. The improvement of any of clauses 226-230, wherein the at least one type of anti-SARS-CoV-2 antibody detected specifically binds to a variant:

a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with

tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

c) from a SARS-CoV-2 virus comprising any combination of a) and b).

Clause 232. In an improvement of a method or system for determining an amount of an anti-SARS-CoV-2 IgG antibody in a subject based on the amount of detectable signal assessed, wherein the improvement comprises: (a) a linear assay range of up to 50,000 AU/mL; (b) determining an amount in arbitrary units rather than an index; and/or (c) improved detection of positive samples as compared to other assays.

Clause 233. A method of predicting outcome in a subject that is or was infected with SARS-CoV-2, the method comprising the steps of:

a) detecting an anti-SARS-CoV-2 IgG antibody in at least one biological sample obtained from the subject within a first ten days after onset of symptoms of SARS-CoV-2 infection;

b) detecting an anti-SARS-CoV-2 IgM antibody in the at least one biological sample obtained from the subject within the first ten days after onset of symptoms of SARS-CoV-2 infection;

c) determining which of the anti-SARS-CoV-2 IgG or anti-SARS-CoV-2 IgM antibody detected in a) and b) first appears in the subject;

d) predicting that the subject is more likely to have an unfavorable outcome if anti-SARS-CoV-2 IgG antibody first appears in the subject prior to the appearance of anti-SARS-CoV-2 IgM antibody; and

e) predicting that the subject is more likely to have a favorable outcome if anti-SARS-CoV-2 IgG antibody first appears in the subject at the same time or after the appearance of anti-SARS-CoV-2 IgM antibody.

Clause 234. The method of clause 233, wherein the subject is hospitalized for symptoms of SARS-CoV-2.

Clause 235. The method of clause 233 or clause 234, wherein (i) the anti-SARS-CoV-2 IgG antibody and the one anti-SARS-CoV-2 IgM antibody are detected in the same biological

sample; or (ii) the anti-SARS-CoV-2 IgG antibody and the anti-SARS-CoV-2 IgM antibody are detected in different biological samples.

Clause 236. The method of any of clauses 233-235, wherein the anti-SARS-2 IgG antibody and the anti-SARS-CoV-2 IgM antibody are (i) detected on the same day within the first ten days after onset of symptoms; (ii) detected on different days within the first ten days after the onset of symptoms.

Clause 237. The method any of clauses 233-236, wherein the anti-SARS-CoV-2 IgG antibody is detected in at least one biological sample obtained from the subject within the first day after the onset of symptoms, within the second day after the onset of symptoms, within the third day after the onset of symptoms, within the fourth day after the onset of symptoms, within the fifth day after the onset of symptoms, within the sixth day after the onset of symptoms, within the seventh date after the onset of symptoms, within the eighth day after the onset of symptoms or within the ninth day after the onset of symptoms.

Clause 238. The method of any of clauses 233-237, wherein the anti-SARS-CoV-2 IgM antibody is detected in at least one biological sample obtained from the subject within the first day after the onset of symptoms, within the second day after the onset of symptoms, within the third day after the onset of symptoms, within the fourth day of infection, within the fifth day after the onset of symptoms, within the sixth day after the onset of symptoms, within the seventh date after the onset of symptoms, within the eighth day after the onset of symptoms or within the ninth day after the onset of symptoms.

Clause 239. The method of any of clauses 233-238, wherein the unfavorable outcome is death.

Clause 240. A method of predicting outcome in a subject that is or was infected with SARS-CoV-2, the method comprising the steps of:

a) obtaining a signal-to-calibrator ratio (S/CO) from an assay of at least one anti-SARS-CoV-2 IgM antibody in at least one biological sample obtained from the subject at least ten days after onset of symptoms of SARS-CoV-2; and

b) predicting that the subject is more likely than not to have an unfavorable outcome if the S/CO ratio of anti-SARS-CoV-2 IgM antibody determined in the biological sample is equal to or greater than about 10 S/CO.

Clause 241. The method of clause 240, wherein the subject is hospitalized for symptoms of SARS-CoV-2.

Clause 242. The method of clause 240 or clause 241, further comprising that the subject is more likely than not to have an unfavorable outcome if the level of anti-SARS-CoV-2 IgM antibody determined in the biological sample is between about 10 S/CO to about 15 S/CO.

Clause 243. The method of any of clauses 240-242, wherein the method is selected from the group consisting of an immunoassay, or a clinical chemistry assay.

Clause 244. The method of any of clauses 240-243, wherein the method is performed using single molecule detection, lateral flow assay or a point-of-care assay.

Clause 245. A method of determining SARS-CoV-2 immune status of a subject, the method comprising the steps of:

a) determining an amount of at least one anti-SARS-CoV-2 IgG antibody and/or anti-SARS-CoV-2 IgG neutralizing antibody in at least one biological sample obtained from a subject; and
b) determining the subject's SARS-CoV-2 immune status, wherein the subject is determined to have immunity to SARS-CoV-2 when the amount of anti-SARS-CoV-2 IgG antibody and/or anti-SARS-CoV-2 IgG neutralizing antibody is (a) from at least about 550 BAU/mL to about 650 BAU/mL; and/or (b) from at least about 100 BAU/mL to about 490 BAU/mL,

wherein the method is performed irrespective of (i) the subject's prior infection and/or vaccination history with SARS-CoV-2, and/or (ii) whether there is any knowledge of the subject's prior infection and/or vaccination history with SARS-CoV-2.

Clause 246. A method of determining SARS-CoV-2 immune status of a subject, the method comprising the steps of:

a) determining an amount of at least one anti-SARS-CoV-2 IgG antibody and/or anti-SARS-CoV-2 IgG neutralizing antibody in at least one biological sample obtained from a subject; and

b) determining the subject's SARS-CoV-2 immune status, wherein the subject is determined to have immunity to SARS-CoV-2 when the amount of anti-SARS-CoV-2 IgG antibody and/or anti-SARS-CoV-2 IgG neutralizing antibody is from at least about 550 BAU/mL to about 650 BAU/mL,

wherein the method is performed irrespective of (i) the subject's prior infection and/or vaccination history with SARS-CoV-2, and/or (ii) whether there is any knowledge of the subject's prior infection and/or vaccination history with SARS-CoV-2.

Clause 247. A method of determining SARS-CoV-2 immune status of a subject, the method comprising the steps of:

a) determining an amount of at least one anti-SARS-CoV-2 IgG antibody and/or anti-SARS-CoV-2 IgG neutralizing antibody in at least one biological sample obtained from a subject; and

b) determining the subject's SARS-CoV-2 immune status, wherein the subject is determined to have immunity to SARS-CoV-2 when the amount of anti-SARS-CoV-2 IgG antibody and/or anti-SARS-CoV-2 IgG neutralizing antibody is from at least about 100 BAU/mL to about 490 BAU/mL,

wherein the method is performed irrespective of (i) the subject's prior infection and/or vaccination history with SARS-CoV-2, and/or (ii) whether there is any knowledge of the subject's prior infection and/or vaccination history with SARS-CoV-2.

Clause 248. The method of any of clauses 245-247, wherein the subject has not previously been infected with SARS-CoV-2.

Clause 249. The method of an of clauses 245-247, wherein the subject has previously been infected with SARS-CoV-2.

Clause 250. The method of any of clauses 245-249, wherein the subject has not previously been vaccinated for SARS-CoV-2.

Clause 251. The method of any of clauses 245-249, wherein the subject has previously been vaccinated for SARS-CoV-2.

Clause 252. The method of any of clauses 245-251, wherein the immune status of the subject is that the subject has immunity to SARS-CoV-2.

Clause 253. The method of any of clauses 245-252, wherein the immune status of the subject is that the subject does not have immunity to SARS-CoV-2.

Clause 254. The method of any of clauses 245-253, wherein the method is selected from the group consisting of an immunoassay, or a clinical chemistry assay.

Clause 255. The method of any of clauses 245-254, wherein the method is performed using single molecule detection, lateral flow assay or a point-of-care assay.

Clause 256. A system for assessing whether a subject is likely to have immunity from infection from SARS-CoV-2 in a biological sample obtained from a subject, the system comprising:

at least one type of first specific binding partner comprising at least one type of β-coronavirus isolated polypeptide or variant thereof selected from a C-terminal domain nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain nucleocapsid protein and a receptor binding domain (RBD) of a spike protein from at least one type β-coronavirus that specifically binds to at least one type of anti-SARS-CoV-2 antibody and at least one type of second specific binding partner comprising at least one detectable label;

at least one device for detecting the at least one label from the complex, wherein the amount of signal from the label indicates the presence or amount of anti-SARS-CoV-2 antibody in the sample; and

a means for assigning a differentiative rating indicating whether the subject is likely to have immunity from infection from SARS-CoV-2 based on the presence or amount of at least one type of anti-SARS-CoV-2 antibody detected in the sample.

Clause 257. The system of clause 256, wherein the differentiative rating is a color and/or number rating.

Clause 258. The system of clause 257, wherein the color and/or number rating is displayed on a mobile device through a mobile application.

Clause 259. The system of any of clauses 256-258, wherein the variant:

a) is of the C-terminal domain of a nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2

comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

b) is the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

c) comprises any combination of a) and b).

Clause 260. The system of any of clauses 256-259, wherein the device for detecting the label from the complex is automated or semi-automated.

Clause 261. The system of clause 260, wherein the device for detecting the label from the complex is semi-automated and comprises uploading the differentiative rating using a bar code.

Clause 262. The system of any of clauses 256-261, wherein the at least one type of anti-SARS-CoV-2 antibody is an anti-SARS-CoV-2 IgG antibody, an anti-SARS-CoV-2 IgM antibody, or any combination thereof.

Clause 263. The system of any of clauses 256-262, wherein the at least one type of second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM (e.g., anti-human-IgM IgG) antibody, or an anti-species IgG (e.g., anti-human-IgG IgG) antibody, and anti-species IgM (e.g., anti-human-IgM IgG) antibody.

Clause 264. The system of any of clauses 256-263, wherein the at least one type of first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

Clause 265. The system of any of clauses 256-264, wherein the at least one type of first specific binding partner binds to (a) an anti-SARS-CoV-2 IgG antibody, (b) an anti-SARS-CoV-2 IgM antibody, or (c) combinations of (a) and (b).

What is claimed is:

1. A method for detecting a presence or determining an amount of at least one type of anti-SARS-CoV-2 antibody in a subject, the method comprising the steps of:

a) contacting at least one biological sample from the subject, either simultaneously or sequentially, in any order, with

at least one type of first specific binding partner comprising at least one type of β-coronavirus isolated polypeptide or variant thereof, selected from a C-terminal domain of a nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain of a nucleocapsid protein and a receptor binding domain (RBD) of a spike protein, wherein the polypeptide specifically binds to at least one type of anti-SARS-CoV-2 antibody in the sample, and

at least one type of second specific binding partner comprising a detectable label,

thereby producing one or more types of first complexes comprising the first specific binding partner-anti-SARS-CoV-2 antibody-second specific binding partner; and

b) assessing a signal from the one or more types of first complexes, wherein the amount of detectable signal from the detectable label indicates the presence or amount of at least one type of anti-β-coronavirus antibody in the sample.

2. The method of claim 1, wherein the at least one type of anti-SARS-CoV-2 antibody specifically binds to a variant:

a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine

with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

c) from a SARS-CoV-2 virus comprising any combination of a) and b).

3. The method of claims 1 or 2, wherein the biological sample is whole blood, serum, plasma, saliva, a nasal mucus specimen, an anal swab specimen, an oropharyngeal specimen, or a nasopharyngeal specimen.

4. The method of any of claims 1-3, wherein the at least one type of anti-SARS-CoV-2 antibody is an IgG antibody, an IgM antibody, or IgG antibody and an IgM antibody.

5. The method of any of claims 1-4, wherein the at least one type of first specific binding partner comprises at least one isolated polypeptide from a C-terminal domain of a nucleocapsid protein from a β-coronavirus, wherein the method is carried out so that said specific binding partner specifically binds to (a) an anti-β-coronavirus IgG antibody; (b) an anti-β-coronavirus IgM antibody; or (b) both an anti-β-coronavirus IgG and an anti-β-coronavirus IgM antibody.

6. The method of any of claims 1-4, wherein the at least one type of first specific binding partner comprises at least one isolated polypeptide from a receptor binding domain (RBD) of a spike protein from a β-coronavirus, wherein the method is carried out so that said specific binding partner specifically binds to (a) an anti-β-coronavirus IgG antibody; (b) an anti-β-coronavirus IgM antibody; or (b) both an anti-β-coronavirus IgG antibody and anti-β-coronavirus IgM antibody.

7. The method of any of claims 1-6, wherein the at least one type of second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM (e.g., anti-human-IgM IgG) antibody, or an anti-species IgG (e.g., anti-human-IgG IgG) and an anti-species IgM (e.g., anti-human-IgM IgG) antibody.

8. The method of any of claims 1-7, wherein the at least one type of first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that

(1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

9. The method of any of claims 1-4 or 6, wherein the at least one type of first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

10. The method of claim 8 or claim 9, wherein the method is carried out so that the at least one type of first specific binding partner binds to (a) an anti-SARS-CoV-2 IgG antibody; (b) an anti-SARS CoV-2 IgM antibody; or (c) both an anti-SARS-CoV-2 IgG antibody and anti-SARS-CoV-2 IgM antibody.

11. The method of any of claims 1-10, wherein the method comprises detecting:

(a) at least one anti-SARS-CoV-2 IgG antibody in at least one biological sample obtained from a subject wherein the at least one type of first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2 and specifically binds to at least one anti-SARS-CoV-2 IgG antibody; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2, and/or

(b) at least one anti-SARS-CoV-2 IgM antibody in at least one biological sample obtained in a subject wherein the at least one first specific binding partner wherein the at least one first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17 and specifically binds to at least one anti-SARS-CoV-2 IgM antibody; and

wherein the at least one anti-SARS-CoV-2 IgG antibody and/or at least one anti-SARS-CoV-2 IgM antibody are detected in a single biological sample obtained from the subject or in multiple biological samples obtained from the subject; and

further wherein, when the at least one anti-SARS-CoV-2 IgG antibody and at least one anti-SARS-CoV-2 IgM antibody are detected simultaneously or sequentially, in any order, in a single biological sample obtained from the subject or in multiple biological samples obtained from the subject.

12. The method of any of claims 4-11, wherein the method quantifies up to (a) about 91% or (b) about 99% of anti-SARS-CoV-2 IgG antibodies.

13. The method of any of claims 1-12, wherein the method further comprises detecting SARS-CoV-2 from at least one biological sample obtained from the subject, said at least one biological sample being a single biological sample or multiple biological samples.

14. The method of claim 13, wherein the SARS-CoV-2 detected comprises a variant:

a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

c) from a SARS-CoV-2 virus comprising any combination of a) and b).

15. The method of any of claims 12-14, wherein the SARS-CoV-2 is detected by its viral RNA using polymerase chain reaction, or by its viral antigen.

16. The method of any of claims 12-14, wherein said multiple biological samples are obtained at the same or different times.

17. The method of any of claims 12-16, wherein the method further comprises detecting whether the subject is in:

(a) an initial period of infection without any antibodies being produced, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen, negative for anti-SARS-CoV-2 IgM antibodies, and negative for anti-SARS-CoV-2 IgG antibodies; and/or

(b) an early acute phase of infection, and is developing an immune response to the virus and producing antibodies, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen, positive for an anti-SARS-CoV-2 IgM antibodies, and negative for anti-SARS-CoV-2 IgG antibodies; and/or

(c) an early acute phase of infection or had a false negative viral RNA result or viral antigen result, or a false positive anti-SARS-CoV-2 IgM antibody result, when the at least one biological sample obtained from the subject is negative for viral RNA, positive for anti-SARS-CoV-2 IgM antibodies, and negative for anti-SARS-CoV-2 IgG antibodies; and/or

(d) an acute phase of infection, and is progressing in an immune response, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen, positive for anti-β-coronavirus IgM antibodies, and positive for anti-SARS-CoV-2 IgG antibodies; and/or

(e) a late acute phase of infection, or has developed a recurrent infection with SARS-CoV-2, when the at least one biological sample obtained from the subject is positive for viral RNA or viral antigen, negative for anti-SARS-CoV-2 IgM antibodies, and positive for anti-SARS-CoV-2 IgG antibodies;

(f) a late acute phase of infection or recovery phase, or had a false negative viral RNA result, when the at least one biological sample obtained from the subject is negative for viral RNA or viral antigen, positive for anti-β-coronavirus IgM antibodies, and positive for anti-SARS-CoV-2 IgG antibodies;

(g) recovery phase when the at least one biological sample obtained from the subject is negative for viral RNA or viral antigen, negative for anti-SARS-CoV-2 IgM antibodies and positive for anti-SARS-CoV-2 IgG antibodies; and/or

(h) recovery phase when the at least one biological sample obtained from the subject is negative for viral RNA or viral antigen and negative for anti-SARS-CoV-2 IgM antibodies and anti-SARS-CoV-2 IgG antibodies.

18. The method any of claims 1-17, wherein the method further comprises a pre-treatment step done at the same time as, or prior to, contacting the at least one type of first specific binding partner, the at least one type of second specific binding partner, or the at least one type of first specific binding partner and the at least one type of second specific binding partner, with the biological sample, and wherein the pretreatment step optionally comprises treatment with anti-human IgG, anti-human IgM, or anti-human IgG and anti-human IgM.

19. The method of any of claims 1-18, wherein the at least one type of first specific binding partner is immobilized on a solid support.

20. The method of any of claims 1-19, wherein the method is performed in from about 5 to about 20 minutes, less than about 20 minutes, and optionally is performed in less than about 5 minutes, less than about 10 minutes or less than about 15 minutes.

21. The method of any of claims 1-20, wherein the method further comprises use with at least one calibrator reagent, at least one control reagent, or at least one calibrator reagent and at least one control reagent.

22. The method of any of claims 1-21, wherein the method further employs use of at least one calibrator reagent or a control reagent comprising a sequence of monoclonal antibody CR3018 or CR3022.

23. The method of any of claims 1-22, wherein the method is selected from the group consisting of an immunoassay, or a clinical chemistry assay.

24. The method of any of claims 1-23, wherein the method is performed using single molecule detection, lateral flow assay, or a point-of-care assay.

25. The method of any of claims 1-24, wherein the method further comprises identifying a subject having one or more anti-SARS-CoV-2 IgG and/or anti-SARS-CoV-2 IgM antibodies as a candidate subject to provide a biological sample for use in convalescent therapy againstSARS-CoV-2.

26. The method of claim 25, wherein the subject is identified as a candidate to provide a biological sample for use in convalescent therapy if the level of one or more SARS-CoV-2 IgG and/or SARS-CoV-2 IgM antibodies is from at least about 100 BAU/mL to about 490 BAU/mL; at least about 110 BAU/mL to about 490 BAU/mL; at least about 200 BAU/mL to about 490 BAU/mL; at least from about 300 BAU mL to about 490 BAU/mL; at least about 400 BAU mL to about 490 BAU/mL; at least about 100 BAU/mL; at least about 110 BAU/mL; at least about 120 BAU/mL; at least about 130 BAU/mL; at least about 140 BAU/mL; at least about 150 BAU/mL; at least about 160 BAU/mL; at least about 170 BAU/mL; at least about 180 BAU/mL; at least about 190 BAU/mL; at least about 200 BAU/mL; at least about 210 BAU/mL; at least about 220 BAU/mL; at least about 230 BAU/mL; at least about 240 BAU/mL; at least about 250 BAU/mL; at least about 260 BAU/mL; at least about 270 BAU/mL; at least about 280 BAU/mL; at least about 290 BAU/mL; at least about 300 BAU/mL; at least about 300 BAU/mL; at least about 310 BAU/mL; at least about 320 BAU/mL; at least about 330 BAU/mL; at least about 340 BAU/mL; at least about 350 BAU/mL; at least about 360 BAU/mL; at least about 370 BAU/mL; at least about 380 BAU/mL; at least about 390 BAU/mL; at least about 400 BAU/mL; at least about 400 BAU/mL; at least about 410 BAU/mL; at least about 420 BAU/mL; at least about 430 BAU/mL; at least about 440 BAU/mL; at least about 450 BAU/mL; at least about 460 BAU/mL; at least about 470 BAU/mL; at least about 480 BAU/mL; or at least about 490 BAU/mL.

27. The method of claim 26, wherein the subject is identified as a candidate to provide a biological sample for use in convalescent therapy if the level of one or more SARS-CoV-2 IgG and/or anti-β-coronavirus IgM antibodies is from at least about 500 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 500 BAU/mL; from at least about 510 BAU/mL; at least about 520 BAU/mL; at least about 530 BAU/mL; at least about 540 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL; at least about 560 BAU/mL; at least about 570 BAU/mL; at least about 580 BAU/mL; at least about 590 BAU/mL; at least about 600 BAU/mL; at least about 610 BAU/mL; at least about 620 BAU/mL; at least about 630 BAU/mL; at least about 639 BAU/mL; at least about 640 BAU/mL; or at least about 650 BAU/mL.

28. The method of any of claims 1-27, wherein the method:

(a) further comprises detecting at least one type of anti-SARS-CoV-2 IgG neutralizing antibody;

(b) demonstrates high qualitative agreement with a plaque reduction neutralization assay;

(c) detects increasing amounts of anti-SARS-CoV-2 IgG antibodies as detected by the method that are associated with increasing amounts of anti-SARS-CoV-2 IgG neutralizing antibodies;

(d) has a probability profile that corresponds to high titer levels in the plaque reduction neutralization assay such that there is a high probability of the levels of anti-SARS-CoV-2 IgG antibodies determined by the method being at or above the levels of anti-SARS-CoV-2 IgG neutralizing antibodies determined in the plaque reduction neutralization assay; or

(e) demonstrates high qualitative agreement with an ACE2 binding inhibition assay.

29. The method of claim 28, wherein a level of anti-SARS-CoV-2 IgG antibodies of at least about 4160 AU/mL (about 590 BAU/mL) when used as a cut-off or threshold correspond to about a 0.95 probability of obtaining a plaque reduction neutralization assay ID50 at 1:250 dilution.

30. The method of claim 28, wherein the anti-SARS-CoV-2 neutralizing antibody is an anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof.

31. The method of any of claims 1-30, wherein the method comprises obtaining the at least one biological sample from the subject at a critical time of from about fourteen (14) days to about thirty-five (35) days after onset of symptoms of SARS-CoV-2.

32. The method of claim 31, wherein the method further comprises determining that the subject: (i) more likely than not will develop or experience at least one of a cytokine storm, acute respiratory distress syndrome (ARDS), or a combination of a cytokine storm and ARDS if anti-SARS-CoV-2 IgM antibody, anti-SARS-CoV-2 IgG antibody, or anti-SARS-CoV-2 IgM antibody and anti-SARS-CoV-2 IgG antibody are not detected in the biological sample within the critical time; or (ii) more likely than not will not develop or experience at least one of a cytokine storm, ARDS, or a combination of a cytokine storm and ARDS if anti-SARS-CoV-2 IgM antibody, anti-SARS-CoV-2 IgG antibody, or anti-SARS-CoV-2 IgM antibody and anti-SARS-CoV-2 IgG antibody are not detected in the biological sample within the critical time.

33. The method of any of claims 1-32, wherein the subject: (1) is naïve and was not previously vaccinated against SARS-CoV-2; (2) is naïve and was previously vaccinated against

SARS-CoV-2; (3) is currently infected with SARS-CoV-2 and was not previously vaccinated against SARS-CoV-2; (4) is currently infected with SARS-CoV-2 and was previously vaccinated against SARS-CoV-2; (5) was previously infected with SARS-CoV-2, recovered, and was not previously vaccinated against SARS-CoV-2; or (6) was previously infected with SARS-CoV-2, recovered, and then was vaccinated against SARS-CoV-2.

34. The method of claim 33, wherein the method is performed to: (a) determine whether the subject can be administered a current vaccine for SARS-CoV-2; or (b) monitor the subject following the current vaccine or previous vaccination, based on detecting the presence of at least one type of anti-SARS-CoV-2 antibody in the sample.

35. The method of claim 34, performed regardless of variation in timing and/or severity of prior infection with SARS-CoV-2.

36. The method of any of claim 34 or 35, wherein the method comprises determining that the current vaccine for SARS-CoV-2:

(a) can be administered to the subject when no anti-SARS-CoV-2 antibodies, no anti-SARS-CoV-2 IgG neutralizing antibodies, or an amount of anti-SARS-CoV-2 IgG antibody or anti-SARS-CoV-2 IgG neutralizing antibody that is insufficient to impart immunity, are detected in the biological sample; and/or

(b) should not be administered to the subject if an amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 IgG neutralizing antibody that is sufficient to impart immunity is detected in the biological sample.

37. The method of claim 36, wherein the amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 IgG neutralizing antibody sufficient to impart immunity is from at least about 100 BAU/mL to about 490 BAU/mL; at least about 110 BAU/mL to about 490 BAU/mL; at least about 200 BAU/mL to about 490 BAU/mL; at least from about 300 BAU mL to about 490 BAU/mL; at least about 400 BAU mL to about 490 BAU/mL; at least about 100 BAU/mL; at least about 110 BAU/mL; at least about 120 BAU/mL; at least about 130 BAU/mL; at least about 140 BAU/mL; at least about 150 BAU/mL; at least about 160 BAU/mL; at least about 170 BAU/mL; at least about 180 BAU/mL; at least about 190 BAU/mL; at least about 200 BAU/mL; at least about 210 BAU/mL; at least about 220 BAU/mL; at least about 230 BAU/mL; at least about 240 BAU/mL; at least about 250 BAU/mL; at least about 260 BAU/mL; at least about 270

BAU/mL; at least about 280 BAU/mL; at least about 290 BAU/mL; at least about 300 BAU/mL; at least about 300 BAU/mL; at least about 310 BAU/mL; at least about 320 BAU/mL; at least about 330 BAU/mL; at least about 340 BAU/mL; at least about 350 BAU/mL; at least about 360 BAU/mL; at least about 370 BAU/mL; at least about 380 BAU/mL; at least about 390 BAU/mL; at least about 400 BAU/mL; at least about 400 BAU/mL; at least about 410 BAU/mL; at least about 420 BAU/mL; at least about 430 BAU/mL; at least about 440 BAU/mL; at least about 450 BAU/mL; at least about 460 BAU/mL; at least about 470 BAU/mL; at least about 480 BAU/mL; or at least about 490 BAU/mL.

38. The method of claim 36, wherein the amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 IgG neutralizing antibody sufficient to impart immunity is: (a) from at least about 550 BAU/mL to about 650 BAU/mL; (b) at least about 550 BAU/mL; (c) at least about 560 BAU/mL; (d) at least about 570 BAU/mL; (e) at least about 580 BAU/mL; (f) at least about 590 BAU/mL; (g) at least about 600 BAU/mL; (h) at least about 610 BAU/mL; (i) at least about 620 BAU/mL; (j) at least about 630 BAU/mL; (k) at least about 639 BAU/mL; (l) at least about 640 BAU/mL; or (m) at least about 650 BAU/mL.

39. The method of claim 34, wherein the method comprises obtaining the biological sample within a time frame after the subject has received either the current vaccine or previous vaccination for SARS-CoV-2 of at least one day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, at least 15 days, at least 16 days, at least 17 days, at least 18 days, at least 19 days, at least 20 days, at least 21 days, at least 22 days, at least 23 days, at least 24 days, at least 25 days, at least 26 days, at least 27 days, at least 28 days, at least 29 days, at least 30 days, at least 31 days, at least 32 days, at least 33 days, at least 34 days, at least 35 days, at least 36 days, at least 37 days, at least 38 days, at least 39 days, at least 40 days, at least 41 days, at least 42 days, at least 43 days, at least 44 days, at least 45 days, at least 46 days, at least 47 days, at least 48 days, at least 49 days, or at least 50 days.

40. The method of claim 34, wherein the biological sample is obtained within about 7 to about 21 days after the subject has received either the current vaccine or previous vaccination.

41. The method of claim 39 or claim 40, wherein the method comprises determining that at least one further vaccine for SARS-CoV-2:

(a) can be administered to the subject after the current vaccine if no anti-SARS-CoV-2 antibodies, no anti-SARS-CoV-2 IgG neutralizing antibodies, and/or an amount of anti-SARS-CoV-2 IgG antibody or anti-SARS-CoV-2 IgG neutralizing antibody that is insufficient to impart immunity, is detected in the biological sample obtained from the subject; or

(b) should not be administered to the subject after the current vaccine if an amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 IgG neutralizing antibody that is sufficient to impart immunity is detected in the biological sample obtained from the subject.

42. The method of claim 41, wherein the amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 IgG neutralizing antibody sufficient to impart immunity is from at least about 100 BAU/mL to about 490 BAU/mL; at least about 110 BAU/mL to about 490 BAU/mL; at least about 200 BAU/mL to about 490 BAU/mL; at least from about 300 BAU mL to about 490 BAU/mL; at least about 400 BAU mL to about 490 BAU/mL; at least about 100 BAU/mL; at least about 110 BAU/mL; at least about 120 BAU/mL; at least about 130 BAU/mL; at least about 140 BAU/mL; at least about 150 BAU/mL; at least about 160 BAU/mL; at least about 170 BAU/mL; at least about 180 BAU/mL; at least about 190 BAU/mL; at least about 200 BAU/mL; at least about 210 BAU/mL; at least about 220 BAU/mL; at least about 230 BAU/mL; at least about 240 BAU/mL; at least about 250 BAU/mL; at least about 260 BAU/mL; at least about 270 BAU/mL; at least about 280 BAU/mL; at least about 290 BAU/mL; at least about 300 BAU/mL; at least about 300 BAU/mL; at least about 310 BAU/mL; at least about 320 BAU/mL; at least about 330 BAU/mL; at least about 340 BAU/mL; at least about 350 BAU/mL; at least about 360 BAU/mL; at least about 370 BAU/mL; at least about 380 BAU/mL; at least about 390 BAU/mL; at least about 400 BAU/mL; at least about 400 BAU/mL; at least about 410 BAU/mL; at least about 420 BAU/mL; at least about 430 BAU/mL; at least about 440 BAU/mL; at least about 450 BAU/mL; at least about 460 BAU/mL; at least about 470 BAU/mL; at least about 480 BAU/mL; or at least about 490 BAU/mL.

43. The method of claim 41, wherein the amount of anti-SARS-CoV-2 antibody or anti-SARS-CoV-2 IgG neutralizing antibody sufficient to impart immunity is from at least about 500 BAU/mL to about 650 BAU/mL; at least about 550 BAU/mL to about 650 BAU/mL; at least about 500 BAU/mL; from at least about 510 BAU/mL; at least about 520 BAU/mL; at least about 530 BAU/mL; at least about 540 BAU/mL; at least about 550 BAU/mL to about 650

BAU/mL; at least about 550 BAU/mL; at least about 560 BAU/mL; at least about 570 BAU/mL; at least about 580 BAU/mL; at least about 590 BAU/mL; at least about 600 BAU/mL; at least about 610 BAU/mL; at least about 620 BAU/mL; at least about 630 BAU/mL; at least about 639 BAU/mL; at least about 640 BAU/mL; or at least about 650 BAU/mL.

44. The method of any of claims 28, 30, 36, 37, 38, 41, 42 or 43 wherein the anti-SARS-CoV-2 neutralizing antibody is an anti-SARS-CoV-2 IgG antibody that is an anti-nucleocapsid (N) antibody, an anti-spike antibody, or any combination thereof.

45. The method of any of claims 1-44, wherein the method further comprises (a) monitoring the subject for at least one type of anti- SARS-CoV-2 antibody, optionally, IgG and/or IgM antibodies; (b) treating the subject for SARS-CoV-2; (c) monitoring the subject for at least one type of anti- SARS-CoV-2, optionally, IgG and/or IgM antibodies and treating the subject for SARS-CoV-2; or (d) treating the subject for a SARS-CoV-2 and monitoring the subject for at least one type of anti- SARS-CoV-2 antibody, optionally, IgG and/or IgM antibodies.

46. The method of any of claims 1-45, wherein the volume of the biological sample used in the method is from about 0.30 μL to about 0.40 μL.

47. The method of any of claims 1-46, wherein the method comprises (a) diluting the biological sample prior to or during the method and further wherein the diluting comprises mixing about 10 μL of the biological sample with about 290 μL of at least one buffer to form a biological sampling mixture; and (b) removing about 10 μL from the biological sampling mixture for use in the method.

48. The method of any of claims 1-47, wherein the method is adapted for use in an automated system or a semi-automated system.

49. A kit for performing the method of claim 1, wherein the kit comprises:

a. at least one type of specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof selected from a C-terminal domain nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain nucleocapsid protein and a receptor binding domain (RBD) of a spike protein from a β-coronavirus; and

b. at least one type of second specific binding partner comprising at least one detectable label.

50. The kit of claim 49, wherein the at least one type of anti-SARS-CoV-2 antibody detected or determined specifically binds to a variant:

a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

c) from a SARS-CoV-2 virus comprising any combination of a) and b).

51. The kit of claim 49 or claim 50, wherein the kit further comprises, or is configured to be used with, at least one calibrator reagent, at least one control reagent, or at least one calibrator reagent and at least one control reagent.

52. The kit of any of claims 49-51, wherein the kit further comprises at least one solid support.

53. The kit of any of claims 49-52, wherein the kit further comprises, or is configured to be used with, at least one pretreatment reagent.

54. The kit of any of claims 49-53, wherein the isolated polypeptide is a fusion polypeptide.

55. The kit of any of claims 49-54, wherein the at least one type of second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM

(e.g., anti-human-IgM IgG) antibody, or an anti-species IgG (e.g., anti-human-IgG IgG) antibody and an anti-species IgM (e.g., anti-human-IgM IgG) antibody.

56. The kit of any of claims 49-55, wherein the kit further comprises at least one calibrator reagent or control reagent comprising a sequence of monoclonal antibody CR3018 or CR3022.

57. The kit of any of claims 49-56, wherein the at least one type of first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

58. The kit of any of claims 49-57, wherein the at least one type of first specific binding partner is a receptor binding domain (RBD) of a spike protein that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

59. The kit of any of claims 49-58, wherein the at least one type of first specific binding partner binds to (a) an anti-SARS-CoV-2 IgG antibody, (b) an anti-SARS-CoV-2 IgM antibody, or (c) combinations of (a) and (b).

60. The kit of any of claims 49-59, wherein the kit is adapted for use with an automated or semi-automated system.

61. A system for detecting a presence or determining an amount of anti-SARS-CoV-2 antibody in a biological sample obtained from a subject comprising:

at least one type of first specific binding partner comprising at least one β-coronavirus isolated polypeptide or variant thereof selected from a C-terminal domain nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain nucleocapsid protein and a receptor binding domain (RBD) of a spike protein from a β-coronavirus that specifically binds to at least one type of anti-SARS-CoV-2 antibody and at least one type of second specific binding partner comprising at least one detectable label; and

at least one device for detecting the at least one label from the complex, wherein the amount of signal from the label indicates the presence or amount of anti- SARS-CoV-2antibody in the sample.

62. The system of claim 61, wherein the at least one type of anti-SARS-CoV-2 antibody detected specifically binds to a variant:

a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

c) from a SARS-CoV-2 virus comprising any combination of a) and b).

63. The system of claim 61 or claim 62, wherein the device for detecting the label from the complex is automated or semi-automated.

64. The system of any of claims 61-63, wherein the at least one type of anti-SARS-CoV-2 antibody is an IgG antibody, an IgM antibody, or any combination thereof.

65. The system of any of claims 61-64, wherein the at least one type of second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM (e.g., anti-human-IgM IgG) antibody, or an anti-species IgG (e.g., anti-human-IgG IgG) antibody, and anti-species IgM (e.g., anti-human-IgM IgG) antibody.

66. The system of claims 61-65, wherein the at least one type of first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

67. The system of any of claims 61-66, wherein the at least one type of first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

68. The system of any of claims 61-67, wherein the at least one type of first specific binding partner binds to (a) an anti-SARS-CoV-2 IgG antibody, (b) an anti-SARS-CoV-2 IgM antibody, or (c) combinations of (a) and (b).

69. In an improvement of a method of detecting at least one type of anti-SARS-CoV-2 antibody in a biological sample, wherein the method comprises detecting a complex comprising a first type of specific binding partner, said sample anti-SARS-CoV-2 antibody, and a second type of specific binding partner comprising at least one detectable label, wherein the improvement comprises using at least one type of first specific binding partner comprising at least one isolated polypeptide from a C-terminal domain of a β-coronavirus nucleocapsid protein or a variant thereof.

70. In the improvement of claim 69, wherein the at least one type of first specific binding partner is a C-terminal domain nucleocapsid protein or variant thereof that (1) has an amino acid sequence of SEQ ID NO:1; (2) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQNQ (SEQ ID NO:21), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; (3) is a fusion protein comprising an initiator amino acid methionine and the sequences GPQSNQ (SEQ ID NO:22), RSAPRITFG (SEQ ID NO:7), GPTDST (SEQ ID NO:23), and amino acids 211 to 419 of SEQ ID NO:2; or (4) has an amino acid sequence of amino acids 210 to 419 of SEQ ID NO:2.

71. In an improvement of a method of detecting at least one type of anti- SARS-CoV-2 antibody in a biological sample, wherein the method comprises detecting a complex

comprising a first type of specific binding partner, said sample anti- SARS-CoV-2 antibody, and a second type of specific binding partner comprising at least one detectable label, wherein the improvement comprises using a first specific binding partner comprising at least one isolated polypeptide from a receptor binding domain (RBD) of a β-coronavirus spike protein or a variant thereof.

72. In the improvement of claim 71, wherein the at least one type of first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

73. In the improvement of claim 70 or claim 71, wherein the at least one first type of specific binding partner binds to (a) an anti-SARS-CoV-2 IgG antibody, (b) an anti-SARS-CoV-2 IgM antibody, or (c) combinations of (a) and (b).

74. The improvement of any of claims 69-73, wherein the at least one type of anti-SARS-CoV-2 antibody detected specifically binds to a variant:

a) of the nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

b) of the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

c) from a SARS-CoV-2 virus comprising any combination of a) and b).

75. In an improvement of a method or system for determining an amount of an anti-SARS-CoV-2 IgG antibody in a subject based on the amount of detectable signal assessed, wherein the improvement comprises: (a) a linear assay range of up to 50,000 AU/mL; (b) determining an amount in arbitrary units rather than an index; and/or (c) improved detection of positive samples as compared to other assays.

76. A method of predicting outcome in a subject that is or was infected with SARS-CoV-2, the method comprising the steps of:

a) detecting an anti-SARS-CoV-2 IgG antibody in at least one biological sample obtained from the subject within a first ten days after onset of symptoms of SARS-CoV-2 infection;

b) detecting an anti-SARS-CoV-2 IgM antibody in the at least one biological sample obtained from the subject within the first ten days after onset of symptoms of SARS-CoV-2 infection;

c) determining which of the anti-SARS-CoV-2 IgG or anti-SARS-CoV-2 IgM antibody detected in a) and b) first appears in the subject;

d) predicting that the subject is more likely to have an unfavorable outcome if anti-SARS-CoV-2 IgG antibody first appears in the subject prior to the appearance of anti-SARS-CoV-2 IgM antibody; and

e) predicting that the subject is more likely to have an favorable outcome if anti-SARS-CoV-2 IgG antibody first appears in the subject at the same time or after the appearance of anti-SARS-CoV-2 IgM antibody.

77. The method of claim 76, wherein the subject is hospitalized for symptoms of SARS-CoV-2.

78. The method of claim 76 or claim 77, wherein (i) the anti-SARS-CoV-2 IgG antibody and the one anti-SARS-CoV-2 IgM antibody are detected in the same biological sample; or (ii) the anti-SARS-CoV-2 IgG antibody and the anti-SARS-CoV-2 IgM antibody are detected in different biological samples.

79. The method of any of claims 76-78, wherein the anti-SARS-2 IgG antibody and the anti-SARS-CoV-2 IgM antibody are (i) detected on the same day within the first ten days

after onset of symptoms; (ii) detected on different days within the first ten days after the onset of symptoms.

80. The method any of claims 76-79, wherein the anti-SARS-CoV-2 IgG antibody is detected in at least one biological sample obtained from the subject within the first day after the onset of symptoms, within the second day after the onset of symptoms, within the third day after the onset of symptoms, within the fourth day after the onset of symptoms, within the fifth day after the onset of symptoms, within the sixth day after the onset of symptoms, within the seventh date after the onset of symptoms, within the eighth day after the onset of symptoms or within the ninth day after the onset of symptoms.

81. The method of any of claims 76-80, wherein the anti-SARS-CoV-2 IgM antibody is detected in at least one biological sample obtained from the subject within the first day after the onset of symptoms, within the second day after the onset of symptoms, within the third day after the onset of symptoms, within the fourth day of infection, within the fifth day after the onset of symptoms, within the sixth day after the onset of symptoms, within the seventh date after the onset of symptoms, within the eighth day after the onset of symptoms or within the ninth day after the onset of symptoms.

82. The method of any of claims 76-81, wherein the unfavorable outcome is death.

83. A method of predicting outcome in a subject that is or was infected with SARS-CoV-2, the method comprising the steps of:

a) obtaining a signal-to-calibrator ratio (S/CO) from an assay of at least one anti-SARS-CoV-2 IgM antibody in at least one biological sample obtained from the subject at least ten days after onset of symptoms of SARS-CoV-2; and

b) predicting that the subject is more likely than not to have an unfavorable outcome if the S/CO ratio of anti-SARS-CoV-2 IgM antibody determined in the biological sample is equal to or greater than about 10 S/CO.

84. The method of claim 83, wherein the subject is hospitalized for symptoms of SARS-CoV-2.

85. The method of claim 83 or claim 84, further comprising that the subject is more likely than not to have an unfavorable outcome if the level of anti-SARS-CoV-2 IgM antibody determined in the biological sample is between about 10 S/CO to about 15 S/CO.

86. The method of any of claims 76-85, wherein the method is selected from the group consisting of an immunoassay, or a clinical chemistry assay.

87. The method of any of claims 76-86, wherein the method is performed using single molecule detection, lateral flow assay or a point-of-care assay.

88. A method of determining SARS-CoV-2 immune status of a subject, the method comprising the steps of:

a) determining an amount of at least one anti-SARS-CoV-2 IgG antibody and/or anti-SARS-CoV-2 IgG neutralizing antibody in at least one biological sample obtained from a subject; and

b) determining the subject's SARS-CoV-2 immune status, wherein the subject is determined to have immunity to SARS-CoV-2 when the amount of anti-SARS-CoV-2 IgG antibody and/or anti-SARS-CoV-2 IgG neutralizing antibody is (a) from at least about 550 BAU/mL to about 650 BAU/mL; or (b) from at least about 100 BAU/mL to about 490 BAU/mL,

wherein the method is performed irrespective of (i) the subject's prior infection and/or vaccination history with SARS-CoV-2, and/or (ii) whether there is any knowledge of the subject's prior infection and/or vaccination history with SARS-CoV-2.

89. The method of claim 88, wherein the subject has not previously been infected with SARS-CoV-2.

90. The method of claim 88, wherein the subject has previously been infected with SARS-CoV-2.

91. The method of any of claims 88-90, wherein the subject has not previously been vaccinated for SARS-CoV-2.

92. The method of any of claims 88-90, wherein the subject has previously been vaccinated for SARS-CoV-2.

93. The method of any of claims 88-92, wherein the immune status of the subject is that the subject has immunity to SARS-CoV-2.

94. The method of any of claims 88-92, wherein the immune status of the subject is that the subject does not have immunity to SARS-CoV-2.

95. The method of any of claims 88-94, wherein the method is selected from the group consisting of an immunoassay, or a clinical chemistry assay.

96. The method of any of claims 88-95, wherein the method is performed using single molecule detection, lateral flow assay or a point-of-care assay.

97. A system for assessing whether a subject is likely to have immunity from infection from SARS-CoV-2 in a biological sample obtained from a subject, the system comprising:

at least one type of first specific binding partner comprising at least one type of β-coronavirus isolated polypeptide or variant thereof selected from a C-terminal domain nucleocapsid protein, a receptor binding domain (RBD) of a spike protein, or a C-terminal domain nucleocapsid protein and a receptor binding domain (RBD) of a spike protein from at least one type β-coronavirus that specifically binds to at least one type of anti-SARS-CoV-2 antibody and at least one type of second specific binding partner comprising at least one detectable label;

at least one device for detecting the at least one label from the complex, wherein the amount of signal from the label indicates the presence or amount of anti-SARS-CoV-2 antibody in the sample; and

a means for assigning a differentiative rating indicating whether the subject is likely to have immunity from infection from SARS-CoV-2 based on the presence or amount of at least one type of anti-SARS-CoV-2 antibody detected in the sample.

98. The system of claim 97, wherein the differentiative rating is a color and/or number rating.

99. The system of claim 98, wherein the color and/or number rating is displayed on a mobile device through a mobile application.

100. The system of any of claims 97-99, wherein the variant:

a) is of the C-terminal domain of a nucleocapsid protein having one or more substitutions, deletions or a substitution and deletion at positions 210-419 of SEQ ID NO:2 comprising: (1) replacing serine with phenylalanine at amino acid position 235 (S235F); (2) replacing methionine with isoleucine at amino acid position 234 (M234I); (3) replacing lysine

with asparagine at amino acid position 373 (K373N); (4) replacing aspartic acid with tyrosine at amino acid position 377 (D377Y); (5) replacing alanine with threonine at amino acid position 376 (A376T); or (6) any combinations of (1)-(5), either alone or combined with any other substitutions and/or deletions in amino acids 210-419 of SEQ ID NO:2 other than those recited in (1)-(5);

b) is the RBD of a spike protein having one or more substitutions, deletions or a substitution and deletion at positions 319-542 of SEQ ID NO:15 comprising (1) replacing lysine with asparagine at amino acid position 417 (K417N); (2) replacing lysine with threonine at amino acid position 417 (K417T); (3) replacing leucine with arginine at amino acid position 452 (L452R); (4) replacing serine with asparagine at amino acid position 477 (S477N); (5) replacing glutamic acid with lysine at amino acid position 484 (E484K); (6) replacing asparagine with tyrosine at amino acid position 501 (N501Y); or (7) any combinations of (1)-(6), either alone or combined with any other substitutions and/or deletions in amino acids 319-542 of SEQ ID NO:15 other than those recited in (1)-(6); or

c) comprises any combination of a) and b).

101. The system of any of claims 97-100, wherein the device for detecting the label from the complex is automated or semi-automated.

102. The system of claim 101, wherein the device for detecting the label from the complex is semi-automated and comprises uploading the differentiative rating using a bar code.

103. The system of any of claims 97-102, wherein the at least one type of anti-SARS-CoV-2 antibody is an anti-SARS-CoV-2 IgG antibody, an anti-SARS-CoV-2 IgM antibody, or any combination thereof.

104. The system of any of claims 97-103, wherein the at least one type of second specific binding partner is an anti-species IgG (e.g., anti-human-IgG IgG) antibody, an anti-species IgM (e.g., anti-human-IgM IgG) antibody, or an anti-species IgG (e.g., anti-human-IgG IgG) antibody, and anti-species IgM (e.g., anti-human-IgM IgG) antibody.

105. The system of any of claims 97-104, wherein the at least one type of first specific binding partner is a receptor binding domain (RBD) of a spike protein or variant thereof that has an amino acid sequence of amino acids 319 to 542 of SEQ ID NO:15 or SEQ ID NO:17.

106. The system of any of claims 97-105, wherein the at least one type of first specific binding partner binds to (a) an anti-SARS-CoV-2 IgG antibody, (b) an anti-SARS-CoV-2 IgM antibody, or (c) combinations of (a) and (b).