

SARS-CoV-2 and influenza can help to inform time-critical medical decision-making, facilitate infection control efforts, promote efficient resourcing, optimize use of targeted therapies and antimicrobials, and reduce ancillary testing or procedures. Thus, there is a need in the art for a quick, reliable, specific, and sensitive method to detect and differentiate SARS-CoV-2, influenza A, and influenza B.

SUMMARY OF THE INVENTION

The present disclosure provides methods for the rapid detection of the presence or absence of SARS-CoV-2 in a biological or non-biological sample, for example, multiplex detection of SARS-CoV-2 by qualitative or quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR) in a single test tube or a single well. The present disclosure also provides methods for the rapid and simultaneous detection of the presence or absence of SARS-CoV-2, influenza A and influenza B in a biological or non-biological sample, for example, multiplex detection of SARS-CoV-2, influenza A and influenza B by qualitative or quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR) in a single test tube or a single well. Embodiments Examples include methods of detection of SARS-CoV-2, influenza A and influenza B comprising performing a reverse transcription step and at least one cycling step, which may include an amplifying step and a hybridizing step. Furthermore, embodimentexamples include primers, probes, and kits that are designed for the detection of SARS-CoV-2 or for the multiplex detection of SARS-CoV-2, influenza A and influenza B in a single tube or a single well. The detection methods are designed to target various regions of each of the target genomes. For example, the methods are designed to target the region of the SARS-CoV-2 genome that encodes the structural envelope (E) region, and/or the non-structural Open Reading Frame (ORF1a/b gene coding for the ORF1a polyprotein and the ORF1a/b polyprotein) region. The methods may also be designed to target other regions of the SARS-CoV-2 genome, alone or in combination, such as S gene (coding for spike protein responsible for binding to cell receptor), ORF3ab, E gene (coding for envelope protein), and M gene (coding for membrane protein). In addition, there are 265 bases of non-coding region at the 5' terminal end and 229 bases of non-coding region at the 3' terminal end of the SARS-CoV-2 genome, and these may be targeted as well.

With respect to influenza A and B, the methods may be designed to target any gene or non-coding regions within the eight segments that make up their genomes. For example, for influenza A, the methods may be designed to target the influenza A Segment 7 matrix protein 2 (M2) and matrix protein 1 (M1) sequences. For influenza B, the methods may be designed to target the influenza B Segment 8 nuclear export protein (NEP) and nonstructural protein 1 (NS1) sequences.

The methods for the simultaneous detection of SARS-CoV-2, influenza A and influenza B by RT-PCR in a single test tube or single well may also include simultaneously detecting by RT-PCR in a single test tube or single well additional viruses that may cause respiratory disease in humans, especially in the upper respiratory tract. Examples of these viruses include but are not limited to:

5 Coronavirus (229E, NL63, OC43, HKU1), Respiratory Syncytial Virus, Human Metapneumovirus, Adenovirus (B,E,U,C), Enterovirus, Rhinovirus, and Human Parainfluenza Virus (1, 2, 3, 4).

~~In one embodiment, a~~ method for detecting SARS-CoV-2 in a sample is provided, comprising performing an amplifying step including contacting the sample with a set of primers to produce an amplification product if SARS-CoV-2 is present in the sample; performing a hybridizing step

10 including contacting the amplification product with one or more detectable probes; and detecting the presence or absence of the amplified product, wherein the presence of the amplified product is indicative of the presence of SARS-CoV-2 in the sample and wherein the absence of the amplified product is indicative of the absence of SARS-CoV-2 in the sample; wherein the set of primer comprises or consists of an oligonucleotide sequence selected from the group consisting of SEQ

15 ID NOs: 1-20, 27-31, and 40-41 or a complement thereof; and wherein the one or more detectable probes comprises or consists of an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-26, 32, and 42-43 or a complement thereof.

In one aspect, the present disclosure provides a method of detecting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a sample, the method comprising:

- 20 - performing an amplifying step comprising contacting the sample with a set of primers to produce an amplification product if a SARS-CoV-2 nucleic acid is present in the sample;
- performing a hybridizing step comprising contacting the amplification product with one or more detectable probes; and
- 25 - detecting the presence or absence of the amplification product, wherein the presence of the amplification product is indicative of the presence of SARS-CoV-2 in the sample and wherein the absence of the amplification product is indicative of the absence of SARS-CoV-2 in the sample;
- wherein the set of primers comprises a first primer comprising a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-3 and 27-31, 40, and a second primer
- 30 comprising a second oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-14, and 41; and

wherein the detectable probe of the one or more detectable probes comprises a third oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-23 and 42-43 or a complement thereof.

In one embodimentexample, a multiplex method for detecting SARS-CoV-2 in a sample is provided, comprising performing an amplifying step including contacting the sample with a first set of primers to produce an amplification product if SARS-CoV-2 is present in the sample and a second set of primers to produce an amplification product if SARS-CoV-2 is present in the sample; performing a hybridizing step including contacting the amplification products with at least one detectable probe hybridizing to an amplification product produced by the first primer pair and with at least one second detectable probe hybridizing to an amplification product produced by the second primer pair; and detecting the presence or absence of the amplified product(s), wherein the presence of the amplified product(s) is indicative of the presence of SARS-CoV-2 in the sample and wherein the absence of the amplified product is indicative of the absence of SARS-CoV-2 in the sample; wherein the first set of primers comprises or consists of a forward primer oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-3, and 27-31, and a reverse primer oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-14; and the second set of primers comprises or consists of a forward primer oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, and 40 and a reverse primer oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20, and 41; and wherein the at least one detectable probe hybridizing to an amplification product produced by the

first primer pair comprises or consists of an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-23, and 42, or a complement thereof and wherein the at least one detectable probe hybridizing to an amplification product produced by the second primer pair comprises or consists of an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 24-26, 32, and 43 or a complement thereof.

In some [embodimentsexamples](#), the first primer pair specifically hybridizes to and amplifies the SARS-CoV-2 target nucleic acid and the second primer pair hybridizes to and amplifies the SARS-CoV-2 target nucleic acid. In some [embodimentsexamples](#) the second primer pair hybridizes to and amplifies the SARS-CoV-2 target nucleic acid and other coronavirus target nucleic acids from the subgenus Sarbecovirus. In some [embodimentsexamples](#), the at least one detectable probe hybridizing to an amplification product produced by the first primer pair specifically hybridizes to the SARS-CoV-2 target nucleic acid. In some [embodimentsexamples](#), the at least one detectable probe hybridizing to an amplification product produced by the second primer pair hybridizes to the SARS-CoV-2 target nucleic acid. In some [embodimentsexamples](#), the at least one detectable probe hybridizing to an amplification product produced by the second primer pair hybridizes to the SARS-CoV-2 target nucleic acid and other coronavirus target nucleic acids from the subgenus Sarbecovirus.

In one [embodimentexample](#), the set of primers for amplification of the SARS-CoV-2 target includes a first primer comprising or consisting a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6, 27-31, and 40 or a complement thereof, and a second primer comprising or consisting a second oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-20, and 41, or a complement thereof, and the one or more detectable probes for detection of the amplification product comprises or consists of an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-26, 32, 42, and 43 or a complement thereof. In one [embodimentexample](#), the set of primers for specific amplification of the SARS-CoV-2 target includes a first primer comprising or consisting a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-3, and 27-31, and a second primer comprising or consisting a second oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-14, and the one or more detectable probes for detection of the amplification product comprises or consists of an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-23, and 42, or a complement thereof.

In one [embodimentexample](#), the set of primers for specific amplification of the SARS-CoV-2 target includes a first primer comprising or consisting a first oligonucleotide sequence of SEQ ID NO: 40, and a second primer comprising or consisting a second oligonucleotide sequence of SEQ ID

NO: 41, and the one or more detectable probes for detection of the amplification product comprises or consists of an oligonucleotide sequence of SEQ ID NO: 43, or a complement thereof.

In one [embodimentexample](#), the set of primers for amplification of the SARS-CoV-2 target includes a first primer comprising or consisting a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, and a second primer comprising or consisting a second oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20, and the one or more detectable probes for detection of the amplification product comprises or consists of an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 24-26 and 32, or a complement thereof. In some [embodimentexamples](#), the set of primers is suitable for amplification of SARS-CoV-2 target nucleic acid and other coronavirus target nucleic acids from the subgenus Sarbecovirus.

In another [embodimentexample](#), the set of primers for amplification of the SARS-CoV-2 target includes a first primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 1, and a second primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 7, and a detectable probe that comprises or consists of an oligonucleotide sequence of SEQ ID NO: 21. In another [embodimentexample](#), the first primer comprises or consists of an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 27 and 30, the second primer comprises or consists of an oligonucleotide sequence of SEQ ID NO: 7, and a detectable probe that comprises or consists of an oligonucleotide sequence of SEQ ID NO: 21. In another [embodimentexample](#), the set of primers for amplification of the SARS-CoV-2 target includes a first primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 1, and a second primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 7, 8, or 9, and a detectable probe that comprises or consists of an oligonucleotide sequence of SEQ ID NO: 21. In another [embodimentexample](#), the set of primers for amplification of the SARS-CoV-2 target includes a first primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 2, and a second primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 9, 10, or 11, and a detectable probe that comprises or consists of an oligonucleotide sequence of SEQ ID NO: 22.

In another [embodimentexample](#), the first primer comprises or consists of an oligonucleotide sequence of SEQ ID NO: 5, the second primer comprises or consists of an oligonucleotide sequence of SEQ ID NO: 15, and the detectable probe comprises or consists of an oligonucleotide sequence selected from the group consisting of SEQ ID NO: 25 and 32. In yet another [embodimentexample](#), the first primer comprises or consists of an oligonucleotide sequence of SEQ ID NO: 6, the second primer comprises or consists of an oligonucleotide sequence of SEQ ID NO: 18, and the detectable probe comprises or consists of an oligonucleotide sequence of SEQ ID NO: 26.

In one [embodimentexample](#), the set of primers for amplification of the SARS-CoV-2 target includes a first primer comprising or consisting a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6 and 27-31, and 40, and a second primer comprising or consisting a second oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-20, and 41.

In one [embodimentexample](#), the set of primers for amplification of the SARS-CoV-2 target includes a first primer comprising or consisting a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-3 and 27-31, and a second primer comprising or consisting a second oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-14. In

another [embodimentexample](#), the set of primers for amplification of the SARS-CoV-2 target includes a first primer comprising or consisting a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, or a complement thereof, and a second primer comprising or consisting a second oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20, or a complement thereof. In another [embodimentexample](#), the set of primers for

amplification of the SARS-CoV-2 target includes a first primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 1, and a second primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 7. In another [embodimentexample](#), the first primer

comprises or consists of an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 27 and 30, the second primer comprises or consists of an oligonucleotide sequence of

SEQ ID NO: 7. In another [embodimentexample](#), the first primer comprises or consists of an oligonucleotide sequence of SEQ ID NO: 5, the second primer comprises or consists of an oligonucleotide sequence of SEQ ID NO: 15. In yet another [embodimentexample](#), the first primer

comprises or consists of an oligonucleotide sequence of SEQ ID NO: 6, the second primer comprises or consists of an oligonucleotide sequence of SEQ ID NO: 18. In yet another

[embodimentexample](#), the first primer comprises or consists of an oligonucleotide sequence of SEQ ID NO: 40, the second primer comprises or consists of an oligonucleotide sequence of SEQ ID NO: 41.

In another [embodimentexample](#), the set of primers for amplification of the SARS-CoV-2 target includes a plurality of first primers, a plurality of second primers, and a plurality of detectable probes, wherein the plurality of first primers is the combination of a first primer comprising the oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1 and 27, and a first primer comprising the oligonucleotide sequence of SEQ ID NO: 5; wherein the plurality of second primers is the combination of a second primer comprising the oligonucleotide sequence of SEQ ID NO: 7 and a second primer comprising the oligonucleotide sequence of SEQ ID NO: 15; and wherein the plurality of detectable probes is the combination of an oligonucleotide probe

comprising the oligonucleotide sequence of SEQ ID NO: 21 and an oligonucleotide probe comprising the oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 25 and 32. In still another embodiment example, the plurality of first primers is the combination of a first primer comprising the oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1 and 27, a first primer comprising the oligonucleotide sequence of SEQ ID NO: 5, and a first primer comprising the oligonucleotide sequence of SEQ ID NO: 6; the plurality of second primers is the combination of a second primer comprising the oligonucleotide sequence of SEQ ID NO: 7, a second primer comprising the oligonucleotide sequence of SEQ ID NO: 15, and a second primer comprising the oligonucleotide sequence of SEQ ID NO: 18; and the plurality of detectable probes is the combination of an oligonucleotide probe comprising the oligonucleotide sequence of SEQ ID NO: 21, an oligonucleotide probe comprising the oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 25 and 32, and an oligonucleotide probe comprising the oligonucleotide sequence of SEQ ID NO: 26. In still another embodiment example, the plurality of first primers is the combination of a first primer comprising the oligonucleotide sequence of SEQ ID NO: 27, and a first primer comprising the oligonucleotide sequence of SEQ ID NO: 40; the plurality of second primers is the combination of a second primer comprising the oligonucleotide sequence of SEQ ID NO: 7 and a second primer comprising the oligonucleotide sequence of SEQ ID NO: 41; and wherein the plurality of detectable probes is the combination of an oligonucleotide probe comprising the oligonucleotide sequence of SEQ ID NO: 42 and an oligonucleotide probe comprising the oligonucleotide sequence of SEQ ID NO: 43.

~~In another aspect, a~~ method for simultaneously detecting SARS-CoV-2, influenza A, and influenza B in a sample is provided, comprising performing an amplifying step including contacting the sample with a first set of primers, a second set of primers, and a third set of primers to produce one or more amplification products if SARS-CoV-2, and/or influenza A, and/or influenza B is present in the sample; wherein the first set of primers produces an amplification product if SARS-CoV-2 is present in the sample, the second set of primers produces an amplification product if influenza A is present in the sample, the third set of primers produces an amplification product if influenza B is present in the sample; performing a hybridizing step including contacting the amplification product(s) with three or more detectable probes, wherein the three or more detectable probes includes at least one probe specific for the amplification products of each of the first, the second, and the third sets of primers; and detecting the presence or absence of the amplified products, wherein the presence of the amplified product is indicative of the presence of SARS-CoV-2, influenza A, and/or influenza B in the sample and wherein the absence of the amplified product is indicative of the absence of SARS-CoV-2, influenza A, and/or influenza B in the sample.

In some [embodimentsexamples](#), the method further comprises providing a fourth set of set of primers and a fourth detectable probe. Herein, the fourth set of primers may produce an amplification product if SARS-CoV-2 or other coronavirus target nucleic acids from the subgenus Sarbecovirus is present in the sample and the detectable fourth probe detects the presence or absence of the amplified products, wherein the presence of the amplified product is indicative of the presence of SARS-CoV-2 or other coronavirus target nucleic acids from the subgenus Sarbecovirus in the sample. In another [embodimentexample](#), the fourth set of primers may produce an amplification product if SARS-CoV-2 is present in the sample and the detectable fourth probe detects the presence or absence of the amplified products, wherein the presence of the amplified product is indicative of the presence of SARS-CoV-2 in the sample.

In another [embodimentexample](#), the first set of primers used in the method(s) comprises a forward primer comprising or consisting of an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-3 and 27-31, and a reverse primer comprising or consisting of an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-14; the second set of primers comprises a forward primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 33, and a reverse primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 34; and the third set of primers comprises a forward primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 36, and a reverse primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 37; and wherein the first detectable probe comprises or consists of an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-23, and 42, the second detectable probe comprises or consists of an oligonucleotide sequence of SEQ ID NO: 35 or 44, and the third detectable probe comprises or consists of an oligonucleotide sequence of SEQ ID NO: 38 or 45. In another [embodimentexample](#), the method further comprises providing a fourth set of primers that produces an amplification product if SARS-CoV-2 or SARS-CoV-2 and other coronavirus target nucleic acids from the subgenus Sarbecovirus is present in the sample. In certain [embodimentsexamples](#), the fourth set of primers comprises a forward primer comprising or consisting of an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, and 40, and a reverse primer comprising or consisting of an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20, and 41; the fourth detectable probe comprises or consists of an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 24-26, 32 and 43. In some [embodimentsexamples](#), the fourth set of primers comprises a forward primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, and a reverse primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20, and the fourth detectable probe comprises an oligonucleotide sequence

selected from the group consisting of SEQ ID NOs: 24-26, and 32. In some [embodimentexamples](#), the fourth set of primers that produces a fourth amplification product if SARS-CoV-2 target nucleic acids are present in the sample, wherein the fourth set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 40 and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 41 and the fourth detectable probe for detecting the amplification product comprises of an oligonucleotide sequence of SEQ ID NO: 43

In one [embodimentexample](#), the first set of primers that produces an amplification product if SARS-CoV-2 is present in the sample comprises a forward primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 27 and a reverse primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 7 and the first detectable probe for detecting the amplification product comprises or consists of an oligonucleotide sequence of SEQ ID NO: 21 or 42. In another [embodimentexample](#), the fourth set of primers that produces an amplification product if SARS-CoV-2 or SARS-CoV-2 and other coronavirus target nucleic acids from the subgenus Sarbecovirus is present in the sample comprises a forward primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 5 or 40, and a reverse primer comprising or consisting of an oligonucleotide sequence of SEQ ID NO: 15 or 41, and the fourth detectable probe for detecting the amplification product comprises or consists of an oligonucleotide sequence of SEQ ID NO: 32 or 43.

The disclosure provides oligonucleotides comprising or consisting of a sequence of nucleotides selected from SEQ ID NOs: 1-45, or a complement thereof, which oligonucleotides have 100 or fewer nucleotides. Further, the present disclosure provides an oligonucleotide that includes a nucleic acid having at least 70% sequence identity (e.g., at least 75%, 80%, 85%, 90% or 95%, etc.) to one of SEQ ID NOs: 1-45, or a complement thereof, which oligonucleotide has 100 or fewer nucleotides. Generally, these oligonucleotides may be primer nucleic acids, probe nucleic acids, or the like in these [embodimentexamples](#). In certain [aspectsexamples](#), the oligonucleotides have 40 or fewer nucleotides (e.g., 35 or fewer nucleotides, 30 or fewer nucleotides, 25 or fewer nucleotides, 20 or fewer nucleotides, 15 or fewer nucleotides, etc.). In some [aspectsexamples](#), the oligonucleotides comprise at least one modified nucleotide, e.g., to alter nucleic acid hybridization stability relative to unmodified nucleotides. Optionally, the oligonucleotides comprise at least one label and optionally at least one quencher moiety. In some [aspectsexamples](#), the oligonucleotides include at least one conservatively modified variation. “Conservatively modified variations” or, simply, “conservative variations” of a particular nucleic acid sequence refers to those nucleic acids, which encode identical or essentially identical amino acid sequences, or, where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. One of skill in the art will recognize

that individual substitutions, deletions or additions which alter, add or delete a single nucleotide or a small percentage of nucleotides (typically less than 5%, more typically less than 4%, 2% or 1%) in an encoded sequence are “conservatively modified variations” where the alterations result in the deletion of an amino acid, addition of an amino acid, or substitution of an amino acid with a chemically similar amino acid.

In one [aspectexample](#), amplification can employ a polymerase enzyme having 5' to 3' nuclease activity. Thus, the donor fluorescent moiety and the acceptor moiety, e.g., a quencher, may be within no more than 5 to 20 nucleotides (e.g., within 8 or 10 nucleotides) of each other along the length of the probe. In another [aspectexample](#), the probe includes a nucleic acid sequence that permits secondary structure formation. Such secondary structure formation may result in spatial proximity between the first and second fluorescent moiety. According to this method, the second fluorescent moiety on the probe can be a quencher.

In one [aspectexample](#), the specific SARS-CoV-2, influenza A and/or influenza B probes may be labeled with a fluorescent dye, which acts as a reporter. The probe may also have a second dye, which acts as a quencher. The reporter dye is measured at a defined wavelength, thus permitting detection and discrimination of the amplified SARS-CoV-2, influenza A and/or influenza B target. The fluorescent signal of the intact probes is suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage by the 5' to 3' nuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye is concomitantly increased. Optionally, one or more additional probes (e.g., such as an internal reference control or other targeted probe (e.g., other viral nucleic acids) may also be labeled with a reporter fluorescent dye, unique and distinct from the fluorescent dye label associated with the SARS-CoV-2, influenza A and/or influenza B probe. In such case, because the specific reporter dyes are measured at defined wavelengths, simultaneous detection and discrimination of the amplified SARS-CoV-2, influenza A and/or influenza B target and the one or more additional probes is possible.

The present disclosure also provides for methods of detecting the presence or absence of SARS-CoV-2, or SARS-CoV-2 nucleic acid, in a biological sample from an individual. The present disclosure also provides for methods of detecting the presence or absence of SARS-CoV-2, influenza A and/or influenza B or SARS-CoV-2, influenza A and/or influenza B nucleic acid(s), in a biological sample from an individual. These methods can be employed to detect the presence or absence of SARS-CoV-2 or SARS-CoV-2, influenza A and/or influenza B nucleic acid(s) in

nasopharyngeal (NSP) and oropharyngeal swab samples, for use in diagnostic testing. Additionally, the same test may be used by someone experienced in the art to assess other sample types to detect SARS-CoV-2 or SARS-CoV-2, influenza A and/or influenza B nucleic acid(s). Such methods generally include performing a reverse transcription step and at least one cycling step, which includes an amplifying step and a dye-binding step. Typically, the amplifying step includes contacting the sample with a plurality of pairs of oligonucleotide primers to produce one or more amplification products if a nucleic acid molecule is present in the sample, and the dye-binding step includes contacting the amplification product with a double-stranded DNA binding dye. Such methods also include detecting the presence or absence of binding of the double-stranded DNA binding dye into the amplification product, wherein the presence of binding is indicative of the presence of SARS-CoV-2 or SARS-CoV-2, influenza A and/or influenza B nucleic acid(s) in the sample, and wherein the absence of binding is indicative of the absence of SARS-CoV-2 or SARS-CoV-2, influenza A and/or influenza B nucleic acid(s) in the sample. A representative double-stranded DNA binding dye is ethidium bromide. Other nucleic acid-binding dyes include DAPI, Hoechst dyes, PicoGreen[®], RiboGreen[®], OliGreen[®], and cyanine dyes such as YO-YO[®] and SYBR[®] Green. In addition, such methods also can include determining the melting temperature between the amplification product and the double-stranded DNA binding dye, wherein the melting temperature confirms the presence or absence of SARS-CoV-2 or SARS-CoV-2, influenza A and/or influenza B nucleic acid(s).

~~In a further embodiment, a~~ kit for detecting one or more nucleic acids of SARS-CoV-2 is provided. The kit can include one or more sets of primers specific for amplification of the gene target; and one or more detectable oligonucleotide probes specific for detection of the amplification products. In another ~~embodiment~~example, a kit for simultaneously detecting one or more nucleic acids of SARS-CoV-2, one or more nucleic acids of influenza A and one or more nucleic acids of influenza B is provided. The kit can include one or more sets or primers specific for amplification of the SARS-CoV-2 gene target, the influenza A gene target and the influenza B gene target, as well as one or more detectable oligonucleotide probes specific for detection of the amplification products for SARS-CoV-2, influenza A and influenza B.

In another aspect, the present disclosure provides a kit for detecting a nucleic acid of SARS-CoV-2 comprising:

- a first primer comprising a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-3 and 27-31, and 40;
- a second primer comprising a second oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-14, and 41; and
- a third fluorescently detectably labeled oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-23 and 42-43, or a complement, the third detectably labeled oligonucleotide sequence configured to hybridize to an amplicon generated by the first primer and the second primer.

In one ~~aspect~~example, the kit can include probes already labeled with donor and corresponding acceptor moieties, e.g., another fluorescent moiety or a dark quencher, or can include fluorophoric moieties for labeling the probes. The kit can also include nucleoside triphosphates, nucleic acid polymerase, and buffers necessary for the function of the nucleic acid polymerase. The kit can also include a package insert and instructions for using the primers, probes, and fluorophoric moieties to detect

the presence or absence of SARS-CoV-2 nucleic acid or SARS-CoV-2, influenza A and/or influenza B nucleic acid(s) in a sample.

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 to which this ~~invention~~disclosure belongs.

5 Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present subject matter, suitable methods and materials are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

10 The details of one or more ~~embodiment~~examples of the ~~invention~~disclosure are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the ~~invention~~disclosure will be apparent from the drawings and detailed description, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

15 FIG. 1 shows the genome organization of SARS-CoV-2 (labeled here as Wuhan-Hu-1) and SARS-CoV and the locations of the target regions of the SARS-CoV-2 primer and probes of the present invention. E: envelope protein gene; M: membrane protein gene; N: nucleocapsid protein gene; ORF1a/b: ORF for non-structural genes; S: spike protein gene; Numbers below amplicon are genome position according to Wuhan-Hu-1, GenBank MN908947.

20 FIG. 2 shows PCR growth curves of experiments, where the primers and probes for the nCoV1 assay are tested against various concentrations of linearized SARS-CoV-2 DNA template.

FIG. 3 shows PCR growth curves of experiments, where the primers and probes for the nCoV1 assay are tested against various concentrations of RNA transcript of SARS-CoV-2.

25 FIG. 4 shows PCR growth curves of experiments, where the primers and probes for the Pan-Sarbeco-2 assay are tested against various concentrations of linearized SARS-CoV-2 DNA template.

FIG. 5 shows the growth curves (left) and Ct charts with dynamic range plotted (right) in the multiplex PCR test described in Example 5 for the nCoV1 assay (top) and the Pan-Sarbecovirus-1 assay (bottom) and across the indicated levels ($1e+8$ to $1e+1$) tested using the synthetic *in vitro* transcripts.

30 FIG. 6 displays the summary of the Limit of Detection (LOD) Data from the experimental data shown in FIG. 5

FIG. 7 shows the growth curves generated from the SARS-CoV-2 Test using isolated genomic RNA from a patient sample at the indicated levels diluted in Specimen Diluent (SD) in a multiplex PCR test comprising the nCoV1 assay (left) and Pan-Sarbecovirus-1 assay (right).

CLAIMS:

1. A method of detecting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a sample, the method comprising:
 - performing an amplifying step comprising contacting the sample with a set of primers to produce an amplification product if a SARS-CoV-2 nucleic acid is present in the sample;
 - performing a hybridizing step comprising contacting the amplification product with one or more detectable probes; and
 - detecting the presence or absence of the amplification product, wherein the presence of the amplification product is indicative of the presence of SARS-CoV-2 in the sample and wherein the absence of the amplification product is indicative of the absence of SARS-CoV-2 in the sample;

wherein the set of primers comprises a first primer comprising a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-~~6-3~~ and 27-31, 40, ~~or a complement thereof~~, and a second primer comprising a second oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-~~2014~~, and 41, ~~or a complement thereof~~; and wherein the detectable probe of the one or more detectable probes comprises a third oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-~~2326, 32,~~ and 42-43 or a complement thereof.

2. The method of claim 1, wherein:
 - the hybridizing step comprises contacting the amplification product with the detectable probe that is labeled with a donor fluorescent moiety and a corresponding acceptor moiety; and
 - the detecting step comprises detecting the presence or absence of fluorescence resonance energy transfer (FRET) between the donor fluorescent moiety and the acceptor moiety of the probe, wherein the presence or absence of fluorescence is indicative of the presence or absence of SARS-CoV-2 in the sample.
3. The method of claim 2, wherein said amplification step employs a polymerase enzyme having 5' to 3' nuclease activity.
4. The method of any one of claims 1 to 3, wherein the sample is a biological sample selected from a nasopharyngeal sample or an oropharyngeal sample.

~~5. The method of any one of claims 1 to 4, further comprising a method of detecting a nucleic acid from one or more other viruses, in parallel, wherein the one or more other viruses is selected from the group consisting of influenza virus, bat coronavirus, severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) and Middle East respiratory syndrome (MERS)~~

coronavirus (MERS-CoV), Coronavirus (229E, NL63, OC43, HKU1), Respiratory Syncytial Virus, Human Metapneumovirus, Adenovirus (B,E,U,C), Enterovirus, Rhinovirus, and Human Parainfluenza Virus (1, 2, 3, 4), and any combinations of the above.

6. ~~The method of any one of claims 1 to 5, wherein the first primer comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-3 and 27-31, or a complement thereof; the second primer comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-14, or a complement thereof; and the detectable probe comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-23 and 42, or a complement thereof.~~

7. ~~The method of claim 6 further comprising a first primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, and 40, or a complement thereof; a second primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20, and 41, or a complement thereof; and a detectable probe comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 24-26, 32 and 43, or a complement thereof.~~

5. The method of any one of claims 1 to 4, wherein the first primer comprises an oligonucleotide sequence of SEQ ID NO: 1 or 27-29; the second primer comprises an oligonucleotide sequence of SEQ ID NO: 7; and the detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 21 or 42, or a complement thereof; or

wherein the first primer comprises an oligonucleotide sequence of SEQ ID NO: 40; the second primer comprises an oligonucleotide sequence of SEQ ID NO: 41; and the detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 43, or a complement thereof.

8.6. The method of any one of claims 1 to 54, further comprising performing the amplifying step in the presence of a second set of primers comprising a wherein the first primer comprises comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, or a complement thereof; the and a second primer comprises comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20, or a complement thereof to produce a second amplification product if SARS-CoV-2 or SARS-CoV-2 and other coronavirus target nucleic acids from the subgenus Sarbecovirus is present in the sample; and performing the hybridizing step in the presence of a the detectable probe comprises comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 24-26 and 32, or a complement thereof, wherein detecting the presence of the second amplification product is indicative of the presence of SARS-CoV-2 or SARS-CoV-2 and other

coronavirus target nucleic acids from the subgenus Sarbecovirus in the sample and wherein the absence of the amplification product is indicative of the absence of SARS-CoV-2 or SARS-CoV-2 and other coronavirus target nucleic acids from the subgenus Sarbecovirus in the sample.

- 5 9.7. The method of any one of claims 1 to ~~54~~, wherein the set of primers for amplification of the SARS-CoV-2 includes a plurality of first primers, a plurality of second primers, and a plurality of detectable probes,
wherein the plurality of first primers is the combination of a first primer comprising the oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1 and 27, and a
10 first primer comprising the oligonucleotide sequence of SEQ ID NO: 5;
wherein the plurality of second primers is the combination of a second primer comprising the oligonucleotide sequence of SEQ ID NO: 7 and a second primer comprising the oligonucleotide sequence of SEQ ID NO: 15; and
wherein the plurality of detectable probes is the combination of an oligonucleotide probe
15 comprising the oligonucleotide sequence of SEQ ID NO: 21 or 42, or a complement thereof
and an oligonucleotide probe comprising the oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 25 and 32, or a complement thereof.
- 20 8. The method of any one of claims 1 to 7, further comprising a method of detecting a nucleic acid from one or more other viruses, in parallel, wherein the one or more other viruses is selected from the group consisting of influenza virus, bat-coronavirus, severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) and Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV), Coronavirus (229E, NL63, OC43, HKU1), Respiratory Syncytial Virus, Human Metapneumovirus, Adenovirus (B,E,U,C), Enterovirus, Rhinovirus, and Human Parainfluenza Virus (1, 2, 3, 4), and any combinations of the above.
- 25 9. The method of claim 8, wherein the method is for simultaneously detecting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), influenza A, and influenza B in a sample further comprising performing the amplifying step in the presence of a set of influenza A primers to produce an amplification product if influenza A is present in the sample; and a set of influenza B primers to produce an amplification product if influenza B is present in the
30 sample; and performing the hybridizing step in the presence of one or more detectable influenza A probes and one or more influenza B probes, wherein detecting the presence of the influenza A amplification product is indicative of the presence of influenza A in the sample and wherein the absence of the influenza A amplification product is indicative of the absence

of influenza A in the sample; and wherein detecting the presence of the influenza B amplification product is indicative of the presence of influenza B in the sample and wherein the absence of the influenza B amplification product is indicative of the absence of influenza A in the sample.

5 10. The method of claim 9, wherein the set of influenza A primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 33, and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 34; and one of the one or more detectable influenza A probes comprises an oligonucleotide sequence of SEQ ID NO: 35 or 44, or a complement thereof.

10 11. The method of any one of claims 9 to 10, wherein the set of influenza B primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 36, and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 37; and one of the one or more detectable influenza B probes comprises an oligonucleotide sequence of SEQ ID NO: 38 or 45, or a complement thereof.

15 ~~10. The method of any one of claims 1 to 5, wherein the first primer comprises an oligonucleotide sequence of SEQ ID NO: 40, or a complement thereof; the second primer comprises an oligonucleotide sequence of SEQ ID NO: 41, or a complement thereof; and the detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 43, or a complement thereof.~~

11.12. A kit for detecting a nucleic acid of SARS-CoV-2 comprising:

- 20 - a first primer comprising a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-~~6~~3 and 27-31, and 40-~~or a complement thereof~~;
- a second primer comprising a second oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-~~20~~14, and 41-~~or a complement thereof~~; and
- 25 - a third fluorescently detectably labeled oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-~~23~~26, 32, and 42-43, or a complement, the third detectably labeled oligonucleotide sequence configured to hybridize to an amplicon generated by the first primer and the second primer.

12.13. The kit of claim ~~11~~12, wherein the third detectably labeled oligonucleotide sequence comprises a donor fluorescent moiety and a corresponding acceptor moiety.

30 ~~13. The kit of any one of claims 11 and 12, wherein the sample is a biological sample selected from a nasopharyngeal sample or an oropharyngeal sample.~~

14. The kit of any one of claims ~~11-12~~ to 13 further comprising nucleoside triphosphates, nucleic acid polymerase, and buffers necessary for the function of the nucleic acid polymerase.
15. The kit of any one of claims ~~11-12~~ to 14, wherein at least one of the first, second, and third oligonucleotide sequences comprises at least one modified nucleotide.
- 5 16. The kit of any one of claims ~~11-12~~ to 15, wherein the first primer comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-~~3~~ and 27-~~31-29~~, ~~or a complement thereof~~; the second primer comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-~~14~~, ~~or a complement thereof~~; and the detectable probe comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-~~23~~, and 42, or a complement thereof; or
10 wherein the first primer comprises an oligonucleotide sequence of SEQ ID NO: 40; the second primer comprises an oligonucleotide sequence of SEQ ID NO: 41; and the detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 43, or a complement thereof.
- 15 17. The kit of ~~claim 16~~ any one of claims 12 to 16 further comprising a first primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, ~~and 40~~, ~~or a complement thereof~~; a second primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20, ~~and 41~~, ~~or a complement thereof~~; and a detectable probe comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 24-26, and 32, ~~and 43~~, or a complement thereof.
- 20 18. The kit of ~~any one of claims 11 to 15~~ claim 17, wherein the first primer comprises an oligonucleotide sequence ~~selected from the group consisting of~~ SEQ ID NOs: 4-6~~5~~, ~~or a complement thereof~~; the second primer comprises an oligonucleotide sequence ~~selected from the group consisting of~~ SEQ ID NOs: 15-20, ~~or a complement thereof~~; and the detectable probe comprises an oligonucleotide sequence ~~selected from the group consisting of~~ SEQ ID
25 NOs: ~~24-26 and~~ 25 or 32, or a complement thereof.
- 30 19. The kit of any one of claims 12 to 18, wherein the kit is for simultaneously detecting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), influenza A, and influenza B and further comprises a set of influenza A primers and one or more detectable influenza A probes and a set of influenza B primers and one or more detectable influenza B probes.
20. The kit of claim 19, wherein the set of influenza A primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 33, and a reverse primer comprising

an oligonucleotide sequence of SEQ ID NO: 34; and one of the one or more detectable influenza A probes comprises an oligonucleotide sequence of SEQ ID NO: 35 or 44; and/or the set of influenza B primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 36, and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 37; and one of the one or more detectable influenza B probes comprises an oligonucleotide sequence of SEQ ID NO: 38 or 45.

~~19. The kit of any one of claims 11 to 15, wherein the first primer comprises an oligonucleotide sequence of SEQ ID NO: 40, or a complement thereof; the second primer comprises an oligonucleotide sequence of SEQ ID NO: 41, or a complement thereof; and the detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 43, or a complement thereof.~~

~~20. A method for simultaneously detecting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), influenza A, and influenza B in a sample comprising:~~

~~—performing an amplifying step, wherein the sample is contacted with a first set of primers, a second set of primers, and a third set of primers to produce one or more amplification products if SARS-CoV-2, and/or influenza A, and/or influenza B is present in the sample; wherein the first set of primers produces an amplification product if SARS-CoV-2 is present in the sample, the second set of primers produces an amplification product if influenza A is present in the sample, the third set of primers produces an amplification product if influenza B is present in the sample;~~

~~—performing a hybridizing step wherein the amplification product(s) are contacted with three or more detectable probes, wherein the three or more detectable probes includes at least one probe specific for the amplification products of each of the first, the second and the third sets of primers; and detecting the presence or absence of the amplified products, wherein the presence of the amplified product is indicative of the presence of SARS-CoV-2, influenza A, and/or influenza B in the sample and wherein the absence of the amplified product is indicative of the absence of SARS-CoV-2, influenza A, and/or influenza B in the sample.~~

~~21. The method of claim 20, wherein the first set of primers comprises a first primer comprising a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6 and 27-31, 40, or a complement thereof, and a second primer comprising a second oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-20, and 41, or a complement thereof; and wherein the first detectable probe of the one or more detectable probes comprises~~

a third oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-26, 32, and 42-43 or a complement thereof.

22. The method of claim 21, wherein the first set of primers comprises a forward primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-3, 27-31, and a reverse primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-14; and wherein the first detectable probe comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-23, and 42.

23. The method of claim 22, wherein the first set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 1 or 27 and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 7, and the first detectable probe for detecting the amplification product comprises an oligonucleotide sequence of SEQ ID NO: 21 or 42.

24. The method of any one of claims 20 to 23, wherein the second set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 33, and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 34; and the third set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 36, and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 37; and wherein the second detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 35 or 44, and the third detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 38 or 45.

25. The method of any one of claims 20 and 22 to 24 further comprising a fourth set of primers that produces an amplification product if SARS-CoV-2 or SARS-CoV-2 and other coronavirus target nucleic acids from the subgenus Sarbecovirus is present in the sample and a fourth detectable probe that specifically hybridizes to the fourth amplification product.

26. The method of claim 25, wherein the fourth set of primers comprises a forward primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, and a reverse primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20; the fourth detectable probe comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 24-26 and 32.

27. The method of claims 26, wherein the fourth set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 5 and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 15 and the fourth detectable probe for detecting the amplification product comprises an oligonucleotide sequence of SEQ ID NO: 25 or 32.

28. ~~The method of any one of claims 20 and 22 to 24 further comprising a fourth set of primers that produces an amplification product if SARS-CoV-2 target nucleic acids are present in the sample, wherein the fourth set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 40 and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 41 and the fourth detectable probe for detecting the amplification product comprises an oligonucleotide sequence of SEQ ID NO: 43.~~
29. ~~A kit for simultaneously detecting one or more nucleic acids of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), one or more nucleic acids of influenza A and one or more nucleic acids of influenza B in a sample comprising:~~
- a) ~~a first set of primers that produces a first amplification product if SARS-CoV-2 is present in the sample, a second set of primers that produces a second amplification product if influenza A is present in the sample, and a third set of primers that produces a third amplification product if influenza B is present in the sample;~~
- b) ~~three or more detectable probes comprising a first detectable probe that specifically hybridizes to the first amplification product, a second detectable probe that specifically hybridizes to the second amplification product, and a third detectable probe that specifically hybridizes to the third amplification product.~~
30. ~~The kit of claim 29, wherein the first set of primers comprises a first primer comprising a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6 and 27-31, 40, or a complement thereof, and a second primer comprising a second oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-20, and 41, or a complement thereof; and wherein the first detectable probe of the one or more detectable probes comprises a third oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-26, 32, and 42-43 or a complement thereof.~~
31. ~~The kit of claims 30, wherein the first set of primers comprises a forward primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-3, 27-31, and a reverse primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-14; and wherein the first detectable probe comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-23, and 42.~~
32. ~~The kit of claim 31, wherein the first set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 1 or 27 and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 7, and the first detectable probe for detecting the amplification product comprises an oligonucleotide sequence of SEQ ID NO: 21 or 42.~~

33. ~~The kit of any one of claims 29 to 32, wherein the second set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 33, and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 34; and the third set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 36, and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 37; and wherein the second detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 35 or 44, and the third detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 38 or 45.~~

34. ~~The kit of any one of claims 29 and 31 to 33 further comprising a fourth set of primers that produces a fourth amplification product if SARS-CoV-2 or SARS-CoV-2 and other coronavirus target nucleic acids from the subgenus Sarbecovirus is present in the sample and a fourth detectable probe that specifically hybridizes to the fourth amplification product.~~

35. ~~The kit claim 34, wherein the fourth set of primers comprises a forward primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, and a reverse primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20, and the fourth detectable probe comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 24-26, and 32.~~

36. ~~The kit of claim 35, wherein the fourth set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 5 and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 15 and the fourth detectable probe for detecting the amplification product comprises an oligonucleotide sequence of SEQ ID NO: 25 or 32.~~

37. ~~The kit of any one of claims 29 and 31 to 33 further comprising a fourth set of primers that produces a fourth amplification product if SARS-CoV-2 target nucleic acids are present in the sample, wherein the fourth set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 40 and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 41 and the fourth detectable probe for detecting the amplification product comprises of an oligonucleotide sequence of SEQ ID NO: 43.~~