

---

Methods and reagents for diagnosis of SARS-CoV-2 infection

---

The present invention relates to a method for diagnosing a SARS-CoV-2 infection comprising the step of detecting the presence or absence of an antibody to SEQ ID NO: 1, preferably IgA class antibody, in a sample from a subject, a method for the differential diagnosis of a coronavirus infection, a use of  
5 an antibody to SEQ ID NO:1, preferably IgA class antibody for diagnosing a SARS-CoV-2 infection or for the differential diagnosis of a coronavirus infection, preferably for distinguishing between a SARS-CoV-2, MERS, NL63, 229E, OC43 and HKU1 infection, and a kit comprising a polypeptide comprising SEQ ID NO:1 or a variant thereof, preferably coated to a diagnostically useful carrier and one or more, preferably all reagents from the group comprising an antibody to SEQ ID NO:1, a washing buffer, a  
10 means for detecting the presence of an antibody, preferably IgA class antibody, preferably a secondary antibody binding specifically to IgA class antibodies, preferably comprising a detectable label, and a dilution buffer.

At the end of 2019, a rising number of pneumonia patients with unknown pathogen emerged from  
15 Wuhan, the capital of Hubei province, China, to nearly the entirety of China. A novel coronavirus was isolated and based on its phylogeny, taxonomy and established practice, the Coronavirus Study Group (CSG) recognized it as a sister to severe acute respiratory syndrome coronavirus (SARS-CoV-1) and labeled it as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although SARS-CoV-2 is generally less pathogenic than SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV), it has a relatively high transmissibility. Since symptoms may be mild and may be  
20 confused with a cold, there is the danger that patients may be unaware that they have been infected and may help the virus spread further.

SARS-CoV-2 is a coronavirus which has four major structural proteins, specifically the spike (S),  
25 envelope (E), membrane (M) and nucleocapsid (N) proteins. The N protein holds the RNA genome, while the other three structural proteins are components of the viral envelope. The S protein is responsible for allowing the virus to attach and fuse to the membrane of a host cell. It comprises an S1 domain which mediates the attachment and an S2 domain which mediates the fusion of the viral cellular membrane with the host cell. The S1 domain comprises the receptor binding domain (RBD),  
30 the binding site to the receptor angiotensin converting enzyme 2 (ACE2) on human host cells. Therefore, the RBD is a binding site of neutralizing antibodies which block the interaction between the virus and its host cells, thus conferring immunity. By contrast to SARS-CoV-1 and SARS-CoV-2, which are associated with a high mortality and severe illness, other coronaviruses exist which are associated with a mild and passing illness, such as coronaviruses 229E, NL63, OC43 and HKU1.  
35 These coronaviruses are frequently associated with common cold, in particular among children.

Owing to the high transmissibility and health impact, reliable methods for the diagnosis of SARS-CoV-2 infection are paramount. In the past, a combination of serological and real-time reverse transcrip-

tion-polymerase chain reaction (RT-PCR) methods were used to detect and confirm infections with coronaviruses.

RT-PCR methods are based on the detection of one or more nucleic acids of the coronavirus of interest, more specifically of the viral ribonucleic acid (RNA). RT-PCR-based methods are rapid and, based on samples from throat or nasal pharyngeal swabs, can be used during the first week of illness for the detection of SARS-CoV-2. However, their usefulness very much depends on how long the nucleic acid is detectable in samples, which varies from virus to virus. In particular, a negative result from a diagnostic test performed on a sample obtained at an early stage of the disease may be meaningless. Moreover, generally a sample from the upper respiratory tract of the patient is required. Improper or insufficient recovery of such a sample, prolonged transportation time and associated degradation of the viral RNA, or instrument malfunction may also lead to false-negative results. Therefore, several samples should be examined, one from week 1 of the infection and a follow-up sample obtained 3 to 4 weeks later.

Corman *et al.* published a real-time RT-PCR based assay for the detection of SARS-CoV-2 (Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill.* 2020;25(3):2000045. doi:10.2807/1560-7917.ES.2020.25.3.2000045), first published in January 2020 by the WHO on their webpage.

Serological tests are also frequently used. These tests are based on the detection of antibodies directed against certain virus-specific antigens. However, serological assays also suffer from severe impediments:

One frequently observed issue is their limited sensitivity. In order for serological assays to yield a positive result, the presence of virus-antigen specific antibodies in the patient's blood is required.

Generally, the time point from which an antibody is first produced and detectable after an infection with a new virus cannot be predicted, because it depends on a multitude of factors, including the virus itself, the viral load *i.e.*, the quantity of virus in a given amount of patient's blood, patient-related factors such as age, gender, health condition, immune status, etc., the immunoglobulin class of the antibody of interest, and the antigen target chosen to set up a diagnostic test. Particularly at very early stages of the disease, *i.e.* before the patient's body has been able to mount a specific antibody response in detectable quantities, serological assays may give a negative result, despite an ongoing infection. Further serological testing, taking into account whether an antibody to be detected belongs to a certain immunoglobulin (Ig) class, may help monitor the course of the disease and distinguish an acute from a past infection or a vaccination. Most serological assays currently available for detection of viral infections including SARS-CoV-1 infection hence focus on the detection of IgG, or IgG and IgM class antibodies. However, besides their risk of yielding false-negative results specifically in very early stages of infection due to the intrinsic delay of antibody response, serological assays for diagnosing SARS-CoV-1 infection are also prone to false-positive results, presumably due to a high seroprevalence in the population of antibodies against common seasonal coronaviruses (CoV) and the associated presence of cross-reactive antibodies against conserved parts of immunogenic virus proteins. This cross-reactivity against such antigenically closely related seasonal coronaviruses also disallowed

- a differential diagnosis, *for example* a differentiation of the life-threatening variant SARS-CoV-1 from other coronaviruses. Challenges and pitfalls of serological assays for diagnosing and differentiating coronaviruses, specifically for diagnosing SARS-CoV-1, have been reviewed in Meyer, Drosten & Müller, *Virus Research* 194 (2014); 175-186. These multiple challenges associated with the serological diagnosis of earlier coronaviruses, e.g. poor sensitivity in early stages of infection; and poor specificity caused by the presence of cross-reactive antibodies, also apply to the development of diagnostic assays for detection of the recently emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
- In view of the current severe threat posed by the ongoing global SARS-CoV-2 pandemic and the above mentioned issues of available assays for diagnosing a corresponding infection, there is an urgent need in the art for improved, in particular more sensitive, more specific, and hence more reliable means and methods for diagnosing a SARS-CoV-2 infection, particularly for the early diagnosis of a SARS-CoV-2 infection, and for differentiation thereof from infections with other coronaviruses, such as the common seasonal coronaviruses, in order to enable a more targeted virus-specific and hence more effective treatment.

- The present invention addresses this need by providing diagnostic means and methods overcoming these previous impediments.
- Specifically, the present invention addresses, without intended to be limiting, the following problems:
- One problem addressed by the present invention is to provide an assay and reagents for the early serological detection of SARS-CoV-2. This is particularly important, as patients may still suffer from active disease and be contagious, even if a negative PCR result was obtained. N.B. that the Center for Disease Control and Prevention (CDC) recommends that patients remain isolated for at least 10 days since symptom onset and up to 20 days in cases of severe illness.
- Another problem addressed by the present invention is to provide an assay that may be used to distinguish known coronavirus infections, in particular a SARS-CoV-2 infection from other coronavirus infections, preferably coronaviruses associated with mild cold-like symptoms or other pathogens or causes of such symptoms. Preferably, the infections can be distinguished at an early stage.
- Another problem addressed by the present invention is to provide an assay with high optimized reliability, in particular with regard to sensitivity and/or specificity, preferably sensitivity.
- Another problem addressed by the present invention is to provide an assay with high sensitivity, for patients lacking antibodies to the SARS-CoV-2 N protein or lacking IgM class antibodies.
- Another problem addressed by the present invention is to provide an assay with long-lasting high sensitivity.

- Previous assays for diagnosing coronavirus infections are described, for example, in the following documents: WO2014/045254 discloses assays for diagnosing a Middle East respiratory syndrome-related coronavirus (MERS-CoV) infection, including serological assays wherein antibodies against viral proteinaceous antigens are detected in a sample. However, no practical evidence of such an assay is shown. US2005/0112559 discloses SARS-CoV-1-related methods and agents. The nucle-

ocapsid (N) protein is considered the major diagnostic antigen. WO2005/118813 discloses a variant of SARS-CoV-1-related S protein and the detection of its presence, or the presence of antibodies binding to it, in samples. US2006/0188519 discloses peptides for the diagnosis of a SARS-CoV-1 infection. Hsueh *et al.* reported that IgG could be detected as early as four days after the onset of a SARS-CoV-1 infection, simultaneously as or one day *earlier* than IgM and IgA (Hsue, P. R., Huang, L. M., Chen, P. J., Kao, C. L., and Yang P. C. (2004) Chronological evolution of IgM, IgA, IgG and neutralization antibodies after infection with SARS-associated coronavirus, Clinical Microbiology and Infection, 10(12), 1062-1066). Reusken *et al.* disclose the specific, but not necessarily sensitive detection of antibodies to a SARS-CoV-2 spike protein fragment (Specific serology for emerging human coronaviruses by protein microarray. Euro Surveill. 2013;18(14). Yu *et al.* (2020) Measures for diagnosing and treating infections by a novel coronavirus responsible for a pneumonia outbreak originating in Wuhu, China, Microbes and Infection 22 (2020), 74-79 disclose, without pointing to specific antigens, that immunological methods may be used for detecting a SARS-CoV-2 infection, but are associated with poor sensitivity and specificity.

The above-discussed problems are solved by the present invention, as described in the present specification and in the claims.

In a first aspect, the invention provides a method for diagnosing a SARS-CoV-2 infection, comprising the step of detecting the presence or absence of an antibody to SEQ ID NO:1, preferably IgM, IgG and/or IgA class antibody, more preferably IgA class antibody, in a sample from a subject. Whenever reference to a SEQ ID NO is made throughout this specification and the SEQ ID NO denotes an amino acid sequence, it is intended to denote a polypeptide comprising said corresponding amino acid string even if in that connection the term "polypeptide" is not specifically mentioned. The term "comprising" has two meanings in connection with this invention, namely "containing" and "consisting of."

In a second aspect, the invention provides a method for the differential diagnosis of a coronavirus infection, preferably for distinguishing between a SARS-CoV; preferably SARS-CoV-2; MERS, NL63, 229E, OC43 and HKU1 infection, comprising the step of detecting the presence or absence of an antibody to SEQ ID NO: 1 in a sample from a subject, preferably an IgA and/or IgG class antibody, more preferably an IgA class antibody.

In a preferred embodiment, the presence of an IgG and/or IgM class antibody to SEQ ID NO: 1 is detected in addition to an IgA class antibody to SEQ ID NO: 1.

In accordance with the present invention, the "subject", or interchangeably referred to herein as "patient", refers to a mammal, preferably a human, but may alternatively also refer to a different mammal, such as a non-human primate or other mammalian animal or even a non-mammalian animal capable of producing an antibody to SEQ ID NO: 1 or variant thereof.

In accordance with the present invention, the term "sample" from a subject may be a sample of any bodily fluid or tissue of said subject that may comprise an antibody. Exemplary bodily fluids include for example blood, saliva, nasal mucus or lymph fluid. In a preferred embodiment, the sample is a blood sample, preferably selected from the group comprising whole blood, serum, plasma, capillary blood, arterial blood, venous blood or any mixture thereof. The capillary blood is preferably in the form of a

dried blot spot, which may be prepared by the patient, sent to the lab, followed by extraction of the blood. The skilled person is aware of various means and methods that may be applied to obtain a sample from a subject suitable for the purposes of the herein disclosed methods, products and uses and in the required quantities. In certain embodiments, the sample may be obtained from the subject  
5 by a physician, whereas in other cases, the sample may be obtained by the subject itself, for example, by using minimal invasive means, such as finger pricking to draw blood ("finger-stick blood").

It is also understood that, for the purposes of the herein disclosed invention, the sample may originate from a single subject, *i.e.*, a single individual, but may alternatively also comprise samples from more  
10 than one subject, wherein said samples from more than one subject are pooled into a single sample. For example, in certain cases, it might be more efficient in terms of resources and experimental time, to first analyze a pooled sample comprising samples from a group of subjects (e.g. members of a common household, school class, sports team, or same company department), and only in case of the detection of the presence of an antibody to SEQ ID NO: 1 or a variant thereof, to conduct a second  
15 analysis wherein samples from individual subjects are assayed separately.

Various methods or uses according to the invention can be conducted with a sample from a subject as described herein. These methods or uses can also be characterized as "*in vitro*" methods or "*in vitro*"  
20 uses.

In accordance with the present invention, the term "secondary antibody" in its broadest sense is to be understood to refer to any kind of "binding moiety", preferably binding protein, capable of specific binding to an IgA, IgG and/or IgM class antibody or a fragment thereof such as a constant domain of a particular Ig class of a selected species, preferably human species. Non-limiting examples of binding  
25 moieties include antibodies, for example antibodies immunologically or genetically derived from any species, for example human, chicken, camel, llama, lamprey, shark, goat, rodent, cow, dog, rabbit, etc., antibody fragments, domains or parts thereof, for example Fab, Fab', F(ab')<sub>2</sub>, scFab, Fv, scFv, VH, VHH, VL, VLRs, and the like, diabodies, monoclonal antibodies (mAbs), polyclonal antibodies (pAbs), mAbdAbs, phage display-derived binders, affibodies, heteroconjugate antibodies, bispecific  
30 antibodies, evibodies, lipocalins, anticalins, affibodies, avimers, maxibodies, heat shock proteins such as GroEL and GroES, trans-bodies, DARPin, aptamers, C-type lectin domains such as tetranectins; human  $\gamma$ -crystallin and human ubiquitin-derived binders such as affilins, PDZ domain-derived binders; scorpion toxin and/or Kunitz-type domain binders, fibronectin-derived binders such as adnectins, receptors, ligands, lectins, streptavidin, biotin, including derivatives and/or combinations thereof such  
35 as bi-/multi-specific formats formed from two or more of these binding molecules. Various antibody-derived and alternative (*i.e.* non-antibody) binding protein scaffolds including methods of generation thereof are known in the art (e.g. reviewed in Chiu ML *et al.*, Antibodies (Basel), (2019);8(4):55; Simeon R. & Chen Z., Protein Cell. (2018);9(1):3-14; and Chapter 7 – Non-Antibody Scaffolds from Handbook of Therapeutic Antibodies (2007) edited by Stefan Dübel.  
40 All these types of molecules are well known in the art. Some examples are described in more detail herein below.

"Eivobodies" are engineered binding proteins derived from the variable(V)-set Ig-like scaffold of the T-cell surface receptor Cytotoxic T Lymphocyte-associated Antigen 4 (CTLA-4). Loops corresponding to CDRs of antibodies can be substituted with heterologous sequences to confer different binding properties. Methods of making Eivobodies are known in the art and are described, for example, U.S. Patent No. 7,166,697.

"Lipocalins" are a family of extracellular proteins which transport small hydrophobic molecules such as steroids, bilins, retinoids and lipids. They have a rigid beta-sheet secondary structure with a number of loops at the open end of the conical structure which can be engineered to bind to different target antigens. "Anticalins", also termed "Affilins", in accordance with the present invention, are between 160-180 amino acids in size, and are derived from lipocalins (Rothe C & Skerra A., *BioDrugs*. (2018);32(3):233-243; Gebauer M & Skerra A, *Curr Opin Biotechnol*. (2019); 60:230-241).

"Affibodies" are a family of antibody mimetics that is derived from the Z-domain of staphylococcal protein A. Affibodies are structurally based on a three-helix bundle domain. An affibody has a molecular mass of around 6 kDa and is stable at high temperatures and under acidic or alkaline conditions. Target specificity is obtained by randomization of amino acids located in two alpha-helices involved in the binding activity of the parent protein domain (Feldwisch, J & Tolmachev, V. (2012) *Methods Mol. Biol.* 899:103-126). Methods of making affibodies are known in the art and are described in Wikman M *et al.*, *Protein Eng Des Sel.* (2004);17(5):455-62.

"Avimers", short for avidity multimers), are a class of artificial multi-domain proteins which specifically bind certain antigens via multiple binding sites. This protein is also known as "maxibody" or low-density lipoprotein receptor (LDLR) domain A. It consists of two or more (poly)peptide sequences, which are based on A domains. The A domains are 30 to 35 amino acids scaffolds (~4 kDa) derived from extracellular cysteine-rich cell surface receptor proteins and stabilized by disulfide bond formation and complexation of calcium ions. The scaffold structure is maintained by 12 conserved amino acids, leaving all the remaining non-conserved residues amenable to randomization and ligand binding. Avimers are highly thermostable. Due to their small size, avimers often consist of multiple A-domains with each binding to a different site on the target, thereby achieving increased affinity through avidity (Silverman J *et al.* (2005), *Nat Biotechnol* 23:1556–1561).

"DARPPins" are designed ankyrin repeat domains and based on tightly packed ankyrin repeats, each forming a  $\beta$ -turn and two antiparallel  $\alpha$ -helices. DARPPins usually carry three repeats corresponding to an artificial consensus sequence, whereby a single repeat typically consists of 33 amino acids, six of which form the binding surface. During recombinant library design, these sites are used to introduce the codons of random amino acids. DARPPins are typically formed by two or three of the binding motifs contained between the N- and C-terminal motifs shielding the hydrophobic regions. DARPPins are small proteins (~14-18 kDa) that are extremely thermostable and resistant to proteases and denaturing agents (Plückthun A., *Annu Rev Pharmacol Toxicol*. (2015);55:489-511).

"Kunitz-type domain binders" are ~60-amino-acid polypeptides (~7 kDa) derived from the active motif of Kunitz-type protease inhibitors such as aprotinin (bovine pancreatic trypsin inhibitor), Alzheimer's amyloid precursor protein, and tissue factor pathway inhibitor. The hydrophobic core of the Kunitz domain is composed of a twisted two-stranded antiparallel  $\beta$ -sheet and two  $\alpha$ -helices stabilized by three pairs of disulfide bonds. Residues in the three loops can be substituted without destabilizing the

structural framework (Hosse RJ *et al.* (2006). Protein Sci 15:14-27; Simeon R. & Chen Z. Protein Cell. (2018);9(1):3-14).

“Adnectins” is a class of binding proteins having a scaffold which consists of a backbone of the natural amino acid sequence of the 10th domain of the 15 repeating units of human fibronectin type III (FN3).

5 The molecule adopts a  $\beta$ -sandwich fold with seven strands connected by six loops similar like an immunoglobulin domain, but without any disulfide bonds. Three loops at one end of the  $\beta$ -sandwich can be engineered to enable an adnectin to specifically recognize a target of interest. Non-loop residues have also been found to expand the available binding footprint. Ligand-binding adnectin variants with binding affinities in the nanomolar to picomolar range have been selected via mRNA,  
10 phage, and yeast display (Hackel BJ, *et al.* (2008) J Mol Biol 381:1238–1252).

Means and methods for developing, screening and identification of suitable binding molecules of various scaffolds, including, without being limiting, those described herein above, toward desired target structures, such as the IgA and/or IgG class antibodies, are well known and routinely employed in the art. Exemplary nowadays routinely-performed methods include, without intended to being  
15 limiting, high-throughput (HT) combinatorial library-based display and selection methods, such as phage display, ribosome display, mRNA display, and cell surface display (e.g. yeast display).

In a preferred embodiment, the secondary antibody is an immunoglobulin (Ig), preferably IgG raised in a non-human species, wherein said secondary antibody specifically binds immunoglobulins of one or  
20 more specific Ig classes or fragments thereof (e.g. a constant domain of a particular Ig class) of another selected species, preferably human species. An example is a polyclonal antibody raised in goat that specifically recognizes human IgA (*i.e.*, a polyclonal goat anti-human IgA antibody). In another preferred embodiment, the secondary antibody is a monoclonal antibody that specifically binds immunoglobulins of one or more specific Ig classes or fragments thereof (e.g. a constant  
25 domain of a particular Ig class) of another selected species, preferably human species. Means and methods for producing (mono- or polyclonal) antibodies capable of specific binding of one or more selected target antigens are well known in the art.

In certain embodiments, the secondary antibody may be chosen to specifically bind to only one, only  
30 two, or all three classes of Ig antibodies, *i.e.* IgA, IgG and/or IgM. In certain embodiments, instead of using only one single kind of secondary antibody, a mixture of several different secondary antibodies may be used, wherein the different secondary antibodies either bind to the same one or different Ig classes (e.g., a mixture of different antibodies (e.g., polyclonal antibodies) all binding to IgA), or to, e.g. IgA and IgG or IgM or wherein the different secondary antibodies bind to different individual target  
35 structures (e.g. one kind of secondary antibody specifically binding to IgA, and another specifically binding to IgG).

In a preferred embodiment, the antibody to SEQ ID NO: 1 is detected using a labeled secondary antibody, preferably a labeled secondary antibody binding to IgA, IgG and/or IgM class antibodies.

As used herein, the term "labeled", with regard to the secondary antibody, is intended to embrace such embodiments wherein the secondary antibody is labelled by coupling, preferably physically linking, a detectable substance, such as a radioactive agent or a other molecule providing a detectable signal, such as, without intended to being limiting, a fluorophore, such as a small organic chemical fluorophore or fluorescent protein, or an enzymatically active label, i.e. an enzyme, such as alkaline phosphatase, whose presence can be assessed and optionally be quantified based on its reactivity with, and/or conversion of, a substrate substance. Various suitable detectable labels are known in the art and some of which are also described herein below.

10 In a preferred embodiment, the antibody, preferably IgA, IgG or IgM class antibody, is detected using a method selected from the group comprising colorimetry, immunofluorescence, detection of enzymatic activity, chemiluminescence and radioactivity.

In a preferred embodiment, the infection is detected at an early stage. In a preferred embodiment, the term "early stage", as referred to herein in the context of the course of a SARS-CoV-2 infection, refers to, in such cases where the time-point of infection is known, e.g. infection of a subject in a laboratory experiment, any time point within less than 14 days, preferably less than six days after the time-point of infection, i.e. the time-point of initial contact between the virus and the subject's body. In another preferred embodiment, the term "early stage", as referred to herein in the context of the course of a SARS-CoV-2 infection, refers to any time-point within less than 14 days, preferably less than six days after the onset of illness (also termed in the field of virology as "days post onset of illness" (dpoi)), i.e. after the first occurrence of one or more typical SARS-CoV-2 infection-associated clinical symptoms, such as defined herein. The person skilled in the art is aware that upon infection, components of a corona virus can be detected directly, for example using PCR for detecting nucleic acid and immunoassays for detecting virus antigens in samples. After the peak of the infection with the maximum virus load, possibly only few days after the first contact of the patient with the virus, the concentration of virus decreases which makes the direct detection increasingly difficult and finally impossible, at the latest when the virus is absent in samples. Meanwhile, as soon as virus components are present in the blood of the patient, the production of antibodies against virus components is triggered. In another preferred embodiment, the term "early stage" refers to the time window between the first contact between virus and patient or the onset of illness, preferably the onset of illness, and the production of detectable IgG antibodies to the virus, more preferably before IgG antibodies are the dominant immunoglobulin class, i.e. are present at a higher concentration than IgA and IgM, most preferably before the peak of IgG concentration is reached. Thus, the detection of IgA and IgM antibodies can be a contribution to the diagnosis of an infection that is still acute, with detectable symptoms or not, but before the full IgG immune response is developed.

In a 3<sup>rd</sup> aspect, the invention provides a use of an antibody to SEQ ID NO: 1, preferably IgA class antibody, for diagnosing a SARS-CoV-2 infection or for the differential diagnosis of a coronavirus infection, preferably for distinguishing between a SARS-CoV, preferably SARS-CoV-2, MERS, NL63, 229E, OC43 and HKU1 infection.

In a preferred embodiment, the use is for the early diagnosis or early stage diagnosis of a SARS-CoV-2 infection.

In a 4<sup>th</sup> aspect, the invention provides a kit comprising a polypeptide comprising SEQ ID NO: 1 or a variant thereof, preferably coated to a diagnostically useful carrier and one or more, preferably all  
5 reagents from the group comprising an antibody to SEQ ID NO: 1, a washing buffer, a means for detecting the presence or absence of an antibody to SEQ ID NO: 1, preferably an IgA, IgG and/or IgM class antibody, more preferably an IgA class antibody, preferably a secondary antibody binding specifically to IgA IgG and/or IgM, more preferably IgA class antibodies, preferably comprising a detectable label, and a dilution buffer.

10

In a preferred embodiment, the diagnostically useful carrier is selected from the group comprising a bead, preferably a magnetic or paramagnetic bead, a test strip, a microtiter plate, a membrane, preferably from the group comprising a nitrocellulose membrane, western blot, line blot and dot blot, a lateral flow device, a glass surface, a slide, a microarray, a chromatography column and a biochip and  
15 is preferably a microtiter plate.

In a preferred embodiment, the kit comprises two or more, preferably three or more calibrators.

In a preferred embodiment, each calibrator is a recombinant antibody binding to SEQ ID NO: 1 which is preferably recognized by a secondary antibody binding to IgA class antibodies. In certain preferred  
20 embodiments, the calibrator is a recombinant antibody binding to SEQ ID NO: 1 or variant thereof, and said recombinant antibody is preferably an immunoglobulin (Ig) of the same Ig class as the Ig class to which the secondary antibody binds.

In a 5<sup>th</sup> aspect, the invention provides a use of a polypeptide comprising SEQ ID NO: 1 or a variant thereof and/or an antibody to SEQ ID NO: 1, preferably IgA class antibody, for the manufacture of a diagnostic kit. In accordance with this aspect, the invention particularly relates to a use of a polypeptide  
25 comprising SEQ ID NO: 1 or a variant thereof for the manufacture of a diagnostic kit. Moreover, the invention also provides a use of an antibody to SEQ ID NO: 1, preferably IgA, IgM and/or IgG, more preferably IgA class antibody, for the manufacture of a diagnostic kit.

The diagnostic kit is preferably for the diagnosis of a SARS-CoV-2 infection, or for the differential  
30 diagnosis of a coronavirus infection, preferably for distinguishing between a SARS-CoV, preferably SARS-CoV-2, MERS, NL63, 229E, OC43 and HKU1 infection. Accordingly, the invention relates to a use of a polypeptide comprising SEQ ID NO: 1 or a variant thereof for the manufacture of a diagnostic kit for the diagnosis of a SARS-CoV-2 infection. Said diagnosis of a SARS-CoV-2 infection may, for example, comprise a step of detecting the presence or absence of an antibody to SEQ ID NO: 1,  
35 preferably an IgA IgM and/or IgG, more preferably IgA class antibody to SEQ ID NO: 1, in a sample from a subject. Said diagnosis of a SARS-CoV-2 infection may also comprise a step of obtaining a sample from a subject, and a step of detecting the presence or absence of an antibody to SEQ ID NO: 1, preferably an IgA, IgM and/or IgG, more preferably IgA class antibody to SEQ ID NO: 1, in the sample from the subject. The invention further relates to a use of a polypeptide comprising SEQ ID  
40 NO: 1 or a variant thereof for the manufacture of a diagnostic kit for the differential diagnosis of a coronavirus infection, preferably for distinguishing between a SARS-CoV, preferably SARS-CoV-2,

- MERS, NL63, 229E, OC43 and HKU1 infection. Said differential diagnosis may, for example, comprise a step of detecting the presence or absence of an antibody to SEQ ID NO: 1 in a sample from a subject, preferably an IgA, IgM and/or IgG, more preferably IgA and/or IgG class antibody to SEQ ID NO: 1, most preferably an IgA class antibody to SEQ ID NO: 1. Said differential diagnosis may also
- 5 comprise a step of obtaining a sample from a subject, and a step of detecting the presence or absence of an antibody to SEQ ID NO: 1 in the sample from the subject, preferably an IgA, IgM and/or IgG, more preferably IgA and/or IgG class antibody to SEQ ID NO: 1, most preferably an IgA class antibody class antibody to SEQ ID NO: 1.
- 10 In accordance with this aspect, the invention likewise provides a polypeptide comprising SEQ ID NO: 1 or a variant thereof for use in diagnosis, for example for use in a diagnostic method practised on the human or animal body. In particular, the invention provides a polypeptide comprising SEQ ID NO: 1 or a variant thereof for use in the diagnosis of a SARS-CoV-2 infection. Said diagnosis of a SARS-CoV-2 infection may, for example, comprise a step of detecting the presence or absence of an antibody to
- 15 SEQ ID NO: 1, preferably an IgA, IgM and/or IgG, more preferably IgA and/or IgG class antibody to SEQ ID NO: 1, most preferably an IgA class antibody to SEQ ID NO: 1, in a sample from a subject. Said diagnosis of a SARS-CoV-2 infection may also comprise a step of obtaining a sample from a subject, and a step of detecting the presence or absence of an antibody to SEQ ID NO: 1, preferably an IgA, IgM and/or IgG, more preferably IgA and/or IgG class antibody to SEQ ID NO: 1, most
- 20 preferably an IgA class antibody to SEQ ID NO: 1, in the sample from the subject. The invention further provides a polypeptide comprising SEQ ID NO: 1 or a variant thereof for use in the differential diagnosis of a coronavirus infection, preferably for distinguishing between a SARS-CoV (preferably SARS-CoV-2), MERS, NL63, 229E, OC43 and HKU1 infection. Said differential diagnosis may, for example, comprise a step of detecting the presence or absence of an antibody to SEQ ID NO: 1 in a
- 25 sample from a subject, preferably an IgA, IgM and/or IgG, more preferably IgA and/or IgG class antibody to SEQ ID NO: 1, most preferably an IgA class antibody to SEQ ID NO: 1. Said differential diagnosis may also comprise a step of obtaining a sample from a subject, and a step of detecting the presence or absence of an antibody to SEQ ID NO: 1 in the sample from the subject, preferably an IgA, IgM and/or IgG, more preferably IgA and/or IgG class antibody to SEQ ID NO: 1, most preferably
- 30 an IgA class antibody to SEQ ID NO: 1.

- The invention further provides an antibody to SEQ ID NO: 1, preferably an IgA, IgM and/or IgG, more preferably IgA and/or IgG class antibody to SEQ ID NO: 1, most preferably an IgA class antibody, for use in diagnosis, for example for use in a diagnostic method practised on the human or animal body.
- 35 In particular, the invention relates to an antibody to SEQ ID NO: 1, preferably an IgA, IgM and/or IgG, more preferably IgA and/or IgG class antibody to SEQ ID NO: 1, most preferably an IgA class antibody, for use in the diagnosis of a SARS-CoV-2 infection. The invention further relates to an antibody to SEQ ID NO: 1, preferably an IgA, IgM and/or IgG, more preferably IgA and/or IgG class antibody to SEQ ID NO: 1, most preferably an IgA class antibody, for use in the differential diagnosis
- 40 of a coronavirus infection, preferably for distinguishing between a SARS-CoV, preferably SARS-CoV-2, MERS, NL63, 229E, OC43 and HKU1 infection.

In a 6<sup>th</sup> aspect, the invention provides a use of a recombinant antibody binding to SEQ ID NO: 1 which is preferably recognized by a secondary antibody binding to IgA class antibodies as a calibrator for the early diagnosis of a SARS-CoV-2 infection.

- 5 In accordance with this aspect, the invention also provides a use of a recombinant antibody, for example a recombinant IgA class antibody, binding to SEQ ID NO: 1 as a calibrator, preferably as a calibrator for the detection of an antibody to SEQ ID NO: 1, preferably an IgA class antibody to SEQ ID NO: 1, in a sample from a subject. Such a calibrator can be used, in particular, in a method of diagnosing a SARS-CoV-2 infection for in a method for the differential diagnosis of a coronavirus
- 10 infection, preferably for distinguishing between a SARS-CoV, preferably SARS-CoV-2, MERS, NL63, 229E, OC43 and HKU1 infection. The invention thus likewise provides a use of a recombinant antibody, for example a recombinant IgA class antibody, binding to SEQ ID NO: 1 as a calibrator, preferably for the diagnosis, particularly for the early diagnosis, of a SARS-CoV-2 infection.

In a preferred embodiment, the human subject is a vaccinated human subject.

- 15 In a preferred embodiment, the method further comprises evaluating the result for the diagnosis. In a preferred embodiment, the method further comprises transferring the result of the diagnosis or the evaluation to a different location.

- Various antigens and antibodies have been used as the basis for the immunological detection of
- 20 coronaviruses, among them the whole virus or any of the structural proteins or fragments thereof as well as antibodies binding to these antigens.

The present invention is based on the inventors' surprising finding that antibodies against SEQ ID NO: 1 can be detected at an early stage of the infection. They may be detected for the purpose of determining the presence of an infection at an early stage of the infection.

- 25 In other words, the results disclosed herein demonstrate a superior sensitivity, preferably as *measured by the number of correctly positive determined samples relative to the total number of samples examined*, for diagnosing a SARS-CoV-2 infection already at early stage of infection. An important contribution to the observed sensitivity increase arises from the surprising finding that IgA class antibodies to SEQ IDNO:1 become detectable earlier than antibodies of other Ig classes, such as IgG antibodies in many patients, including a subpopulation of patients who lack detectable IgM antibodies.
- 30 Moreover, the invention is based on the surprising finding that antibodies to SEQ ID NO: 1, preferably IgG and/or IgA, persist longer and are detectable for a longer period of time than antibodies to SARS-CoV-2 N protein.

- Also, there is surprisingly low cross reactivity with a range of antibodies in samples from patients
- 35 infected with other coronaviruses, preferably other than SARS-CoV-1 and SARS-CoV-2, which contributes to the superior specificity, preferably as *measured by the high number of correctly identified negative samples, i.e. low number of false-positive identified samples relative to the total number of samples examined*, of detection of antibodies to SEQ ID NO: 1. In particular, the inventors have found that the specificity of SEQ ID NO1 as an antigen is superior compared to SEQ ID NO: 33
- 40 (S2 domain) (Okba et al., Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody

Responses in Coronavirus Disease Patients. Emerg Infect Dis. 2020 Jul;26(7):1478-1488, prepublished on medRxiv 2020.03.18.20038059).

In a preferred embodiment, the term „diagnosis“, as used herein, is to be understood in its broadest possible sense and may refer to any kind of procedure aiming to obtain information instrumental in the assessment whether a patient suffered, suffers or is likely or more likely than the average or a comparative subject, the latter preferably having similar symptoms, to suffer from a certain disease or disorder in the past, at the time of the diagnosis or in the future, to find out how the disease is progressing or is likely to progress in the future or to evaluate the responsiveness of a patient or patients in general with regard to a treatment, preferably a vaccine, or to find out whether a sample is from such a patient. Such information may be used for a clinical diagnosis but may also be obtained by an experimental and/or research laboratory for the purpose of general research, for example to determine the proportion of subjects suffering from the disease in a patient cohort or in a population. In other words, the term “diagnosis” comprises not only diagnosing, but also prognosticating and/or monitoring the course of a disease or disorder, including monitoring the response of one or more patients to the administration of a drug or candidate drug, for example to determine its efficacy. Again, the early emergence and/or the long persistence of IgA and/or IgG antibodies to SEQ ID NO:1 may be exploited. While the result may be assigned to a specific patient for clinical diagnostic applications and may be communicated to a medical doctor or institution treating said patient, for example by telephone, fax, letter or in an electronic format such as e-mail or using a data base, this is not necessarily the case for other applications, for example in diagnostics for research purposes, where it may be sufficient to assign the results to a sample from an anonymized patient or a patient cohort. In a preferred embodiment, the person to be diagnosed, *i.e.*, the “subject” or “patient”, is an anonymous blood donor whose blood may be donated or used to obtain therapeutically useful antibodies. Preferably, the disease is a SARS infection, including SARS-CoV-1 and SARS-CoV-2, more preferably a SARS-CoV-2 infection.

In a preferred embodiment, the methods, products and uses according to the present invention may be used for interaction studies, including determining whether a drug candidate or other compound, including a candidate vaccine, or any bodily compound such as a blocking antibody is present and may interfere with the binding of an antibody to SARS-CoV-2 or may affect any downstream process. In a preferred embodiment, they may be used for monitoring the immune response, more preferably the emergence and/or titer of antibodies to a polypeptide comprising, preferably consisting of SEQ ID NO: 1, following the administration of an immunogenic composition comprising a polypeptide comprising SEQ ID NO: 1 or an immunogenic variant thereof, for example to a mammal, which may be a mammal other than a human, such as a laboratory animal. The detection of IgA and/or IgG antibodies, preferably in the late phase of the immunization of a subject, more preferably for the purpose of increasing the sensitivity of the detection or optimizing the sensitivity of the detection, is particularly preferred. In a preferred embodiment, the immunization is the result of a SARS-CoV-2 infection or the result of an administration of a vaccine comprising SEQ ID NO: 1 or a variant thereof. The increase or optimization may be in comparison to other antibodies against SARS-CoV-2 or components thereof,

for example SEQ ID NO: 30 (N protein), preferably IgG class antibodies to SEQ ID NO:30. In a preferred embodiment, the term "late phase", as used herein, refers to the period beginning with day 20, 30, 40, 50, 60, 61, 70, 80 or 90 after the onset of the disease or the first administration of a vaccine, preferably day 60. Preferably it lasts for 28 days, 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 24, 36, 48, 60  
 5 or more months, preferably 3 or more months. In a preferred embodiment, a method or use may be for the long-term monitoring of an immunization. The immunization may be the result of a SARS-CoV-2 infection or of a vaccination, preferably with SEQ ID NO:1 or a variant thereof. At least one sample obtained during the late phase is analyzed, preferably two or more. A sample may be obtained and analyzed at least once a week or once a month during the late phase.

10

The subject is likely or more likely to suffer from a SARS-CoV-2 infection if an IgA and/or IgG and/or IgM antibody to SEQ ID NO: 1 is detected in a sample from them. The person skilled in the art is familiar with general principles in virology regarding the interpretation of results reflecting the presence or absence of antibodies (for example, Doerr, H. W., and Gerlich, W., Medizinische Virologie: Grundlagen, Diagnostik, Prävention und Therapie, Thieme 2010, for example Fig. 9.7 therein). Briefly,  
 15 the first detection of specific antibodies to virus-specific antigens may be used for the initial diagnosis of an infection. IgA antibodies are usually not diagnostically relevant except for specific cases, such as in cases of infections by enteroviruses (Gressner/Arndt, Lexikon der Medizinischen Labordiagnostik, 2. Auflage, Springer, 2013, page 1387), but if they appear, their titer will typically be lower than IgM and IgG class antibodies, and they may appear later. A low concentration of IgG class antibodies,  
 20 preferably in the absence of IgM and IgA class antibodies, indicates an immunization in the past. An increasing IgG class antibody titer or high concentration may be indicative of an acute infection or reinfection. In a preferred embodiment, the presence of an antibody to SEQ ID NO: 1 indicates an immunization, either as a result of a previous or ongoing SARS-CoV-2 infection or a vaccination. In a preferred embodiment, a successful vaccination with a vaccine comprising a polypeptide comprising  
 25 SEQ ID NO: 1 or a variant thereof (or with a nucleic acid encoding a polypeptide comprising SEQ ID NO: 1 or variant thereof, e.g. a RNA-based vaccine), may be distinguished from a SARS-CoV-2 infection by confirming the absence of antibodies to SARS-CoV-2 antigens other than SEQ ID NO: 1, preferably by confirming the absence of antibodies to SEQ ID NO: 30 (SARS-CoV-2 N protein).

30

The sample is preferably a mammalian sample, *i.e.*, a sample from a mammal, more preferably a human sample, *i.e.*, a sample from a human.

The term "diagnosis" does preferably not imply that the diagnostic methods or agents according to the present invention will be definitive and sufficient to finalize the diagnosis on the basis of a single test,  
 35 let alone parameter, but may refer to a contribution to what is referred to as a "differential diagnosis", *i.e.* a systematic diagnostic procedure considering the likelihood of a range of possible conditions on the basis of a range of diagnostic parameters. According to the invention, it can be distinguished if a patient, preferably one already suspected to have a coronavirus infection, suffers from a SARS,  
 40 preferably SARS-CoV-1 and/or SARS-CoV-2 infection, or another coronavirus infection, preferably from the group comprising MERS, NL63, 229E, OC43 and HKU1. NL63, 229E, OC43 and HKU1 are

associated with a significant number of cases of common cold, hence the differential diagnosis may involve distinguishing between SARS-CoV-1 and/or SARS-CoV-2 and a cold. For example, a patient may initially be suspected of suffering from a coronavirus infection owing to a possible exposure to a risk environment and/or based on common symptoms such as fever, cough and shortness of breath.

5 A PCR (for example Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill.* 2020;25(3):2000045. doi:10.2807/1560-7917.ES.2020.25.3.2000045) and/or an immunoassay may then be carried out. If the PCR is negative, for example because the sample was taken a few days after the infection, the inventive method may be used to detect the presence or absence of an antibody to SEQ ID NO: 1. In addition, the

10 presence or absence of an antibody to an antigen from another coronavirus may be detected, preferably from the group comprising SARS-CoV-1, MERS, NL63, 229E, OC43 and HKU1, more preferably MERS, NL63, 229E, OC43 and HKU1. Antibodies to homologues and variants of SEQ ID NO: 1 from SARS-CoV-2 may be detected. Based on specific clinical symptoms such as headache and body pains, which are more characteristic of SARS-CoV-1, or loss of taste and smell and sore

15 throat, which are more characteristic for SARS-CoV-2, the diagnosis may be finalized. It may be considered whether the patient has been exposed to infected patients. For example, SARS-CoV-1 cases are extremely rare, so many patients suffering from common SARS-CoV-1 and SARS-CoV-2 symptoms in a SARS-CoV-2 pandemic can be assumed to suffer from an infection with the latter coronavirus. Distinction between SARS-CoV-1 and SARS-CoV-2 is possible based on the different

20 time-resolved immunoglobulin (Ig) class signature, in particular the later emergence of IgA class antibodies in SARS-CoV-1 (Hsue, P. R., Huang, L. M., Chen, P. J., Kao, C. L., and Yang P. C. (2004) Chronological evolution of IgM, IgA, IgG and neutralization antibodies after infection with SARS-associated coronavirus, *Clinical Microbiology and Infection*, 10(12), 1062-1066). Antibody levels may be monitored, for example over several weeks, for example to detect the disappearance or emer-

25 gence of an antibody of interest, which may help distinguish a primary and a secondary infection or immunization, for example as a result of vaccination, or recognize an infection with more than one coronavirus.

In a preferred embodiment, the term "SARS-CoV-2", as used herein, refers to a virus characterized by

30 the genome deposited on GenBank under accession code MN908947 or SEQ ID NO: 13, preferably as shown in SEQ ID NO: 13, and derivatives thereof having at least 80, preferably 85, preferably 88, preferably 90, preferably 91, preferably 92, preferably 93, preferably 94, preferably 95, preferably 96, preferably 97, preferably 98, preferably 99, preferably 99.5, preferably 99.8, preferably 99.9 or 99.99 percent sequence identity over the entire genome nucleotide sequence. All data base entries or

35 product codes used herein correspond to the version online at the earliest priority or filing date of the application. For example, for the SARS-CoV-2 genome sequence deposited under accession code MN908947, the version MN908947.3 (published on January 17, 2020) was online at the earliest priority or filing date of the present application. The nucleotide sequence disclose in MN908947.3 is identical with SEQ ID NO13. More preferably, mutants such as those from the group comprising the

40 U.K. variant B.1.1.7, the South African variant B.1.351, the Brazilian variant P.1 and the Mink Variant from Denmark are included.

In a preferred embodiment, the SARS-CoV-2 infection to be diagnosed is or may be associated with the U.K. variant B.1.1.7 characterized by a spike protein having, with reference to SEQ ID NO1, one or more mutations, preferably all mutations from the group comprising deletion(s) in His 54 and/or Val55, Gln486Tyr, Ala555Asp, Asp599Gly and Pro666His. Preferably, a variant of SEQ ID NO1 having one or more mutations, preferably all from the group comprising deletion(s) in His 54 and/or Val55, Gln486Tyr, Ala555Asp, Asp599Gly and Pro666His, is used for reagents, methods and uses according to the present invention. A variant of SEQ ID NO1 comprising all these mutations is represented by SEQ ID NO: 51.

In a preferred embodiment, the SARS-CoV-2 infection to be diagnosed is or may be associated with the South African variant B.1.351 characterized by a spike protein having, with reference to SEQ ID NO1, one or more mutations, preferably all mutations from the group comprising Lys402Gln, Glu469Lys, Gln486Tyr and Asp599Gly. Preferably, a variant of SEQ ID NO1 having one or more mutations, preferably all from the group comprising Lys402Gln, Glu469Lys, Gln486Tyr and Asp599Gly, is used for reagents, methods and uses according to the present invention. A variant of SEQ ID NO1 comprising all these mutations is represented by SEQ ID NO: 52.

In a preferred embodiment, the SARS-CoV-2 infection to be diagnosed is or may be associated with the Brazilian variant P.1 characterized by a spike protein having, with reference to SEQ ID NO1, one or more mutations, preferably all mutations from the group comprising Glu469Lys and Gln486Tyr. Preferably, a variant of SEQ ID NO1 having one or more mutations, preferably all from the group comprising Glu469Lys and Gln486Tyr, is used for reagents, methods and uses according to the present invention. A variant of SEQ ID NO1 comprising all these mutations is represented by SEQ ID NO: 53.

In a preferred embodiment, the SARS-CoV-2 infection to be diagnosed is or may be associated with the Mink Variant from Denmark characterized by a spike protein having, with reference to SEQ ID NO1, one or more mutations, preferably all mutations from the group comprising deletion(s) in His 54 and/or Val55, Asp599Gly and Tyr438Phe. Preferably, a variant of SEQ ID NO1 having one or more mutations, preferably all from the group comprising deletion(s) in His 54 and/or Val55, Asp599Gly and Tyr438Phe, is used for reagents, methods and uses according to the present invention. A variant of SEQ ID NO1 comprising all these mutations is represented by SEQ ID NO: 54.

In a preferred embodiment, the term "diagnosis" means that the method or product or use may be used for aiding in the diagnosis of a disease or identifying a subject with a risk of suffering from a disease. The term "diagnosis" may also refer to a method or agent used to choose the most promising treatment regime for a patient. In other words, the method or agent may relate to selecting a treatment regimen for a subject.

35

In a preferred embodiment, the method according to the present invention comprises the step of providing the diagnostically useful carrier and a sample from a patient suspected of being infected, preferably a mammalian, more preferably a human patient. The carrier is coated with the polypeptide comprising SEQ ID NO: 1 or variant thereof. The carrier may then be contacted with the sample under conditions allowing for binding of any antibodies to the polypeptide comprising SEQ ID NO: 1 or variant thereof. The sample may then be removed and the carrier may be washed to remove any

40

remaining sample. A secondary antibody or similar reagent or means binding to the antibody to be detected and carrying a detectable label may then be contacted with the carrier under conditions allowing formation of a complex between any bound antibody and the secondary antibody. The carrier may be washed then to remove non-bound secondary antibody. Finally, the presence of the antibody is detected by checking whether the secondary antibody may be detected.

In a preferred embodiment, the term "SARS-CoV-2 infection" or similar terms, as used herein, refers to an infection of a subject, preferably a human subject, with SARS-CoV-2, i.e., the presence of the virus, at least temporarily, in the body of said subject, more preferably together with detectable levels of the virus itself and/or one or more biomarker from the group comprising a SARS-CoV-2 polypeptide from the group comprising the structural proteins, in particular S and N, more preferably the S1 domain, and antibodies binding to them, and/or a nucleic acid from SARS-CoV-2, the latter detectable by PCR. The subject may have clinical symptoms, preferably one or more, more preferably all from the group comprising fever, tiredness, dry cough, nasal congestion, runny nose, and sore throat. More severe symptoms include breathing difficulties, chest pain or pressure and sudden confusion. The disease may lead to complications such as pneumonia, acute respiratory distress syndrome, sepsis, septic shock and kidney failure. However, many infected subjects have mild symptoms only and may not even be aware of their infection.

In a preferred embodiment, the method is used more than once to examine samples from the same patient, preferably on different days. For example, the presence or absence of antibodies may be detected on a daily basis over one or two weeks. In a preferred embodiment, at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 samples are examined on different days. In a preferred embodiment, samples are taken at least over a period of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 weeks.

The methods, reagents and uses according to the present invention may also be used for screening the potency and the usefulness of an antiviral drug or a vaccine or vaccine candidate. In this regard, it is of note that the method of the invention has been validated in a vaccinated human, i.e. an antibody to the polypeptide comprising the amino acid sequence of SEQ ID NO: 1 could be detected in a human vaccinated with a composition comprising a polypeptide comprising SEQ ID NO:1. They may be used as part of vaccination trials and studies aiming to confirm the quality of vaccines in human and other subjects including laboratory animals or to confirm that a subject's immune system responds to the administration of a vaccine. Preferably the vaccine is based on SEQ ID NO: 1 or a fragment thereof. The methods, reagents and uses may be used to initially diagnose a patient before the initial administration of a vaccine to check whether said patient is already immunized, for example as a result of a previous infection with SARS-CoV-2 or a previous vaccination. For example, the patient may be selected for inclusion in or exclusion from a study or trial and monitored over time. In particular already infected subjects may be excluded from testing a vaccine candidate. It can be confirmed whether a previous vaccination is still effective, preferably based on the detection of IgA and/or IgG class antibodies to SEQ ID NO:1.

The methods and reagents according to the present invention may also be used for screening whether donated blood is contaminated with coronavirus or whether a blood donor produces antibodies to SARS-CoV-2, preferably SEQ ID NO: 1, which may be extracted from his donated blood, for example for therapeutic uses. Following the detection of the presence or absence of IgG, IgM and/or IgA class antibodies, preferably IgG and IgA, more preferably IgA, to SEQ ID NO: 1, an antibody to SARS-CoV-2, preferably to SEQ ID NO: 1 may be purified from the blood donor's blood, for example by affinity chromatography or by contacting the carrier according to the present invention with the donated blood under conditions that are compatible with the formation of a complex comprising the antibody to SEQ ID NO: 1 and the carrier, removal of any remaining donated blood and separation of the antibody from the carrier. In a preferred embodiment, donated blood is selected from the group comprising whole blood, plasma, and serum, and may contain an anti-clotting reagent. Also a compound selected from the group comprising citrate, phosphate, dextrose and adenine, preferably all of them, may be present.

The antibody to be detected binds preferably specifically to SEQ ID NO: 1. Specific binding preferably means that the binding reaction is stronger than a binding reaction characterized by a dissociation constant of  $1 \times 10^{-5}$  M, more preferably  $1 \times 10^{-7}$  M, more preferably  $1 \times 10^{-8}$  M, more preferably  $1 \times 10^{-9}$  M, more preferably  $1 \times 10^{-10}$  M, more preferably  $1 \times 10^{-11}$  M, more preferably  $1 \times 10^{-12}$  M, as determined by surface plasmon resonance using Biacore equipment at 25 °C in PBS buffer at pH 7.

The teachings of the present invention may not only be carried out using polypeptides having the exact sequences referred to in this application explicitly, such as SEQ ID NO: 1, for example by function, name, sequence or accession number, or 6

In accordance with the invention, the term "variant", as used herein, also refers to at least one fragment of the full length sequence referred to, or a polypeptide comprising said fragment, more specifically to one or more amino acid or nucleic acid sequences which are, relative to the full length sequence, truncated at one or both termini by one or more amino acids. Such a fragment comprises or encodes a (poly-)peptide having at least 10, 15, 25, 50, 75, 100, 150, 200, 250, 300, 400, 500 or 600 successive amino acids of the original sequence or a variant thereof. For example, fragments of SEQ ID NO: 1 include SEQ ID NO: 12 and SEQ ID NO: 31. Two or more copies of a fragment may be fused for increased sensitivity.

It is also understood that the term "variant", as used herein, also embraces such polypeptides or fragments thereof comprising amino acid sequences, preferably a fragment comprising at least 25, more preferably 50, more preferably 200 successive amino acids, that are at least 40, 50, 60, 70, 75, 80, 85, 90, 92, 94, 95, 96, 97, 98 or 99 % identical to the reference amino acid sequence referred to or the fragment thereof, wherein amino acids other than those essential for the biological activity, for example the ability to bind specifically to an antibody of interest, or maintain the fold or structure of the polypeptide may be deleted or substituted and/or one or more such essential amino acids may be replaced in a conservative manner and/or amino acids are added or deleted such that the biological

activity of the polypeptide is at least partially preserved. For example, fragments of SEQ ID NO:1 in comprise SEQ ID NO:5, SEQ ID NO:12, SEQ ID NOs:14-29, SEQ ID NOs:35-37 and SEQ ID NOs:40-43. The state of the art comprises various methods that may be used to align two given nucleic acid or amino acid sequences and to calculate the degree of identity, see for example Arthur Lesk (2008), Introduction to bioinformatics, Oxford University Press, 2008, 3<sup>rd</sup> edition. In a preferred embodiment, the ClustalW software (Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., Higgins, D. G. (2007): Clustal W and Clustal X version 2.0. Bioinformatics, 23, 2947-2948) is used applying default settings.

SARS-CoV-2-related publications on specific amino acid sequences such as Beal *et al.* may aid the skilled one in designing variants (Beal, J., Mitechell, T., Wyschogrod, W., Manthey, J. and Clore, A. (2020) Highly Distinguished Amino Acid Sequences of 2019-nCoV (Wuhan Coronavirus) doi: <https://doi.org/10.1101/2020.01.31.929497>), as well as publications relating to SARS-CoV, for example Hua *et al.* (Hua, R., Zhou, Y., Wang, Y., Hua, Y and Tong, T. (2004) Identification of two antigenic epitopes on SARS-CoV spike protein, BBR 319, 929-935), wherein homologous epitopes may be found and SARS-CoV-2 epitopes be identified on account of their homology. For example, possible epitopes may be derived from SEQ ID NO: 5. Dahlke *et al.* present an epitope mapping based on a microarray comprising overlapping 15mer peptides derived from the S1 polypeptide (Dahlke, C., Heidepriem, J., Kobbe, R., Santer R., Koch, T., Fathi, A., Ly, M. L., Schmiedel, S., Seeberger, P. H., ID-UKE COVID-19 study group, Addo, M. M., and Loeffler, F. F. (2020) <https://doi.org/10.1101/2020.04.14.20059733doi>). More specifically, peptides comprising amino acid sequences SSVLHSTQDLFLPFF (SEQ ID NO: 14, which is 30-44 of SEQ ID NO: 1), TWFHAIHVSGTNGTKRFDNPV (SEQ ID NO: 15, which is 48-68), NVVIKVCFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEG (SEQ ID NO: 16, which is 110-166), DLPQGFSALEPLVDL (SEQ ID NO: 17, which is 200-214), LLALHRSYLTPGDSSSGWTAGAAAY (SEQ ID NO: 18, which is 226-250), QPRTFLLKYNENGTITDAVDCALDP (SEQ ID NO: 19, which is 256-277), NATRFASVYAWNRRKR (SEQ ID NO: 20, which is 328-342), TGKIADYNYKLPPDDF (SEQ ID NO: 21, which is 399-414), YNYLYRLFRKSNLKP (SEQ ID NO: 22, which is 434-448), FGRDIADTTDAVRDPQTLEILDI (SEQ ID NO: 23, which is 550-572), SNQVAVLYQDVNCTE (SEQ ID NO: 24, which is 590-604), AGCLIGAEHVNNSEYCDIP (SEQ ID NO: 25, which is 632-650) of S1 protein were identified as IgA-reactive epitopes. Peptides comprising amino acid sequences YNSASFSTFKCYGVS (SEQ ID NO: 26, which is 354-368), STGSNVFQTRAGCLI (SEQ ID NO: 27, which is 622-636) of S1 protein were identified as IgG-reactive epitopes. Peptides comprising amino acid sequences SSVLHSTQDLFLPFF (SEQ ID NO: 28, which is 30-44) and LLALHRSYLTPGDSSSGWTAGAAAY (SEQ ID NO: 29, which is 226-250) of S1 protein were identified as IgM-reactive epitopes. Moreover, the inventors have shown that peptides having sequences RTQLPPAYTNS (SEQ ID NO: 41), LTPGDSSSGWTAG (SEQ ID NO: 35), YQAGSTPCNGV (SEQ ID NO: 36) and YGFQPTNGVGYQ (SEQ ID NO: 37) are reactive with antibodies from SARS-CoV-2 patients. Many other publications can be used by the person skilled in the art as guidance when designing variants (Zhang et al. (2020) Mining of epitopes on spike protein

- of SARS-CoV-2 from COVID-19 patients, *Cell Res* **30**, 702–704 (2020). <https://doi.org/10.1038/s41422-020-0366-x>; Poh, C.M., Carissimo, G., Wang, B. *et al.* Two linear epitopes on the SARS-CoV-2 spike protein that elicit neutralising antibodies in COVID-19 patients. *Nat Commun* **11**, 2806 (2020). <https://doi.org/10.1038/s41467-020-16638-2>; Wang et al. (2020) SARS-CoV-2 Proteome Microarray for Mapping COVID-19 Antibody Interactions at Amino Acid Resolution, *ACS Cent. Sci.* 2020, **6**, 12, 2238–2249). In a preferred embodiment, a polypeptide is used which comprises SEQ ID NO: 1 or a variant thereof and is folded and, more preferably comprises at least 150, 200, 250, 300, 400, 500 or 600 successive amino acids of SEQ ID NO: 1.
- 10 In a preferred embodiment, variants may, in addition, comprise chemical modifications, for example labels such as isotopic labels or detectable labels or covalent modifications such as glycosylation, phosphorylation, acetylation, decarboxylation, citrullination, hydroxylation and the like. The person skilled in the art is familiar with methods for the modification of polypeptides. Moreover, variants may also be generated by way of fusion with other known polypeptides or variants thereof, for example
- 15 artificial linkers, affinity tags, other antigens and the like. For example, SEQ ID NO: 3, SEQ ID NO: 32, SEQ ID NO 34 and SEQ ID NO: 38 are fusion proteins according to the present invention.

According to the present invention, a medical or diagnostic device such as the diagnostically useful carrier may be prepared by expressing a recombinant variant of SEQ ID NO: 1 comprising an affinity

20 tag, optionally with an artificial linker which may include a protease cleavage site, in a cell such as a eukaryotic (such as a CHO or HEK293 cell) or prokaryotic (such as an Ecoli cell) cell, contacting the expressed variant with a ligand binding specifically to the affinity tag, which ligand is immobilized on a solid phase, washing the solid phase such that non-specifically bound material from the cell is removed and eluting the expressed variant from the solid phase, preferably by adding an excess of

25 non-immobilized ligand. The variant may then be immobilized on the device. Optionally, the affinity tag may be removed by contacting the variant with a protease, preferably a protease recognizing the protease cleavage site, preferably before the immobilization. The affinity tag may be selected from the group of tags comprising His, 18A, ACP, aldehyde, Avi, BCCP, calmodulin-binding peptide (CBP), chitin binding domain (CBD), E-Tag, ELK16, FLAG, flash, poly-glutamate, poly-aspartate, GST, Green

30 fluorescent protein, HA, maltose binding protein, myc, nus, NE, ProtA, ProtC, Tho1d4, S-Tag, SnoopTag, SpyTag, SofTag, Streptavidin, Strep-tag II, T7 Epitope Tag, TAP, TC, Thioredoxin, Ty, V5, VSV and Xpress Tag. Useful proteases include, but are not limited to TEV, Thrombin, Faktor Xa or Enteropeptidase. Suitable linkers comprising a protease cleavage site (e.g. between SEQ ID NO: 1 or variant thereof and the affinity tag) may be comprised in the variant, and be part of the coding

35 sequence of commercially available expression vectors, for example pET vector series (Novagen) that may be used for expression of said variant.

The variant of the polypeptide has biological activity. In a preferred embodiment, such biological activity is the ability to bind to the respective antibody. In a preferred embodiment it comprises an

40 epitope having the ability or it has itself the ability to bind to an antibody to SEQ ID NO: 1, preferably an IgA class antibody to SEQ ID NO: 1, preferably from a sample from a patient suffering from SARS-

CoV-2, wherein more preferably the epitope comprises a sequence comprising at least 5, 6, 7 or 8 amino acid residues. More preferably, it does not bind specifically to homologues of SEQ ID NO: 1 from other coronaviruses, preferably from the group comprising MERS (SEQ ID NO: 6), NL63 (SEQ ID NO: 10), 229E (SEQ ID NO: 7), OC43 (SEQ ID NO: 8) and HKU1 (SEQ ID NO: 9), more preferably  
5 from the group comprising SARS-CoV-1 (SEQ ID NO: 11), MERS, NL63, 229E, OC43 and HKU1, wherein specific binding is preferably defined and determined as outlined above, using Biacore equipment.

The person skilled in the art is familiar how to make diagnostically useful reagents based on suitable  
10 amino acid sequences and is able to provide both linear peptides, for example by chemical synthesis, and polypeptides, for example by recombinant expression. In particular, the person skilled in the art is aware that both conformational and sequential epitopes exist, and that a suitable strategy for expression and purification must be chosen to obtain a diagnostically useful polypeptide which may be used to detect an antibody such as the antibody to SEQ ID NO: 1 (for example Reischl, U., Molecular  
15 Diagnosis of Infectious diseases, Humana Press, 1998, for example chapters 12, 15, 16, 18 and 19). The person skilled in the art is also familiar with ways to evaluate the usefulness of recombinant proteins for test systems (for example Reischl, U., Molecular Diagnosis of Infectious diseases, Humana Press, 1998, for example chapters 21-26).

The detection of the antibody or complex for the prognosis, diagnosis, methods or kit according to the present invention comprises the use of a method selected from the group comprising immunodiffusion techniques, immunoelectrophoretic techniques, light scattering immunoassays, agglutination techniques, labeled immunoassays such as those from the group comprising radiolabeled immunoassays, enzyme immunoassays such as colorimetric assays, chemiluminescence immunoassays and immuno-  
25 fluorescence techniques. In a preferred embodiment, the complex is detected using a method selected from the group comprising immunodiffusion techniques, immunoelectrophoretic techniques, light scattering immunoassays, agglutination techniques, labeled immunoassays from the group comprising radiolabeled immunoassays, chemiluminescence immunoassays, immunofluorescence techniques and immunoprecipitation techniques. The person skilled in the art is familiar with these  
30 methods, which are also described in the state of the art, for example in Zane, H. D. (2001): Immunology – Theoretical & Practical Concepts in Laboratory Medicine, W. B. Saunders Company, in particular in Chapter 14. Preferably the test format is an ELISA and a microtiter plate comprising wells is used as a diagnostically useful carrier.

Preferably a polypeptide comprising SEQ ID NO: 1 or a variant thereof is immobilized on a solid phase of the diagnostically useful carrier. It may be directly immobilized on the solid phase when contacted with the sample, but a competitive assay, a capture bridge assay, an immunometric assay, a class-specific second antibody on the solid phase, a class capture assay, direct or indirect, may also be used. The principle of each of these formats is detailed in The Immunoassay Handbook, 3<sup>rd</sup> edition,  
40 edited by David Wild, Elsevier, 2005. More preferably, the solid phase is a test strip or a well of a microtiter plate for ELISA, most preferably a well of a microtiter plate for ELISA.

In a preferred embodiment, a competitive assay format is used, wherein the antibody to be detected competes with another antibody to SEQ ID NO: 1 or another ligand binding specifically to SEQ ID NO: 1. The ligand binding specifically to SEQ ID NO: 1 may be selected from the group comprising an aptamer or antibody binding to SEQ ID NO: 1 and the human ACE2 receptor (SEQ ID NO: 39) or a variant thereof, the natural binding partner of the SARS-CoV-2 spike protein. Such a method may comprise providing a polypeptide comprising SEQ ID NO: 1 or variant thereof and the ligand binding specifically to SEQ ID NO: 1, preferably from the group comprising an antibody, an aptamer and the ACE receptor or a variant thereof. If the antibody to be detected is present, it will interfere with the formation of the complex or partially or fully displace the ligand binding specifically to SEQ ID NO: 1, thus reducing the number of the complexes. Any complex comprising the antibody to be detected may then be detected by precipitating the complex, for example using an affinity ligand attached to the polypeptide comprising SEQ ID NO: 1 or to a molecule binding to the antibody to be detected such as a secondary antibody. Examples of affinity ligands include glutathione and biotin. A binding partner of the affinity ligand may be coated on a solid phase which binds to the affinity ligand such as GST or streptavidin, respectively. Any complex precipitated may then be detected, preferably by detecting a detectable label attached to the ligand binding specifically to the polypeptide comprising SEQ ID NO: 1 or attached to the polypeptide comprising SEQ ID NO: 1, respectively. A specific competitive test has been published by Tan *et al.* (2020) A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein protein interaction, Nature Biotechnology 38 (1073-1078).

Alternatively, the complex may be formed on the surface of a carrier if the ligand binding specifically to SEQ ID NO: 1 or the polypeptide comprising SEQ ID NO: 1 or a variant is coated on said surface. Normally, the presence or absence of IgA, IgM and IgG class antibodies interfering with the complex formation will be detected using such a format. Specific IgA class antibodies may be detected by isolating the antibodies of the Ig class of interest before, preferably from the group comprising IgA, IgM and IgG class antibodies preferably Ig, for example using Protein A or Protein G or a secondary antibody. The person skilled in the art is familiar with the synthesis, selection and use of aptamers (Thivyanathan V, Gorenstein DG. Aptamers and the next generation of diagnostic reagents. *Proteomics Clin Appl.* 2012;6(11-12):563-573. doi:10.1002/prca.201200042) and antibodies (Hust M, Frenzel A, Schirrmann T, Dübel S. Selection of recombinant antibodies from antibody gene libraries. *Methods Mol Biol.* 2014;1101:305-20; Hanack K, Messerschmidt K, Listek M. Antibodies and Selection of Monoclonal Antibodies. *Adv Exp Med Biol.* 2016;917:11-22; Harold F. Stills, in *The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents*, 2012) and with generating and selecting antibodies binding to specific targets (Lottspeich/Engels, *Bioanalytic*, Chapter 6 and references therein, Springer 2012).

In another preferred embodiment, a complex comprising a first and a second polypeptide comprising SEQ ID NO: 1 or a variant thereof, such as RBD, and the antibody to be detected may be formed in a liquid phase if the antibody is present in the sample, since the antibody has two or more binding sites,

each binding to a polypeptide comprising SEQ ID NO: 1. Any complex comprising the antibody to be detected may then be detected by precipitating the complex, for example using an affinity ligand attached to the first polypeptide such as glutathione or biotin and a binding partner coated on a solid phase which binds to the affinity ligand such as GST or streptavidin, respectively, which solid phase may for example be a bead. Any complex precipitated may then be detected, preferably by detecting a detectable label attached to the second polypeptide. Alternatively, the complex may be formed on the surface of a carrier if the first polypeptide comprising SEQ ID NO: 1 or a variant thereof is coated on said surface. Alternatively, the first and the second polypeptide may each be labeled with two different labels which are detectable only if they are in close proximity, for example when bridged by the antibody to be detected. Again, the presence or absence of IgA, IgM and IgG class antibodies will be detected unless the specific Ig class antibodies have been isolated before, preferably from the group comprising IgA, IgM and IgG class antibodies, preferably IgA, for example using Protein A or Protein G or a secondary antibody. Preferably, in an early phase of the infection, when IgG antibodies have not yet been produced, IgA antibodies will predominantly or only be detected.

Any polypeptide, for example a polypeptide comprising SEQ ID NO: 1 or a secondary antibody, may be provided in any form and at any degree of purification, from tissues, fluids or cells comprising said polypeptide in an endogenous form, more preferably cells overexpressing the polypeptide, crude or enriched lysates of such cells, to purified and/or isolated polypeptide which may be essentially pure. In a preferred embodiment, the polypeptide is a native polypeptide, wherein the term "native polypeptide", as used herein, refers to a folded polypeptide, more preferably to a folded polypeptide purified from cells, more preferably from prokaryotic or eukaryotic, preferably mammalian cells. A glycosylated form of the polypeptide may be used. Secondary antibodies are preferably pure, for example following purification based on Protein A or G as an affinity ligand. In a preferred embodiment, any polypeptide used or provided according to the present invention is used or provided in a folded state, in the absence of significant concentrations of denaturing reagents such as thiol-containing compounds such as DTT.

In a preferred embodiment, the presence or absence of an IgA, IgM and/or IgG class antibody to SEQ ID NO: 1 is detected. In another preferred embodiment, the presence or absence of IgA is detected only. In another preferred embodiment, the presence or absence of IgM is detected only. In another preferred embodiment, the presence or absence of IgG is detected only.

In a preferred embodiment, the presence or absence of IgA and the presence or absence of IgG is detected. More preferably, a secondary antibody to IgA class antibodies and a secondary antibody to IgG class antibodies are used or provided for this detection. In another preferred embodiment, the presence or absence of IgM and the presence or absence of IgG is detected. More preferably, a secondary antibody to IgM class antibodies and a secondary antibody to IgG class antibodies are used or provided for this detection. In another preferred embodiment, the presence or absence of IgM and the presence or absence of IgA is detected. More preferably, a secondary antibody to IgM class antibodies and a secondary antibody to IgA class antibodies are used or provided for this detection.

In a preferred embodiment, the presence or absence of an antibody, for example IgA, IgM and/or IgG to SEQ ID NO: 1, may be detected using a polypeptide comprising SEQ ID NO: 1 or a variant thereof, but the polypeptide may comprise additional sequences, preferably artificial sequences for example

5 linkers or binding epitopes. However, such additional sequences are chosen such that the ability of SEQ ID NO: 1 or a variant thereof to bind specifically to the antibody to be detected or the diagnostic reliability, in particular sensitivity and/or specificity, is not significantly altered, let alone abolished. For example, a domain binding to SEQ ID NO: 1 or a fragment thereof, thus masking an epitope, should not be fused to the polypeptide or be present. According to the present invention, a secondary

10 antibody detecting IgA, IgM and/or IgG class immunoglobulins to a SARS-CoV-2 antigen, preferably from the group comprising an antigen comprising SEQ ID NO: 1 or a variant thereof, an antigen comprising SEQ ID NO: 30 or a variant thereof, an antigen comprising SEQ ID NO: 31 or a variant thereof and an antigen comprising SEQ ID NO: 33 or a variant thereof, preferably all, may be detected or used for the diagnosis, preferably early diagnosis of a SARS-CoV-2 infection. An antigen selected

15 from the group comprising an antigen comprising SEQ ID NO: 30 or a variant thereof, an antigen comprising SEQ ID NO: 31 or a variant thereof and an antigen comprising SEQ ID NO: 33 or a variant thereof, preferably all, may be coated on a diagnostically useful carrier, preferably spatially separated or in a mixture, and contacted with a sample for detecting an antibody binding specifically to the respective antigen or antigens. In a preferred embodiment, the detection of an antibody to SEQ ID

20 NO: 1 in addition to the detection of an antibody to such a SARS-CoV-2 antigen other than SEQ ID NO: 1 may increase the overall sensitivity of the assay, in particular diagnosing a SARS-CoV-2 infection at an early stage or for the differential diagnosis at an early stage.

In a preferred embodiment, the products, methods and uses of the present invention are configured

25 such that presence or absence of an antibody to SEQ ID NO: 1 can be distinguished from the presence of an antibody to another SARS-CoV-2 antigen or one or more other antigen, preferably from SARS-CoV-2 N protein, more preferably from the group comprising SARS-CoV-2 N protein, SARS-CoV-2 M protein and SARS-CoV-2 E protein, most preferably from the group comprising SARS-CoV-2 N protein, SARS-CoV-2 M protein SARS-CoV-2 E protein and SARS-CoV-2 S protein

30 epitopes other than those present on SEQ ID NO: 1. In a more preferred embodiment, a polypeptide comprising SEQ ID NO: 1 or a variant thereof is spatially separated from such other SARS-CoV-2 antigens or other coronavirus antigens when used to detect the presence or absence of an antibody to SEQ ID NO: 1. For example, the polypeptide comprising SEQ ID NO: 1 or a variant may be pure and/or isolated on a carrier. For example, it could be on a blot or microtiter well or bead, spatially

35 separate from other antigens.

In a preferred embodiment, the products, methods and uses of the present invention are configured such that presence or absence of an antibody to SEQ ID NO: 1 can be detected, without determining whether the antibody detected belongs to a certain immunoglobulin class. This is often referred to as detecting the "total antibodies" to one or more antigens such as SEQ ID NO: 1. Detecting total

40 antibodies, including IgA and IgG, increases the sensitivity of the assay, since IgA class antibodies present at an early stage and IgA and IgG class antibodies to SEQ ID NO: 1 at a late phase are

detected as part of the total antibodies to SEQ ID NO: 1. Preferably, an antibody to SEQ ID NO: 1 can be distinguished from an antibody to another SARS-CoV-2 antigen such as N protein (SEQ ID NO: 30) or S2 domain (SEQ ID NO: 32), more preferably because other SARS-CoV-2 antigens are absent or spatially separated or an antibody to such another SARS-CoV-2 produces a signal which can be distinguished from a signal produced by an antibody to SEQ ID NO: 1.

In a preferred embodiment, the products, methods and uses of the present invention are configured such that presence or absence of an antibody to SEQ ID NO: 1 can be detected, without determining whether the antibody detected binds to SEQ ID NO: 1 or to another another SARS-CoV-2 antigen such as N protein or S2 protein. This may be accomplished by using a mixture of antigens comprising SEQ ID NO: 1 or a variant thereof in combination with N protein (SEQ ID NO: 30) or a variant thereof or S2 domain (SEQ ID NO: 32) or a variant thereof. In a more preferred embodiment, the whole spike protein comprising S1 and S2 domain (SEQ ID NO: 32) may be used, optionally in combination with N protein (SEQ ID NO: 30). Again, the sensitivity of such an assay is increased since antibodies to SEQ ID NO: 1 including IgA and IgG, increases the sensitivity of the assay, since IgA class antibodies present at an early stage and IgA and IgG class antibodies to SEQ ID NO: 1 at a late phase are detected as part of the total antibodies to SEQ ID NO: 1.

In a preferred embodiment, the products, methods and uses of the present invention are configured such that presence or absence of an antibody to SEQ ID NO: 1 can be detected, without determining whether the antibody detected belongs to a certain immunoglobulin class. This procedure is often referred to as detecting the "total antibodies" to one or more antigens such as SEQ ID NO: 1. Detecting total antibodies, including IgA and IgG, increases the sensitivity of the assay, since IgA class antibodies present at an early stage are detected as part of the antibodies to SEQ ID NO: 1.

In a preferred embodiment, the absence of an antibody to SEQ ID NO: 1 may be detected, using an isolated, pure and/or recombinant polypeptide comprising SEQ ID NO: 1 or a variant thereof.

In a preferred embodiment, the presence or absence of an antibody to SARS-CoV-2 N protein, defined by SEQ ID NO: 30, is determined in addition, preferably an IgA, IgG and/or IgM antibody, more preferably IgG or IgM, most preferably IgG. The carrier according to the invention may be coated with a polypeptide comprising the SARS-CoV-2 N protein defined by SEQ ID NO: 30 or a variant thereof for this purpose.

In a preferred embodiment, the presence or absence of an antibody to the receptor binding domain (RBD) of the SARS-CoV-2 S1 domain defined by SEQ ID NO: 31 is detected. The carrier according to the invention may be coated with a polypeptide comprising SEQ ID NO: 31 or a variant thereof for this purpose.

In a preferred embodiment, the presence or absence of an antibody to the SARS-CoV-2 S2 domain defined by SEQ ID NO: 32 of the SARS-CoV-2 spike protein is detected. The carrier according to the invention may comprise a polypeptide comprising SEQ ID NO: 32 or a variant thereof for this purpose.

In a preferred embodiment, a secondary antibody is an antibody binding to all antibodies from an antibody or immunoglobulin class, preferably a human antibody class, preferably IgA and/or IgG and/or IgM antibodies, preferably IgA. Secondary antibodies typically recognize the constant domain of said class or are polyvalent, with binding sites to various epitopes across the sequence or 3D structure  
5 shared by antibodies of said Ig class. Secondary antibodies are typically from a mammal other than a human or from a bird, preferably from chicken, rabbit, mouse, rat, horse, pig, donkey, goat, cow, camel, llama, or non-human primate. A wide range of them is commercially available.

According to the present invention, the SARS-CoV-2 infection may be detected at increased sensitivity  
10 at an early stage, preferably 5 or fewer days after the onset of disease symptoms.

In a further preferred embodiment, the method the invention further comprises evaluating the result for a diagnosis. The evaluation in accordance with the present invention is carried out to decide whether a treatment for the individual tested is required. The evaluation of the result of the diagnosis may  
15 include involving a physician in order to select an appropriate treatment if the diagnosis was positive. Importantly, the method of the invention excluding the evaluation may be carried out at one location such as a country and the evaluation of the results of the diagnosis may be carried out at a different location

In a different preferred embodiment, the method of the invention further comprises transferring the result of the diagnosis or the evaluation to a different location. This preferred embodiment relates to  
20 cases where the method of the invention, optionally including the evaluation step, are carried out in one location such as one country and the results of the diagnosis and evaluation, respectively, are transferred to a different location such as a different country. The transfer may be effected by any means available to the skilled person. This includes electronic transfer of the data as well as factual  
25 transfer of read material. According to this preferred embodiment, in particular the physician or clinic treating the patient in case of a positive result may take the appropriate steps for successful treatment. The diagnostically useful carrier is preferably selected from the group comprising a glass slide, preferably for microscopy, a biochip, a microarray, a microtiter plate, a lateral flow device, a test strip, a membrane, e.g. a nitrocellulose membrane, preferably a line blot, a chromatography column and a  
30 bead, preferably a microtiter plate.

In a preferred embodiment, the diagnostically useful carrier is a line blot (Raoult, D., and Dasch, G. A. (1989), The line blot: an immunoassay for monoclonal and other antibodies. Its application to the serotyping of gram-negative bacteria. J. Immunol. Methods, 125 (1-2), 57-65; WO2013041540). In a  
35 preferred embodiment, the term "line blot", as used herein, refers to a test strip, more preferably membrane-based, that has been coated with one or more means for specifically capturing an antibody, preferably each of these means is a polypeptide. If two or more means are used, they are preferably spatially separated on the carrier. Preferably, the width of the bands is at least 30, more preferably 40, 50, 60, 70 or 80 % the width of the test strip. The line blot may comprise one or more  
40 control bands for confirming that it has been contacted with sample sufficiently long and under

adequate conditions, in particular in the presence of human serum, or with a secondary antibody, respectively. A line blot is preferably made from a nitrocellulose membrane.

In another preferred embodiment, the diagnostically useful carrier is a bead. Various beads for numerous applications are commercially available, mainly based on carbohydrate, for example  
5 sepharose or agarose, or plastic. They may contain active or activatable chemical groups such as a carboxyl or tosyl or ester group, which can be utilized for the immobilization of a means for specifically capturing an antibody. Preferably, the beads are beads having an average diameter of from 0.1  $\mu\text{m}$  to 10  $\mu\text{m}$ , from 0.5  $\mu\text{m}$  to 8  $\mu\text{m}$ , from 0.75  $\mu\text{m}$  to 7  $\mu\text{m}$  or from 1  $\mu\text{m}$  to 6  $\mu\text{m}$ . The beads can be coated with the means for specifically capturing an antibody directly or *via* affinity ligands, for example biotin  
10 or glutathione and streptavidin or GST, respectively. For example, the bead may be coated with biotin or glutathione and the antigen may be fused with streptavidin or glutathione-S-transferase or a variant thereof, respectively. Preferably, the bead is provided in the form of an aqueous suspension having a bead content of from 10 to 90%, preferably from 20 to 80%, preferably from 30 to 70%, more preferably from 40 to 60% (w/w). The person skilled in the art is familiar with such beads (Diamindis, E. P.,  
15 Chriopoulus, T. K., Immunoassays, 1996, Academic Press), which are commercially available, for example Bio-Plex COOH beads MC10026-01 or 171-506011 from Bio-Rad.

In a particularly preferred embodiment, the beads are paramagnetic beads, which can be easily concentrated on a surface with the aid of a magnet. For this purpose, commercial paramagnetic beads usually contain a paramagnetic mineral, for example iron oxide. A multiplicity of suitable paramagnetic  
20 beads is commercially available. A bead may be labeled with a detectable label.

In a preferred embodiment, a paramagnetic bead is used and washed or incubated in buffer by applying a magnetic field to concentrate and immobilize the beads, following removal of the buffer present and addition of new buffer. The magnetic field may then be discontinued to make the suspension of the beads in the new buffer more efficient. A buffer may be any buffered solution used  
25 according to the present invention including a diluted patient sample, an incubation buffer or a buffer comprising a secondary antibody.

In a preferred embodiment, the antibody is detected using a chemiluminescent label. In a preferred embodiment, this is a chemiluminescent enzyme, preferably selected from the group comprising luciferase, peroxidase, alkaline phosphatase and  $\beta$ -galactosidase or a variant thereof, which may turn  
30 over a chemiluminescent substrate without being consumed itself (Kricka, L. J. (2003). Clinical applications of chemiluminescence. *Analytica chimica acta*, 500(1): 279-286). In another preferred embodiment, the chemiluminescent label is a small organic compound having no enzymatic activity catalyzing a chemiluminescence reaction, which emits a chemiluminescence signal upon being degraded when contacted with a chemiluminescence substrate solution which comprises inorganic  
35 and/or non-enzymatic organic compounds that are required for emitting the signal. Preferably, the small organic compound having no enzymatic activity is selected from the group comprising acridinium esters (Weeks, I., Beheshti, I., McCapra, F., Campbell, A. K., Woodhead, J. S. (1983) Acridinium esters as high specific activity labels in immunoassay. *Clin Chem* 29: 1474-1479) and luminol or a chemiluminescent derivative thereof such as isoluminol. Such small organic compounds may be  
40 coupled to the secondary antibody. In the case of luminol, the substrate solution comprises  $\text{H}_2\text{O}_2$  at a high pH. In the case of an acridinium ester, a mixture of  $\text{H}_2\text{O}_2$  and sodium hydroxide is frequently

used. The small organic compound is consumed upon emission of the chemiluminescence signal. In a preferred embodiment the chemiluminescence of the chemiluminescent label is detected for 1 to 60 seconds, preferably for 2 to 20 seconds, more preferably 3 to 15 seconds following initiation of the chemiluminescent detection reaction. In another preferred embodiment the chemiluminescence of the chemiluminescent label is detected for at least 0.5, 1, 1.5, 2, 2.5 or 3 seconds. In another preferred embodiment, the carrier is a microtiter plate comprising at least 8 wells that may be used for ELISA. At least one of the wells is coated with the means for specifically capturing an antibody, either directly or indirectly, preferably a polypeptide comprising SEQ ID NO: 1 or a variant thereof. At least 3, preferably 4, more preferably 5 calibrators, at defined concentrations may be used to set up a calibration curve for semi-quantitative analysis. When the inventive method is carried out, the calibrators, which typically cover a range of concentrations covering the calibrating curve, may be processed and developed in parallel to the samples. A secondary antibody comprising a detectable label such as an enzymatically active label may be provided, for example a label having horse radish peroxidase activity or alkaline phosphatase activity or an enzyme capable of chemiluminescence.

In another preferred embodiment, the carrier is a microarray. In a preferred embodiment, the term "microarray", as used herein, refers to a chip spotted with a variety of spatially separate antigens, preferably at least 20, preferably 30, 40, 50, 80 or 100. Preferably each antigen is a peptide comprising or consisting of 5 to 25, preferably 7 to 15 successive amino acids spanning a fragment of SEQ ID NO: 1, more preferably spanning the RBD (SEQ ID NO: 31). A secondary antibody comprising a label, preferably a fluorescent label, may be used for the detection. Preferably other antigens are spotted, more preferably from the group of polypeptides comprising SEQ ID NO: 30 (SARS-CoV-2 N protein) and SEQ ID NO: 33 (SARS-CoV-2 S2 protein).

In another preferred embodiment, a glass slide is used, which is on or part of a carrier for microscopic immunofluorescence analysis. A cell, preferably a eukaryotic cell such as a HEK293 cell is on the slide. It may be covered with a mounting buffer. Various compositions and methods are described in the state of the art, for example in "Mountants and Antifades", published by Wright Cell Imaging Facility, Toronto Western Research Institute University Health Network, (<https://de.scribd.com/document/47879592/Mountants-Antifades>), Krenek *et al.* (1989) Comparison of antifading agents used in immunofluorescence, *J. Immunol. Meth* 117, 91-97 and Naim *et al.* (1969) Microphotometry in Immunofluorescence, *Clin. Exp. Immunol.* 4, 697-705. The cell expresses, preferably overexpresses a polypeptide comprising SEQ ID NO: 1 or a variant thereof. The carrier may comprise a mock-transfected cell, which has been transfected with the same vector as the cell overexpressing a polypeptide comprising SEQ ID NO: 1 or a variant thereof, but without the nucleic acid encoding for the latter. Such mock-transfected cell may serve as a negative control. Another cell may comprise an additional coronavirus, preferably SARS, more preferably SARS-CoV-2 antigen, for example N protein, S2 protein or RBD to detect an antibody.

According to the present invention, immunofluorescence may be used to detect an antibody. The person skilled in the art is familiar with the method (Storch, W. B., Immunofluorescence in Clinical Immunology: A Primer and Atlas, Birkhäuser, 2000; Wesseling JG, Godeke GJ, Schijns VE, Prevec L, Graham FL, Horzinek MC, Rottier PJ. Mouse hepatitis virus spike and nucleocapsid proteins expressed by adenovirus vectors protect mice against a lethal infection. *J Gen Virol.* 1993 Oct;74 (Pt

10):2061-9. doi: 10.1099/0022-1317-74-10-2061. PMID: 8409930.). Briefly, an antigen, preferably a polypeptide comprising SEQ ID NO: 1 or a variant thereof, is immobilized on a carrier which may be a cell expressing said antibody and contacted with a sample, followed by detection of the antibody to be detected by fluorescence, preferably using a means for detecting the antibody labeled with a fluorescent label. In a preferred embodiment, the cell is a eukaryotic cell overexpressing the polypeptide, such as a cell selected from the group comprising HEK, Hela, CHO and Jurkat cells and derivatives thereof. In a preferred embodiment, the cell is a recombinant cell overexpressing the polypeptide, which is preferably under the control of a heterologous strong promoter.

According to the present invention, a lateral flow device may be used to detect an antibody. The person skilled in the art is familiar with lateral flow devices for this purpose (Lateral Flow Immunoassay, edited by Raphael Wong, Harley Tse, 2009, Springer; Paper-based diagnostics: Current Status and Future applications, Kevin J. Land, Springer 2019). Briefly, a lateral flow assay may be based on a membrane such as a nitrocellulose membrane which comprises a polypeptide comprising SEQ ID NO: 1 or a variant thereof comprising a detectable label. If the membrane is contacted with a sample, an antibody to be detected will bind to the antigen. The resulting complex will move driven by capillary forces on the membrane and will be immobilized on a test line on the membrane comprising a means for detecting the antibody, typically a secondary antibody binding to the immunoglobulin class or classes of the antibody or the antibodies to be detected such as IgG and/or IgA and/or IgM. Preferably nanoparticles or beads are used as labels, for example gold nanoparticles or latex beads.

According to the present invention, a polypeptide, preferably the polypeptide comprising SEQ ID NO: 1 or a variant thereof, may be a recombinant protein, wherein the term "recombinant", as used herein, refers to a polypeptide produced using genetic engineering approaches at any stage of the production process, for example by fusing a nucleic acid encoding the polypeptide to a strong promoter for overexpression in cells or tissues or by engineering the sequence of the polypeptide itself. The person skilled in the art is familiar with methods for engineering nucleic acids and polypeptides encoded (for example, described in Green M. R. and Sambrook, J. (2012), Molecular Cloning – A Laboratory Manual, Fourth Edition, CSH or in Brown T. A. (1986), Gene Cloning – an introduction, Chapman & Hall) and for producing and purifying native or recombinant polypeptides (for example Handbooks „Strategies for Protein Purification“, „Antibody Purification“, published by GE Healthcare Life Sciences, and in Burgess, R. R., Deutscher, M. P. (2009): Guide to Protein Purification). In another preferred embodiment, the polypeptide is an isolated polypeptide, wherein the term "isolated" means that the polypeptide has been enriched compared to its state upon production using a biotechnological or synthetic approach and is preferably pure, *i.e.* at least 60, 70, 80, 90, 95 or 99 percent of the polypeptide in the respective liquid consists of said polypeptide as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie blue staining and visual inspection. Preferably any polypeptide on a carrier used as a means to capture an antibody is pure.

In a preferred embodiment, a detectable label is used to detect an antibody according to the present invention, which is a label that may be used to distinguish a population of molecules from others using biophysical detection methods. It is preferably selected from the group comprising a fluorescent, a

radioactive, a chemiluminescent label, a heavy metal such as gold label, a nanoparticle, a bead or an enzymatically active label, preferably one catalyzing a colorimetric reaction. In a preferred embodiment, a fluorescent label is selected from the group comprising Alexa dyes, FITC, TRITC and green fluorescent protein (GFP). Iodine-125 may be used as radioactive label. In a preferred embodiment, an enzymatically active label is selected from the group comprising horseradish peroxidase, glucose oxidase, beta galactosidase, alkaline phosphatase and luciferase. The person skilled in the art is able to choose suitable labels and to attach them to proteins, nucleic acids and other molecules (Hassanzadeh L, Chen S, Veedu RN. Radiolabeling of Nucleic Acid Aptamers for Highly Sensitive Disease-Specific Molecular Imaging. *Pharmaceuticals (Basel)*. 2018;11(4):106. Published 2018 Oct 15. doi:10.3390/ph11040106 Hassanzadeh L, Chen S, Veedu RN. Radiolabeling of Nucleic Acid Aptamers for Highly Sensitive Disease-Specific Molecular Imaging. *Pharmaceuticals (Basel)*. 2018;11(4):106. Published 2018 Oct 15. doi:10.3390/ph11040106, *Bioconjugate Techniques, 3rd Edition* (2013) by Greg T. Hermanson, Obermaier C, Griebel A, Westermeier R. Principles of protein labeling techniques. *Methods Mol Biol*. 2015;1295:153-65), and a wide range of labeled molecules are commercially available.

According to the present invention, a means for detecting the presence of antibodies to SEQ ID NO: 1 is provided. In a preferred embodiment, a secondary antibody comprising a detectable label may be used to detect IgA and/or IgG and/or IgM, preferably IgA class antibodies to SEQ ID NO: 1, more preferably SEQ ID NO: 1 and another coronavirus antigen. A protein having peroxidase activity may be used as an enzymatically active label. Preferably the secondary antibody recognizes mammalian, more preferably human antibodies. If antibodies from one Ig class are to be detected, two secondary antibodies may be used, preferably one binding to IgA class antibodies and one binding to IgG class antibodies. The two secondary antibodies may be in a mixture or separate, preferably separate to allow separate detection of antibodies from different Ig classes such as IgA, IgM and IgG antibodies, preferably IgG and IgA for example to obtain information regarding the course of the disease, based on the fact that IgA class antibodies emerge earlier in many patients than IgG class antibodies. Alternatively, one secondary antibody binding to antibodies from more than one Ig class may be used, such as from the group comprising IgG, IgA and IgM, preferably one binding to IgG and IgA class antibodies. A secondary antibody may be a polyclonal or monoclonal antibody. In a preferred embodiment, the secondary antibody is from a mammal other than a human but binds to human antibodies of a certain Ig class, preferably IgA, IgG and/or IgM. The person skilled in the art is familiar with the production and use of secondary antibodies (Kalyuzhny, A., Immunohistochemistry, Essential Elements and Beyond, Springer, 2017, in particular chapter 4; Howard, G. C., and Bethell, D. R., Basic Methods in Antibody Production and Characterization, 2000, CRC press). A variety of secondary antibodies, optionally with labels such as a fluorescent label, is commercially available, for example FITC-labeled secondary antibodies Cat # H15101, Cat # 62-8411 Cat # A24459, horseradish peroxidase labeled secondary antibodies Cat # 31420, Cat # SA1-35467, Cat # SA1-35467, Cat # SA1-35467 and others from Thermo Fisher. Labeled fragments of secondary antibodies or aptamers may also be used. The person skilled in the art is familiar with the synthesis, selection and use of aptamers, which may also be used as means for detecting the presence of antibody to SEQ ID NO: 1,

- for example when binding specifically to the antibody or antibodies to be detected, preferably IgG and/or IgA and/or IgM antibodies (Thiviyanathan V, Gorenstein DG. Aptamers and the next generation of diagnostic reagents. *Proteomics Clin Appl.* 2012;6(11-12):563-573. doi:10.1002/prca.201200042) and the generation of specific antibodies (Hust M, Frenzel A, Schirmann T, Dübel S. Selection of recombinant antibodies from antibody gene libraries. *Methods Mol Biol.* 2014; 1101:305-20; Hanack K, Messerschmidt K, Listek M. Antibodies and Selection of Monoclonal Antibodies. *Adv Exp Med Biol.* 2016;917:11-22; Harold F. Stills, in *The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents*, 2012). Such aptamers may bind specifically to the constant region or epitopes in other parts of the antibody or antibodies to be detected.
- 10 In another preferred embodiment, a polypeptide comprising SEQ ID NO: 1 or a variant thereof, preferably the receptor binding domain (SEQ ID NO: 31), may be used as a means for detecting the presence of an antibody to SEQ ID NO: 1. More specifically, said polypeptide may be coated to a diagnostically useful carrier and may be used to capture any antibody to be detected. Since human antibodies have more than one binding site, only one antigen binding site of a captured antibody may
- 15 be occupied. Subsequent addition of another polypeptide comprising SEQ ID NO: 1 or a variant thereof, which is not coated on the carrier, may lead to the occupation of another binding site by this newly added polypeptide. The complex comprising a coated polypeptide, the antibody to be detected and the other polypeptide may then be detected. More preferably, the other polypeptide carries a detectable label, and this is used to detect the complex. In this embodiment, all antibodies, more
- 20 specifically IgA, IgM and IgG class antibodies may be detected.
- In another preferred embodiment, a polypeptide comprising SEQ ID NO: 39 (ACE2) or a variant thereof binding to the receptor binding domain in SEQ ID NO: 1, optionally in combination with a polypeptide comprising SEQ ID NO: 1 or a variant thereof binding to the ACE2 receptor, may be used as a means for detecting the presence of an antibody to SEQ ID NO: 1. The competitive assay format
- 25 is then used to detect its presence.
- In another preferred embodiment, specific proteins binding to distinct immunoglobulin classes may be labeled with detectable labels and used as a means for detecting the presence of an antibody to SEQ ID NO: 1. For example, protein G, A and L are bacterial proteins which bind to IgG class antibodies, (L. Bjorck, G. Kronvall: *Purification and some properties of streptococcal protein G, a novel IgG-binding reagent.* In: *Journal of Immunology.* 133(2)/1984.), whereas jacalin (Abcam, ThermoFisher) may be
- 30 used for binding of IgA class antibodies (see e.g. Choe et al., *Materials (Basel).* 2016 Dec; 9(12): 994; Wilkinson & Neville *Vet Immunol Immunopathol.* 1988 Mar;18(2):195-8)..
- In a preferred embodiment, a kit according to the present invention comprises a polypeptide comprising SEQ ID NO: 1 or a variant thereof, preferably coated to a diagnostically useful carrier, more
- 35 preferably a microtiter plate, and one or more, preferably all reagents from the group comprising a calibrator, a positive control, a negative control, a washing buffer, a means for detecting the presence of an antibody to SEQ ID NO: 1, preferably an IgA, IgM and/or IgG class antibody, preferably a secondary antibody binding specifically antibodies, more preferably IgA, IgM and/or IgG class
- 40 antibodies, wherein the secondary antibody may comprise a detectable label, a sample buffer, a detection solution, preferably a chromogen/substrate solution, a stop solution and a protective foil.

In a preferred embodiment, a calibrator is a reagent that binds to a polypeptide comprising SEQ ID NO: 1 or a variant thereof and is preferably recognized by secondary antibodies recognizing IgA, IgM and/or IgG class antibodies. The calibrator may be an IgA antibody to SEQ ID NO: 1. Alternatively, the calibrator may be a chimeric antibody, preferably comprising the constant region or other regions or epitopes shared by distinct immunoglobulin classes, preferably IgA and/or IgG and/or IgM, and a variable region, in particular a binding site which is derived from an artificial antibody. The person skilled in the art is familiar with the design, production and use of such calibrators (Lütkecosmann S, Faupel T, Porstmann S, Porstmann T, Micheel B, Hanack K. A cross-reactive monoclonal antibody as universal detection antibody in autoantibody diagnostic assays. Clin Chim Acta. 2019 Dec;499:87-92. doi: 10.1016/j.cca.2019.09.003. Epub 2019 Sep 4. PMID: 31493374, Hackett J Jr, Hoff-Volk J, Golden A, Brashear J, Robinson J, Rapp M, Klass M, Ostrow DH, Mandelki W. Recombinant mouse-human chimeric antibodies as calibrators in immunoassays that measure antibodies to Toxoplasma gondii. J Clin Microbiol. 1998 May;36(5):1277-84. doi: 10.1093/clin/36.5.1277. PMID: 9631165). In a preferred embodiment, a positive control is a solution comprising a compound such as antibody to SEQ ID NO: 1, preferably from the group comprising IgA, IgG and IgM class antibodies, more preferably IgA, from the sample of a patient suffering from SARS-CoV-2 at an amount that a positive result is obtained using the method according to the present invention. A negative control is a reagent that lacks such a compound and could comprise serum from a healthy person. A washing buffer may be used to wash the carrier such as a microtiter plate after the incubation to remove unspecific antibodies and could be PBS. The means for detecting the presence of an antibody could be a secondary antibody binding to the antibody class to be detected, preferably human IgA, IgM and/or IgG class antibodies, and is labeled with a detectable label, preferably with an enzyme, more preferably with an enzyme having peroxidase activity. The sample buffer may be used to dilute patient sample and may be PBS. The detection solution may yield a signal in the presence of the labeled secondary antibody and is preferably a color-developing solution and more preferably 3,3',5,5'-tetramethylbenzidine/H<sub>2</sub>O<sub>2</sub>. The stop solution may be added to a reaction to stop the reaction of the detection solution and may comprise a strong acid, preferably 0.5 M sulphuric acid. The protective foil may be placed on top of the carrier such as a microtiter plate to avoid evaporation.

Any reagent used according to the invention may comprise a preservative, for example azide.

According to the present invention, a use of a polypeptide comprising SEQ ID NO: 1 or a variant thereof or an antibody to SEQ ID NO: 1, preferably an IgA class antibody, for the manufacture of a diagnostic kit is provided. In a preferred embodiment, the polypeptide or antibody is packaged as one of the components of such a kit. The polypeptide or antibody may be used to confirm the quality upon production of the kit. For example, the antibody may be used as a positive control to confirm the reactivity of a polypeptide comprising SEQ ID NO: 1 which is a component of the kit, either separate or coated on a diagnostically useful carrier. The polypeptide may be used to confirm the reactivity of an antibody binding specifically to SEQ ID NO: 1 which is part of the kit. The polypeptide may be used to coat a diagnostically useful carrier as part of the manufacture. Both the polypeptide and antibody may be used to determine the concentration of the antibody or polypeptide, respectively, in buffered

solutions used to make the carrier and the kit or to check whether or not a cell is expressing a polypeptide comprising SEQ ID NO: 1.

According to the present invention, an antibody to SEQ ID NO: 1, preferably IgA class antibody, is used for diagnosing a SARS-CoV-2 infection or for the differential diagnosis according to the present invention. The use may relate to detecting the antibody itself or in combination with other antibodies to SEQ ID NO: 1 or antibodies to other SARS-CoV-2 antigens, thus increasing the overall sensitivity of the diagnostic assay. In a more preferred embodiment, an IgA class antibody is used to increase the sensitivity of a diagnostic assay, in particular at an early stage of the infection. This may be accomplished by detecting not only IgG, but also IgA class antibodies, optionally also IgM class antibodies to SEQ ID NO: 1 or IgA only. In a more preferred embodiment, an IgG class antibody is used to increase the sensitivity of a diagnostic assay, in particular over a period of time which sees a decline in the concentration of antibodies to other antigens, for example IgG class antibodies to the N protein of SARS-CoV-2 such as during the late phase of a SARS-CoV-2 infection. This may be accomplished by detecting not only IgG, but also IgA class antibodies, optionally also IgM class antibodies to SEQ ID NO: 1 or IgG only. In another preferred embodiment, the antibody may be used for the diagnosis by calibrating a device or assay using a calibrator comprising said antibody, preferably an IgA, IgM and/or IgG class antibody, preferably all. In a preferred embodiment, IgA and/or IgG antibody to SEQ ID NO: 1 is used to increase the sensitivity at a late phase of the immunization. The antibody may be used for the validation of an assay, for calibration, for confirming the quality of reagents or assay materials such as a diagnostically useful carrier or as a positive control.

According to the present invention, a polypeptide comprising SEQ ID NO:1 or a variant thereof or an antibody to SEQ ID NO1, preferably an IgA class antibody is used for the manufacture of a diagnostic kit, preferably for the diagnosis of SARS-CoV-2, wherein an IgA class antibody binding specifically to SEQ ID NO: 1 is detected.

A use of an antibody to SEQ ID NO: 1, preferably IgA, IgM and/or IgG, more preferably IgA class antibody, for increasing the sensitivity of the detection of a SARS-CoV-2 infection, preferably at an early stage of the infection, more preferably 5 or fewer days after the onset of disease symptoms. In another preferred embodiment, the use may be at a late phase of the infection. In a preferred embodiment, the presence of or absence IgA, IgM and IgG to SEQ ID NO1 is detected.

The present invention comprises a range of novel nucleic acid and polypeptide sequences, including in particular the sequences described in the following and/or in the sequence listing forming part of the present specification. It will be understood that in case of conflict between any sequence shown herein below and the corresponding sequence described in the sequence listing, the present invention specifically and individually relates to each one of the respective sequences, i.e., to the sequence described herein below and also to the sequence described in the sequence listing.

#### **SEQ ID NO: 1 (SARS-CoV-2 S1 domain from S Protein)**

VNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPV  
LPFNDGVYFASTEKSNIIRGWIFGTTLDSTQSLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSW

MESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGF  
 SALEPLVDLPIGINITRFQTLALHRSYLTGDSGSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVD  
 CALDPLSETKCTLSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRIS  
 NCVADYSVLVNSASFSTFKCYGVSPTKLNLDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLDP  
 5 DFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIQAGSTPCNGVEGFNCYFPLQS  
 YGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLP  
 FQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLT  
 PTWRVYSTGSNVFQTRAGCLIGAEHVNNSECDIPGAGICASYQTQTNSPRRAR

10 **SEQ ID NO: 2 (SARS-CoV-2 S1 domain from S Protein, C-terminally his-tagged, as expressed in cells for the example)**

MFVFLVLLPLVSSQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI  
 HVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSTQSLIVNNATNVVIKVEFCFCN  
 DPFLGVYHKNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIY  
 15 SKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLALHRSYLTGDSGSSGWTAGAAAYVGYLQPR  
 TFLKYNENGTITDAVDCALDPLSETKCTLSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFN  
 ATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGVSPTKLNLDLCFTNVYADSFVIRGDEVQRQIA  
 PGQTGKIADYNYKLDPDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIQAGSTP  
 CNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNG  
 20 LTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQD  
 VNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSECDIPGAGICASYQTQTNSPR  
 RARLEHHHHHHHH

25 **SEQ ID NO: 3 (SARS-CoV-2 S1 domain from S Protein, C-terminally his-tagged, as after cleavage of signal peptide, used in the examples)**

VNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPV  
 LPFNDGVYFASTEKSNIIRGWIFGTTLDSTQSLIVNNATNVVIKVEFCFCNDPFLGVYHKNKSW  
 MESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGF  
 SALEPLVDLPIGINITRFQTLALHRSYLTGDSGSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVD  
 30 CALDPLSETKCTLSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRIS  
 NCVADYSVLVNSASFSTFKCYGVSPTKLNLDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLDP  
 DFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIQAGSTPCNGVEGFNCYFPLQS  
 YGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLP  
 FQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLT  
 35 PTWRVYSTGSNVFQTRAGCLIGAEHVNNSECDIPGAGICASYQTQTNSPRRARLEHHHHHHHH

**SEQ ID NO: 4 (SARS-CoV-2 S1 domain from S Protein, nucleotide sequence encoding SEQ ID NO: 2) – only in sequence listing**

- 40 **SEQ ID NO: 5 (possible SARS-CoV-2 S1 epitope) – only in sequence listing**  
**SEQ ID NO: 6 (S1[MERS\_CoV]) – only in sequence listing**

**SEQ ID NO: 7 (S1[HCoV-229E])** – only in sequence listing

**SEQ ID NO: 8 (S1[HCoV-OC43])** – only in sequence listing

**SEQ ID NO: 9 (S1[HCoV-HKU1])** – only in sequence listing

**SEQ ID NO: 10 (S1[HCoV-NL63])** – only in sequence listing

5 **SEQ ID NO: 11 (S1[SARS\_CoV])** – only in sequence listing

**SEQ ID NO: 12 (fragment of SEQ ID NO: 1)** – only in sequence listing

**SEQ ID NO: 13 (genome of SARS-CoV-2 isolate Wuhan-Hu-1 genome, identical to Genbank MN908947): [ONLY IN SEQUENCE LISTING]**

10 ATTAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCTGTTCTCT  
AAACGAACTTTAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATAA  
TTAATAACTAATTACTGTCTGTGACAGGACACGAGTAACCTCGTCTATCTTCTGCAGGCTGCTTACG  
GTTTCGTCCGTGTTGCAGCCGATCATCAGCACATCTAGGTTTCGTCCGGGTGTGACCGAAAGGT  
AAGATGGAGAGCCTTGTCCCTGGTTTCAACGAGAAAACACACGTCCAACCTCAGTTTGCCTGTTTT  
15 ACAGGTTCCGACGTGCTCGTACGTGGCTTTGGAGACTCCGTGGAGGAGGTCTTATCAGAGGCA  
CGTCAACATCTTAAAGATGGCACCTGTGGCTTAGTAGAAGTTGAAAAAGGCGTTTTGCCTCAACTT  
GAACAGCCCTATGTGTTTCATCAAACGTTTCGGATGCTCGAACTGCACCTCATGGTCATGTTATGGT  
TGAGCTGGTAGCAGAACTCGAAGGCATTCACTACGGTCGTAGTGGTGAGACACTTGGTGTCCTT  
GTCCCTCATGTGGGCGAAATACCAAGTGGCTTACCGCAAGGTTCTTCTTCGTAAGAACGGTAATAA  
20 AGGAGCTGGTGGCCATAGTTACGGCGCCGATCTAAAGTCATTTGACTTAGGCGACGAGCTTGGC  
ACTGATCCTTATGAAGATTTTCAAGAAAACCTGGAACACTAAACATAGCAGTGGTGTTACCCGTGAA  
CTCATGCGTGAGCTTAACGGAGGGGCATACACTCGCTATGTCGATAACAACCTTCTGTGGCCCTG  
ATGGCTACCCTCTTGAGTGCATTAAAGACCTTCTAGCACGTGCTGGTAAAGCTTCATGCACCTTG  
TCCGAACAACCTGGACTTTATTGACACTAAGAGGGGTGTATACTGCTGCCGTGAACATGAGCATGA  
25 AATTGCTTGGTACACGGAACGTTCTGAAAAGAGCTATGAATTGCAGACACCTTTTGAAATTAAATT  
GGCAAAGAAATTTGACACCTTCAATGGGGAATGTCCAAATTTTGTATTTCCCTTAAATCCATAATC  
AAGACTATTCAACCAAGGGTTGAAAAGAAAAAGCTTGATGGCTTTATGGGTAGAAATTCGATCTGTC  
TATCCAGTTGCGTCACCAAATGAATGCAACCAAATGTGCCTTTCAACTCTCATGAAGTGTGATCAT  
TGTGGTGAACTTCATGGCAGACGGGCGATTTTGTAAAGCCACTTGCGAATTTTGTGGCACTGA  
30 GAATTTGACTAAAGAAGGTGCCACTACTTGTGGTACTTACCCCAAATGCTGTTGTTAAATTTA  
TTGTCCAGCATGTCACAATTCAGAAGTAGGACCTGAGCATAGTCTTGCCGAATACCATAATGAAT  
CTGGCTTGAAAACCATCTTCGTAAGGGTGGTCGCACTATTGCCTTTGGAGGCTGTGTGTTCTCT  
TATGTTGGTTGCCATAACAAGTGTGCCTATTGGGTTCCACGTGCTAGCGCTAACATAGGTTGTAA  
CCATACAGGTGTTGTTGGAGAAGGTTCCGAAGGTCTTAATGACAACCTTCTTGAAATACTCCAAA  
35 AAGAGAAAGTCAACATCAATATTGTTGGTGACTTTAACTTAATGAAGAGATCGCCATTATTTGG  
CATCTTTTTCTGCTTCCACAAGTGCTTTTGTGGAACCTGTGAAAGGTTTGGATTATAAAGCATTCA  
AACAAATTGTTGAATCCTGTGGTAATTTTAAAGTTACAAAAGGAAAAGCTAAAAAGGTGCCTGGA  
ATATTGGTGAACAGAAATCAATACTGAGTCCTCTTTATGCATTTGCATCAGAGGCTGCTCGTGTTG  
TACGATCAATTTTCTCCCGCACTCTTGAACTGCTCAAAATTCTGTGCGTGTGTTTACAGAAGGCCG  
40 CTATAACAATACTAGATGGAATTTACAGTATTCACTGAGACTCATTGATGCTATGATGTTACATC  
TGATTTGGCTACTAACAATCTAGTTGTAATGGCCTACATTACAGGTGGTGTGTTTCAGTTGACTTC

GCAGTGGCTAACTAACATCTTTGGCACTGTTTATGAAAACTCAAACCCGTCCTTGATTGGCTTGA  
AGAGAAGTTTAAGGAAGGTGTAGAGTTTCTTAGAGACGGTTGGGAAATTGTAAATTTATCTCAAC  
CTGTGCTTGTGAAATTGTCGGTGGACAAATTGTCACCTGTGCAAAGGAAATTAAGGAGAGTGTTT  
AGACATTCTTTAAGCTTGTAATAAATTTTTGGCTTTGTGTGCTGACTCTATCATTATTGGTGGAGC  
5 TAAACTTAAAGCCTTGAATTTAGGTGAAACATTTGTCACGCACTCAAAGGGATTGTACAGAAAGTG  
TGTTAAATCCAGAGAAGAACTGGCCTACTCATGCCTCTAAAAGCCCCAAAAGAAATTATCTTCTT  
AGAGGGAGAAACACTTCCCACAGAAGTGTTAACAGAGGAAGTTGTCTTGAAAACCTGGTGATTAC  
AACCATTAGAACAACCTACTAGTGAAGCTGTTGAAGCTCCATTGGTTGGTACACCAGTTTGTATTA  
ACGGGCTTATGTTGCTCGAAATCAAAGACACAGAAAAGTACTGTGCCCTTGACCTAATATGATG  
10 GTAACAAACAATACCTTCACACTCAAAGGCGGTGCACCAACAAAGGTTACTTTTGGTGATGACAC  
TGTGATAGAAGTGCAAGGTTACAAGAGTGTAATATCACTTTTGAACCTGATGAAAGGATTGATAA  
AGTACTTAATGAGAAGTGCTCTGCCTATACAGTTGAACCTCGGTACAGAAGTAAATGAGTTCGCCT  
GTGTTGTGGCAGATGCTGTCATAAAACCTTTGCAACCAGTATCTGAATTACTTACCCACTGGGC  
ATTGATTTAGATGAGTGGAGTATGGCTACATACTACTTATTTGATGAGTCTGGTGAGTTAAATTG  
15 GCTTCACATATGTATTGTTCTTTCTACCCTCCAGATGAGGATGAAGAAGAAGGTGATTGTGAAGAA  
GAAGAGTTTGAGCCATCAACTCAATATGAGTATGGTACTGAAGATGATTACCAAGGTAAACCTTTG  
GAATTTGGTGCCACTTCTGCTGCTCTTCAACCTGAAGAAGAGCAAGAAGAAGATTGGTTAGATGA  
TGATAGTCAACAACTGTTGGTCAACAAGACGGCAGTGAGGACAATCAGACAACCTACTATTCAA  
CAATTGTTGAGGTTCAACCTCAATTAGAGATGGAACCTTACCCAGTTGTTTCACTATTGAAGTGA  
20 ATAGTTTTAGTGTTATTTAAACCTTACTGACAATGTATACATTAATAAATGCAGACATTGTGGAAGA  
AGCTAAAAGGTAAAACCAACAGTGTTGTTAATGCAGCCAATGTTTACCTTAAACATGGAGGAG  
GTGTTGCAGGAGCCTTAAATAAGGCTACTAACAATGCCATGCAAGTTGAATCTGATGATTACATAG  
CTACTAATGGACCACTTAAAGTGGGTGGTAGTTGTGTTTTAAGCGGACACAATCTTGCTAAACACT  
GTCTTCATGTTGTCGGCCCAAATGTTAACAAGGTGAAGACATTCAACTTCTTAAGAGTGCTTATG  
25 AAAATTTTAATCAGCACGAAGTTCTACTTGCACCATTATTATCAGCTGGTATTTTTGGTGCTGACC  
CTATACATTCTTTAAGAGTTTGTGTAGATACTGTTTCGCACAAATGTCTACTTAGCTGTCTTTGATAA  
AAATCTCTATGACAAACTTGTTCAGCTTTTTGGAAATGAAGAGTGAAAAGCAAGTTGAACAAAA  
GATCGCTGAGATTCTAAAGAGGAAGTTAAGCCATTTATACTGAAAGTAAACCTTCAGTTGAACA  
GAGAAAACAAGATGATAAGAAAATCAAAGCTTGTGTTGAAGAAGTTACAACAACCTCTGGAAGAAA  
30 CTAAGTTCCTCACAGAAAACCTGTTACTTTATATTGACATTAATGGCAATCTTCATCCAGATTCTGC  
CACTCTTGTTAGTGACATTGACATCACTTTCTTAAAGAAAGATGCTCCATATATAGTGGGTGATGT  
TGTTCAAGAGGGTGTTTTAACTGCTGTGGTTATACCTACTAAAAGGCTGGTGGCACTACTGAAA  
TGCTAGCGAAAGCTTTGAGAAAAGTGCCAACAGACAATTATATAACCACTTACCCGGGTCAGGGT  
TTAAATGGTTACACTGTAGAGGAGGCAAAGACAGTGCTTAAAAAGTGTAAGAGTGCCTTTTACATT  
35 CTACCATCTATTATCTCTAATGAGAAGCAAGAAATCTTGGAACCTGTTTCTTGGAATTTGCGAGAA  
ATGCTTGACATGCAGAAGAAACACGCAATTAATGCCTGTCTGTGTGGAACTAAAGCCATAGT  
TTCAACTATACAGCGTAAATATAAGGGTATTAAAAATACAAGAGGGTGTGGTTGATTATGGTGCTAG  
ATTTTACTTTTACACCAGTAAAACAACCTGTAGCGTCACTTATCAACACACTTAACGATCTAAATGAA  
ACTCTTGTTACAATGCCACTTGGCTATGTAACACATGGCTTAAATTTGGAAGAAGCTGCTCGGTAT  
40 ATGAGATCTCTCAAAGTGCCAGCTACAGTTTCTGTTTCTTCACCTGATGCTGTTACAGCGTATAAT  
GGTTATCTTACTTCTTCTTCTTAAACACCTGAAGAACATTTTATTGAAACCATCTCACTTGCTGGTT

CCTATAAAGATTGGTCCTATTCTGGACAATCTACACAACCTAGGTATAGAATTTCTTAAGAGAGGTG  
ATAAAAGTGTATATTACACTAGTAATCCTACCACATTCCACCTAGATGGTGAAGTTATCACCTTTGA  
CAATCTTAAGACACTTCTTTCTTTGAGAGAAGTGAGGACTATTAAGGTGTTTACAACAGTAGACAA  
CATTAACCTCCACACGCAAGTTGTGGACATGTCAATGACATATGGACAACAGTTTGGTCCAACCTT  
5 ATTTGGATGGAGCTGATGTTACTAAAATAAAACCTCATAATTCACATGAAGGTAAAACATTTTATGT  
TTTACCTAATGATGACACTCTACGTGTTGAGGCTTTTGAGTACTACCACACAACCTGATCCTAGTTT  
TCTGGGTAGGTACATGTCAGCATTAAATCACACTAAAAAGTGGAATACCCACAAGTTAATGGTTT  
AACTTCTATTAAATGGGCAGATAACAACTGTTATCTTGCCACTGCATTGTTAACTCCAACAAATA  
GAGTTGAAGTTTAAATCCACCTGCTCTACAAGATGCTTATTACAGAGCAAGGGCTGGTGAAGCTGC  
10 TAACTTTTGTGCACTTATCTTAGCCTACTGTAATAAGACAGTAGGTGAGTTAGGTGATGTTAGAGA  
AACAATGAGTTACTTGTTCACATGCCAATTTAGATTCTTGCAAAAGAGTCTTGAACGTGGTGTG  
TAAACTTGTGGACAACAGCAGACAACCCCTTAAGGGTGTAGAAGCTGTTATGTACATGGGCACAC  
TTTCTTATGAACAATTTAAGAAAGGTGTTTACAGATACCTTGACGTGGTAAACAAGCTACAAAAT  
ATCTAGTACAACAGGAGTCACCTTTTGTATGATGTCAGCACCACCTGCTCAGTATGAACCTAAGC  
15 ATGGTACATTTACTTGTGCTAGTGAGTACACTGGTAATTACCAGTGTGGTCACTATAAACATATAA  
CTTCTAAAGAAACTTTGTATTGCATAGACGGTGCTTTACTTACAAAGTCCTCAGAATACAAAGGTC  
CTATTACGGATGTTTTCTACAAAGAAAAACAGTTACACAACAACCATAAAACCAGTTACTTATAAATT  
GGATGGTGTGTTTGTACAGAAATTGACCCTAAGTTGGACAATTATTATAAGAAAGACAATTCTTA  
TTTCACAGAGCAACCAATTGATCTTGACCAAACCAACCATATCCAAACGCAAGCTTCGATAATTT  
20 TAAGTTTGTATGTGATAATATCAAATTTGCTGATGATTTAAACCAGTTAACTGGTTATAAGAAACCT  
GCTTCAAGAGAGCTTAAAGTTACATTTTCCCTGACTTAAATGGTGTGTTGGTGGCTATTGATTAT  
AAACACTACACACCCTCTTTAAGAAAGGAGCTAAATTGTTACATAAACCTATTGTTTGGCATGTTA  
ACAAATGCAACTAATAAAGCCACGTATAAACCAAAATACCTGGTGTATACGTTGTCTTTGGAGCACAA  
AACCAGTTGAAACATCAAATTCGTTTGTATGTAAGTCAAGTCAAGGACGCGCAGGGAATGGATAAT  
25 CTTGCCTGCGAAGATCTAAACCAGTCTCTGAAGAAGTAGTGGAAAATCCTACCATACAGAAAGA  
CGTTCTTGAGTGAATGTGAAAACCTACCGAAGTTGTAGGAGACATTATACTTAAACCAGCAAATAA  
TAGTTTAAAAATTACAGAAGAGGTTGGCCACACAGATCTAATGGCTGCTTATGTAGACAATTCTAG  
TCTTACTATTAAGAAACCTAATGAATTATCTAGAGTATTAGGTTTGAACCCCTTGCTACTCATGGT  
TTAGCTGCTGTTAATAGTGCCCTTGGGATACTATAGCTAATTATGCTAAGCCTTTTCTTAACAAA  
30 GTTGTAGTACAACACTACTAACATAGTTACACGGTGTTTAAACCGTGTGTTGACTAATTATATGCCTT  
ATTTCTTTACTTTATTGCTACAATTGTGTACTTTTACTAGAAGTACAAATTCTAGAATTAAAGCATCT  
ATGCCGACTACTATAGCAAAGAATACTGTTAAGAGTGTGCGTAAATTTTGTCTAGAGGCTTCATTT  
AATTATTTGAAGTCACCTAATTTTTCTAACTGATAAATATTATAATTTGGTTTTTACTATTAAGTGT  
TGCCTAGGTTCTTTAATCTACTCAACCGCTGCTTTAGGTGTTTTAATGTCTAATTTAGGCATGCCTT  
35 CTTACTGTACTGGTTACAGAGAAGGCTATTTGAACCTCTACTAATGTCACTATTGCAACCTACTGTA  
CTGGTTCTATACCTTGATGTGTTGTCTTAGTGGTTAGATTCTTTAGACACCTATCCTTCTTTAGA  
AACTATACAAATTACCATTTTCATCTTTTAAATGGGATTTAACTGCTTTTGGCTTAGTTGCAGAGTGG  
TTTTTGGCATATATTCTTTTCACTAGGTTTTCTATGTAAGTTGGCTGCAATCATGCAATTGT  
TTTTTCAGCTATTTTGCAGTACATTTTATTAGTAATCTTGGCTTATGTGGTTAATAATTAATCTTGTA  
40 CAAATGGCCCCGATTTTCACTATGGTTAGAATGTACATCTTCTTGCATCATTTTATTATGTATGGA  
AAAGTTATGTGCATGTTGTAGACGGTTGTAATTCATCAACTTGTATGATGTGTTACAAACGTAATA

GAGCAACAAGAGTCGAATGTACAACCTATTGTTAATGGTGTTAGAAGGTCCTTTTATGTCTATGCTA  
ATGGAGGTAAGGCTTTTGCAAACCTACACAATTGGAATTGTGTTAATTGTGATACATTCTGTGCTG  
GTAGTACATTTATTAGTGATGAAGTTGCGAGAGACTTGCTACTACAGTTTAAAAGACCAATAAATC  
CTACTGACCAGTCTTCTTACATCGTTGATAGTGTTACAGTGAAGAATGGTTCCATCCATCTTTACT  
5 TTGATAAAGCTGGTCAAAAGACTTATGAAAGACATTCTCTCTCATTGTTAACTTAGACAACCT  
GAGAGCTAATAACACTAAAGGTTCAATGCCTATTAATGTTATAGTTTTTGATGGTAAATCAAAATGT  
GAAGAATCATCTGCAAAATCAGCGTCTGTTTACTACAGTCAGCTTATGTGTCAACCTATACTGTTA  
CTAGATCAGGCATTAGTGTCTGATGTTGGTGATAGTGCAGGAAAGTTGCAGTTAAAATGTTTGATGC  
TTACGTTAATACGTTTTTCATCAACTTTTAACTACCAATGGAAAACTCAAAACACTAGTTGCAACT  
10 GCAGAAGCTGAACCTGCAAGAATGTGTCTTAGACAATGTCTTATCTACTTTTATTTTACAGCAGCT  
CGGCAAGGGTTTGTTGATTCAGATGTAGAACTAAAGATGTTGTTGAATGTCTTAAATTGTCACAT  
CAATCTGACATAGAAGTTACTGGCGATAGTTGTAATAACTATATGCTCACCTATAACAAAGTTGAA  
AACATGACACCCCGTGACCTTGGTGCTTGATTGACTGTAGTGCAGCATATTAATGCGCAGGT  
AGCAAAAAGTCACAACATTGCTTTGATATGGAACGTTAAAGATTTTCATGTCATTGTCTGAACAACT  
15 ACGAAAACAAATACGTAGTGCTGCTAAAAAGAATAACTTACCTTTTAAAGTTGACATGTGCAACTAC  
TAGACAAGTTGTTAATGTTGTAACAACAAAGATAGCACTTAAGGGTGGTAAAATTGTTAATAATTG  
GTTGAAGCAGTTAATTAAAGTTACACTTGTGTTCCCTTTTTGTTGCTGCTATTTTCTATTTAATAACAC  
CTGTTTCATGTCATGTCTAAACATACTGACTTTTCAAGTGAAATCATAGGATACAAGGCTATTGATG  
GTGGTGCTACTCGTGACATAGCATCTACAGATACTTGTTTTGCTAACAACATGCTGATTTTGACA  
20 CATGGTTTAGCCAGCGTGGTGGTAGTTATACTAATGACAAAGCTTGCCCATGATTGCTGCAGTC  
ATAACAAGAGAAGTGGGTTTTGTCGTGCCTGGTTTGCCTGGCACGATATTACGCACAACCTAATGG  
TGACTTTTTGCATTTCTTACCTAGAGTTTTTAGTGACAGTTGGTAACATCTGTTACACACCATCAAAA  
CTTATAGAGTACACTGACTTTTGCAACATCAGCTTGTGTTTTGGCTGCTGAATGTACAATTTTTAAA  
GATGCTTCTGGTAAGCCAGTACCATATTGTTATGATACCAATGTACTAGAAGGTTCTGTTGCTTAT  
25 GAAAGTTTACGCCCTGACACACGTTATGTGCTCATGGATGGCTCTATTATTCAATTTCTAACACC  
TACCTTGAAGGTTCTGTTAGAGTGGTAACAACCTTTGATTCTGAGTACTGTAGGCACGGCACTTGT  
GAAAGATCAGAAGCTGGTGTGTTGTATCTACTAGTGGTAGATGGGTACTTAACAATGATTATTAC  
AGATCTTTACCAGGAGTTTTCTGTGGTGTAGATGCTGTAAATTTACTTACTAATATGTTTACACCAC  
TAATTCAACCTATTGGTGCTTTGGACATATCAGCATCTATAGTAGCTGGTGGTATTGTAGCTATCG  
30 TAGTAACATGCCTTGCCCTACTATTTTATGAGGTTTAGAAGAGCTTTTGGTGAATACAGTCATGTAG  
TTGCCTTTAATACTTTACTATTCCCTTATGTCATTCACTGTACTCTGTTTAAACACCAGTTTACTCATTC  
TTACCTGGTGTGTTATTCTGTTATTTTACTTGTACTTGACATTTTATCTTACTAATGATGTTTCTTTTTTA  
GCACATATTCAGTGGATGGTTATGTTTACACCTTTAGTACCTTTCTGGATAACAATTGCTTATATCA  
TTTGATTTCCACAAAGCATTTCTATTGGTTCTTTAGTAATTACCTAAAGAGACGTGTAGTCTTTAA  
35 TGGTGTTCCTTTAGTACTTTTTGAAGAAGCTGCGCTGTGCACCTTTTTGTTAAATAAGAAATGTAT  
CTAAAGTTGCGTAGTGATGTGCTATTACCTCTTACGCAATATAATAGATACTTAGCTCTTTATAATA  
AGTACAAGTATTTTAGTGAGCAATGGATACAACCTAGCTACAGAGAAGCTGCTTGTGTCATCTC  
GCAAAGGCTCTCAATGACTTCAGTAACTCAGGTTCTGATGTTCTTTACCAACCACCACAAACCTCT  
ATCACCTCAGCTGTTTTGCAGAGTGTTTTAGAAAAATGGCATTCCCCTCTGGTAAAGTTGAGGG  
40 TTGTATGGTACAAGTAACTTGTGGTACAACCTACCTTAACGGTCTTTGGCTTGATGACGTAGTTTA  
CTGTCCAAGACATGTGATCTGCACCTCTGAAGACATGCTTAACCCTAATTATGAAGATTTACTCAT

TCGTAAGTCTAATCATAATTTCTTGGTACAGGCTGGTAATGTTCAACTCAGGGTTATTGGACATTC  
TATGCAAAATTGTGTAAGCTTAAGGTTGATACAGCCAATCCTAAGACACCTAAGTATAAGTT  
TGTTTCGCATTCAACCAGGACAGACTTTTTCAAGTGTAGCTTGTACAATGGTTCACCATCTGGTGT  
TTACCAATGTGCTATGAGGCCCAATTTCACTATTAAGGGTTCATTCCTTAATGGTTCATGTGGTAG  
5 TGTGTTGTTTTAACATAGATTATGACTGTGTCTCTTTTTGTTACATGCACCATATGGAATTACCAACT  
GGAGTTCATGCTGGCACAGACTTAGAAGGTAACTTTTATGGACCTTTTGTGACAGGCAAACAGC  
ACAAGCAGCTGGTACGGACACAACCTATTACAGTTAATGTTTTAGCTTGGTTGTACGCTGCTGTTAT  
AAATGGAGACAGGTGGTTTTCTCAATCGATTTACCACAACCTCTTAATGACTTTAACCTTGTGGCTAT  
GAAGTACAATTATGAACCTCTAACACAAGACCATGTTGACATACTAGGACCTCTTTCTGCTCAAAC  
10 TGGAATTGCCGTTTTAGATATGTGTGCTTCATTAAGAATTACTGCAAAATGGTATGAATGGACG  
TACCATATTGGGTAGTGCTTTATTAGAAGATGAATTTACACCTTTTGATGTTGTTAGACAATGCTCA  
GGTGTACTTTCCAAAGTGCAAGTGAAGAACAATCAAGGGTACACACCACTGGTTGTTACTCAC  
AATTTTGACTTCACTTTTAGTTTTAGTCCAGAGTACTCAATGGTCTTTGTTCTTTTTTTGTATGAAA  
ATGCCTTTTTACCTTTTGCTATGGGTATTATTGCTATGTCTGCTTTTGCAATGATGTTGTCAAACA  
15 TAAGCATGCATTTCTCTGTTTGTGTTTTGTTACCTTCTCTTGCCACTGTAGCTTATTTAATATGGTC  
TATATGCCCTGCTAGTTGGGTGATGCGTATTATGACATGGTTGGATATGGTTGATACTAGTTTGTCT  
GGTTTTAAGCTAAAAGACTGTGTTATGTATGCATCAGCTGTAGTGTTACTAATCCTTATGACAGCA  
AGAAGTGTGTATGATGATGGTGCTAGGAGAGTGTGGACACTTATGAATGTCTTGACACTCGTTTA  
TAAAGTTTATTATGGTAATGCTTTAGATCAAGCCATTTCCATGTGGGCTCTTATAATCTCTGTTACT  
20 TCTAACTACTCAGGTGTAGTTACAACCTGTCATGTTTTTGCCAGAGGTATTGTTTTATGTGTGTT  
GAGTATTGCCCTATTTTCTCATAACTGGTAATACACTTCAGTGATAATGCTAGTTTATTGTTTCT  
TAGGCTATTTTTGTACTTGTTACTTTGGCCTCTTTGTTTACTCAACCGCTACTTTAGACTGACTCT  
TGGTGTTTATGATTACTTAGTTTCTACACAGGAGTTAGATATATGAATTCACAGGGACTACTCCC  
ACCCAAGAATAGCATAGATGCCTTCAAACCTCAACATTAAATTGTTGGGTGTTGGTGGCAAACCTT  
25 GTATCAAAGTAGCCACTGTACAGTCTAAAATGTCAGATGTAAAGTGACATCAGTAGTCTTACTCT  
CAGTTTTGCAACAACCTCAGAGTAGAATCATCATCTAAATTGTGGGCTCAATGTGTCCAGTTACACA  
ATGACATTCTCTTAGCTAAAGATACTACTGAAGCCTTTGAAAAATGGTTTCACTACTTTCTGTTTT  
GCTTTCCATGCAGGGTGCTGTAGACATAAACAAGCTTTGTGAAGAAATGCTGGACAACAGGGCAA  
CCTTACAAGCTATAGCCTCAGAGTTTAGTTCCCTTCCATCATATGCAGCTTTTGCTACTGCTCAAG  
30 AAGCTTATGAGCAGGCTGTTGCTAATGGTGATTCTGAAGTTGTTCTTAAAAAGTTGAAGAAGTCTT  
TGAATGTGGCTAAATCTGAATTTGACCGTGATGCAGCCATGCAACGTAAGTTGGAAGATGGCT  
GATCAAGCTATGACCCAAATGTATAACAGGCTAGATCTGAGGACAAGAGGGCAAAGTTACTAG  
TGCTATGCAGACAATGCTTTTCACTATGCTTAGAAAAGTTGGATAATGATGCACTCAACAACATTAT  
CAACAATGCAAGAGATGGTTGTGTTCCCTTGAACATAATACCTCTTACAACAGCAGCCAAACTAAT  
35 GGTTGTCATACCAGACTATAACACATATAAAAAATACGTGTGATGGTACAACATTTACTTATGCATC  
AGCATTGTGGGAAATCCAACAGGTTGTAGATGCAGATAGTAAATTTGTTCAACTTAGTGAAATTAG  
TATGGACAATTACCTAATTTAGCATGGCCTCTTATTGTAAACAGCTTTAAGGGCCAATTCTGCTGT  
CAAATTACAGAATAATGAGCTTAGTCCTGTTGCACTACGACAGATGTCTTGTGCTGCCGGTACTA  
CACAACTGCTTGCACTGATGACAATGCGTTAGCTTACTACAACACAACAAAGGGAGGTAGGTTT  
40 GTACTTGCACTGTTATCCGATTTACAGGATTTGAAATGGGCTAGATTCCTAAGAGTGATGGAAC  
GGTACTATCTATACAGAACTGGAACCACCTTGTAGGTTTGTACAGACACACCTAAAGGTCCTAAA

GTGAAGTATTTATACTTTATTAAGGATTAAACAACCTAAATAGAGGTATGGTACTTGGTAGTTTAG  
CTGCCACAGTACGTCTACAAGCTGGTAATGCAACAGAAGTGCCTGCCAATTCAACTGTATTATCT  
TTCTGTGCTTTTGTCTGTAGATGCTGCTAAAGCTTACAAAGATTATCTAGCTAGTGGGGGACAACC  
AATCACTAATTGTGTTAAGATGTTGTGTACACACACTGGTACTGGTCAGGCAATAACAGTTACACC  
5 GGAAGCCAATATGGATCAAGAATCCTTTGGTGGTGCATCGTGTGTCTGTACTGCCGTTGCCACA  
TAGATCATCCAAATCCTAAAGGATTTTGTGACTTAAAAGGTAAGTATGTACAAATACCTACAACCTG  
TGCTAATGACCCTGTGGGTTTTACACTTAAAAACACAGTCTGTACCGTCTGCGGTATGTGGAAAG  
GTTATGGCTGTAGTTGTGATCAACTCCGCGAACCCATGCTTCAGTCAGCTGATGCACAATCGTTT  
TTAAACGGGTTTGCAGGTGTAAGTGCAGCCCGTCTTACACCGTGCGGCACAGGCACTAGTACTGA  
10 TGTCGTATACAGGGCTTTTGACATCTACAATGATAAAGTAGCTGGTTTTGCTAAATTCCTAAAAAC  
TAATTGTTGTCGCTTCCAAGAAAAGGACGAAGATGACAATTTAATTGATTCTTACTTTGTAGTTAAG  
AGACACACTTTCTCTAACTACCAACATGAAGAAACAATTTATAATTTACTTAAGGATTGTCCAGCTG  
TTGCTAAACATGACTTCTTTAAGTTTAGAATAGACGGTGACATGGTACCACATATATCACGTCAAC  
GTCTTACTAAATACACAATGGCAGACCTCGTCTATGCTTTAAGGCATTTTGATGAAGGTAATTGTG  
15 ACACATTAAGAAATACTTGTACATACAATTGTTGTGATGATGATTATTTCAATAAAAAGGACTG  
GTATGATTTTGTAGAAAACCCAGATATATTACGCGTATACGCCAAGTTAGGTGAACGTGTACGCC  
AAGCTTTGTTAAAAACAGTACAATTCTGTGATGCCATGCGAAATGCTGGTATTGTTGGTGTACTGA  
CATTAGATAATCAAGATCTCAATGGTAACTGGTATGATTTCCGGTGATTTCATACAAACCACGCCAG  
GTAGTGGAGTTCCTGTTGTAGATTCTTATTATTCATTGTTAATGCCTATATTAACCTTGACCAGGG  
20 CTTTAACTGCAGAGTCACATGTTGACACTGACTTAACAAAGCCTTACATTAAGTGGGATTTGTTAA  
AATATGACTTCACGGAAGAGAGGTTAAACTCTTTGACCGTTATTTTAAATATTGGGATCAGACAT  
ACCACCCAAATTGTGTTAACTGTTTGGATGACAGATGCATTCTGCATTGTGCAAACTTTAATGTTT  
TATTCTCTACAGTGTTCACCTACAAGTTTTGGACCACTAGTGAGAAAAATATTTGTTGATGGTG  
TTCCATTTGTAGTTTCAACTGGATAACCACTTCAGAGAGCTAGGTGTTGTACATAATCAGGATGTAA  
25 ACTTACATAGCTCTAGACTTAGTTTTAAGGAATTACTTGTGTATGCTGCTGACCCTGCTATGCACG  
CTGCTTCTGGTAATCTATTACTAGATAAACGCACTACGTGCTTTTCAGTAGCTGCACTTACTAACA  
ATGTTGCTTTTCAAACGTCAAAACCCGGTAATTTTAAACAAAGACTTCTATGACTTTGCTGTGTCTAA  
GGGTTTCTTTAAGGAAGGAAGTTCTGTTGAATTAACAACTTCTTCTTTGCTCAGGATGGTAATGC  
TGCTATCAGCGATTATGACTACTATCGTTATAATCTACCAACAATGTGTGATATCAGACAACCTACTA  
30 TTTGTAGTTGAAGTTGTTGATAAGTACTTTGATTGTTACGATGGTGGCTGTATTAATGCTAACCAA  
GTCATCGTCAACAACCTAGACAAATCAGCTGGTTTTCCATTTAATAAATGGGGTAAGGCTAGACTT  
TATTATGATTCAATGAGTTATGAGGATCAAGATGCACTTTTCGCATATACAAAACGTAATGTCATCC  
CTACTATAACTCAAATGAATCTTAAGTATGCCATTAGTGCAAAGAATAGAGCTCGCACCGTAGCTG  
GTGTCTCTATCTGTAGTACTATGACCAATAGACAGTTTCATCAAAAATTATTGAAATCAATAGCCG  
35 CCACTAGAGGAGCTACTGTAGTAATTGGAACAAGCAAATTCATGGTGGTTGGCACAACATGTTA  
AAAACGTGTTTATAGTGATGTAGAAAACCCCTCACCTTATGGGTTGGGATTATCTAAATGTGATAGA  
GCCATGCCTAACATGCTTAGAATTATGGCCTCACTTGTTCTTGCTCGCAAACATACAACGTGTTGT  
AGCTTGTACACCGTTTCTATAGATTAGCTAATGAGTGTGCTCAAGTATTGAGTGAAATGGTCATG  
TGTGGCGGTTCACTATATGTTAAACCAGGTGGAACCTCATCAGGAGATGCCACAACCTGCTTATGC  
40 TAATAGTGTTTTTAACATTTGTCAAGCTGTACGGCCAATGTTAATGCACTTTTATCTACTGATGGT  
AACAAAATTGCCGATAAGTATGTCCGCAATTTACAACACAGACTTTATGAGTGTCTCTATAGAAAT

AGAGATGTTGACACAGACTTTGTGAATGAGTTTTACGCATATTTGCGTAAACATTTCTCAATGATG  
ATACTCTCTGACGATGCTGTTGTGTGTTTCAATAGCACTTATGCATCTCAAGGTCTAGTGGCTAGC  
ATAAAGAACTTTAAGTCAGTTCTTTATTATCAAAACAATGTTTTATGTCTGAAGCAAAATGTTGGA  
CTGAGACTGACCTTACTAAAGGACCTCATGAATTTGCTCTCAACATACAATGCTAGTTAAACAGG  
5 GTGATGATTATGTGTACCTTCCTTACCCAGATCCATCAAGAATCCTAGGGGCCGGCTGTTTTGTA  
GATGATATCGTAAAAACAGATGGTACACTTATGATTGAACGGTTCGTGTCTTTAGCTATAGATGCT  
TACCCACTTACTAAACATCCTAATCAGGAGTATGCTGATGTCTTTCATTTGACTTACAATACATAA  
GAAAGCTACATGATGAGTTAACAGGACACATGTTAGACATGTATTCTGTTATGCTTACTAATGATA  
ACACTTCAAGGTATTGGGAACCTGAGTTTTATGAGGCTATGTACACACCGCATACAGTCTTACAG  
10 GCTGTTGGGGCTTGTTCTTTGCAATTCACAGACTTCATTAAGATGTGGTGCTTGCATACGTAG  
ACCATTCCTTATGTTGTAAATGCTGTTACGACCATGTCATATCAACATCACATAAATTAGTCTTGTCT  
GTTAATCCGTATGTTTGAATGCTCCAGGTTGTGATGTCACAGATGTGACTCAACTTTACTTAGGA  
GGTATGAGCTATTATTGTAAATCACATAAACCACCCATTAGTTTTCCATTGTGTGCTAATGGACAA  
GTTTTTGGTTTATATAAAATACATGTGTTGGTAGCGATAATGTTACTGACTTTAATGCAATTGCAA  
15 CATGTGACTGGACAAATGCTGGTGATTACATTTTAGCTAACACCTGTAAGTCAAGCTTT  
TTGCAGCAGAAACGCTCAAAGCTACTGAGGAGACATTTAACTGTCTTATGGTATTGCTACTGTAC  
GTGAAGTGCTGTCTGACAGAGAATTACATCTTTCATGGGAAGTTGGTAAACCTAGACCACCACTT  
AACCGAAATTATGTCTTTACTGGTTATCGTGTAACCTAAAAACAGTAAAGTACAAATAGGAGAGTAC  
ACCTTTGAAAAAGGTGACTATGGTGATGCTGTTGTTTACCGAGGTACAACAACCTACAAATTAAT  
20 GTTGGTGATTATTTGTGCTGACATCACATACAGTAATGCCATTAAGTGCACCTACACTAGTGCCA  
CAAGAGCACTATGTTAGAATTACTGGCTTATACCCAACACTCAATATCTCAGATGAGTTTTCTAGC  
AATGTTGCAAATTATCAAAAGGTTGGTATGCAAAAGTATTCTACACTCCAGGGACCACCTGGTACT  
GGTAAGAGTCATTTTGCTATTGGCCTAGCTCTCTACTACCCTTCTGCTCGCATAGTGATACAGCT  
TGCTCTCATGCCGCTGTTGATGCACTATGTGAGAAGGCATTAATAATTTGCCTATAGATAAATGT  
25 AGTAGAATTATACCTGCACGTGCTCGTGAGAGTGTTTTGATAAATCAAAGTGAATTCAACATTA  
GAACAGTATGTCTTTTGTACTGTAAATGCATTGCCTGAGACGACAGCAGATATAGTTGTCTTTGAT  
GAAATTTCAATGGCCACAAATTATGATTGAGTGTTGTCAATGCCAGATTACGTGCTAAGCACTAT  
GTGTACATTGGCGACCCTGCTCAATTACCTGCACCACGCACATTGCTAACTAAGGGCACACTAGA  
ACCAGAATATTTCAATTCAGTGTTAGACTTATGAAAACCTATAGGTCCAGACATGTTCTCGGAAC  
30 TTGTCGGCGTTGTCTGCTGAAATTGTTGACACTGTGAGTGCTTTGGTTTATGATAATAAGCTTAA  
AGCACATAAAGACAAATCAGCTCAATGCTTTAAATGTTTTATAAGGGTGTTATCACGCATGATGT  
TTCATCTGCAATTAACAGGCCACAAATAGGCGTGGTAAGAGAATTCCTTACACGTAACCCTGCTT  
GGAGAAAAGCTGTCTTTATTTACCTTATAATTACAGAAATGCTGTAGCCTCAAAGATTTTGGGAC  
TACCAACTCAAACCTGTTGATTCATCACAGGGCTCAGAATATGACTATGTCATATTTCACTCAAACCA  
35 CTGAAACAGCTCACTCTTGTAATGTAAACAGATTTAATGTTGCTATTACCAGAGCAAAAGTAGGCA  
TACTTTGCATAATGTCTGATAGAGACCTTTATGACAAGTTGCAATTTACAAGCTTGAAATTCACG  
TAGGAATGTGGCAACTTTACAAGCTGAAAATGTAACAGGACTCTTTAAAGATTGTAGTAAGGTAAT  
CACTGGGTTACATCTACACAGGCACCTACACACCTCAGTGTTGACACTAAATTCAAAACCTGAAG  
GTTTATGTGTTGACATACCTGGCATACCTAAGGACATGACCTATAGAAGACTCATCTCTATGATGG  
40 GTTTTAAATGAATTATCAAGTTAATGGTTACCCTAACATGTTTATCAGGCGGAAGAAGCTATAA  
GACATGTACGTGCATGGATTGGCTTCGATGTCGAGGGGTGTCATGCTACTAGAGAAGCTGTTGG

41

TAAGACTAAAAATGTTACAAAAGAAAATGACTCTAAAGAGGGTTTTTCACTTACATTTGTGGGTTT  
ATACAACAAAAGCTAGCTCTTGGAGGTTCCGTGGCTATAAAGATAACAGAACATTCTTGAATGCT  
GATCTTTATAAGCTCATGGGACACTTCGCATGGTGGACAGCCTTTGTTACTAATGTGAATGCGTC  
ATCATCTGAAGCATTTTTAATTGGATGTAATTATCTTGGCAAACCACGCGAACAAATAGATGGTTA  
5 TGCATGCATGCAAATTACATATTTTGGAGGAATACAAATCCAATTCAGTTGTCTTCTTATTCTTTA  
TTTGACATGAGTAAATTTCCCTTAAATTAAGGGTACTGCTGTTATGTCTTTAAAGAAGGTCAA  
ATCAATGATATGATTTTATCTCTTCTTAGTAAAGGTAGACTTATAATTAGAGAAAACAACAGAGTTG  
TTATTTCTAGTGATGTTCTTGTTAACTAAACGAACAATGTTTGTCTTTCTTGTATTGCCACT  
AGTCTCTAGTCAGTGTGTTAATCTTACAACCAGAACTCAATTACCCCTGCATACACTAATTCTTT  
10 CACACGTGGTGTATTACCCTGACAAAGTTTCAGATCCTCAGTTTTACATTCAACTCAGGACTT  
GTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCATGCTATACATGTCTCTGGGACCAATGGTACT  
AAGAGGTTTGATAACCCTGTCTACCATTTAATGATGGTGTATTATTTGCTTCCACTGAGAAGTCT  
AACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTGGAAGACCCAGTCCCTACTTATTGTT  
AATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAATTTTGAATGATCCATTTTGGGTG  
15 TTTATTACCACAAAAACAACAAAAGTTGGATGGAAAGTGAGTTCAGAGTTTATTCTAGTGCGAATA  
ATTGCACTTTTGAATATGTCTCTCAGCCTTTTCTTATGGACCTTGAAGGAAAACAGGGTAATTTCA  
AAAACTTAGGGAATTTGTGTTAAGAATATTGATGGTTATTTTAAATATATTCTAAGCACACGCC  
TATTAATTTAGTGCGTGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTTGCCAAT  
AGGTATTAACATCACTAGGTTTCAAACCTTACTTGCTTTACATAGAAGTTATTTGACTCCTGGTGAT  
20 TCTTCTTCAGGTTGGACAGCTGGTGTCTGCAGCTTATTATGTGGGTTATCTTCAACCTAGGACTTTT  
CTATTAATAATAATGAAAATGGAACCATTACAGATGCTGTAGACTGTGCACCTTGACCCTCTCTCA  
GAAACAAAGTGACGTTGAAATCCTTCACTGTAGAAAAAGGAATCTATCAAACCTTCTAACTTTAGA  
GTCCAACCAACAGAATCTATTGTTAGATTTCTAATATTACAACTTGTGCCCTTTTGGTGAAGTTT  
TTAACGCCACCAGATTTGCATCTGTTTATGCTTGGAACAGGAAGAGAATCAGCAACTGTGTTGCT  
25 GATTATTCTGTCCTATATAATTCGCATCATTTTCCACTTTTAAAGTGTATGGAGTGTCTCTACTA  
AATTAAATGATCTCTGCTTTACTAATGTCTATGCAGATTCATTTGTAATTAGAGGTGATGAAGTCAG  
ACAAATCGCTCCAGGGCAAACCTGGAAAGATTGCTGATTATAATTATAAATTACCAGATGATTTTAC  
AGGCTGCGTTATAGCTTGAATTCTAACAATCTTGATTCTAAGGTTGGTGGTAATTATAATTACCT  
GTATAGATTGTTTAGGAAGTCTAATCTCAAACCTTTTGAAGAGATATTTCAAACGAAATCTATCAG  
30 GCCGGTAGCACACCTTGTAATGGTGTGGAAGGTTTTAATTGTTACTTTCTTTACAATCATATGGT  
TTCCAACCCACTAATGGTGTGGTTACCAACCATACAGAGTAGTAGTACTTTCTTTTGAACCTCTA  
CATGCACCAGCAACTGTTTGTGGACCTAAAAAGTCTACTAATTTGGTTAAAAACAAATGTGTCAAT  
TTCAAACCTCAATGGTTTAAACAGGCACAGGTGTTCTTACTGAGTCTAACAAAAGTTTCTGCCTTTC  
CAACAATTTGGCAGAGACATTGCTGACACTACTGATGCTGTCCGTGATCCACAGACACTTGAGAT  
35 TCTTGACATTACACCATGTTCTTTTGGTGGTGTGAGTGTATAACACCAGGAACAAATACTTCTAA  
CCAGGTTGCTGTTCTTTATCAGGATGTTAACTGCACAGAAGTCCCTGTTGCTATTCATGCAGATCA  
ACTTACTCCTACTTGGCGTGTTTATTCTACAGGTTCTAATGTTTTTCAAACACGTGCAGGCTGTTT  
AATAGGGGCTGAACATGTCAACAACTCATATGAGTGTGACATACCCATTGGTGCAGGTATATGCG  
CTAGTTATCAGACTCAGACTAATTCTCCTCGGCGGGCACGTAGTGTAGCTAGTCAATCCATCATT  
40 GCCTACACTATGTCACCTGGTGCAGAAAATTCAGTTGCTTACTCTAATACTCTATTGCCATACCC  
ACAAATTTTACTATTAGTGTACCACAGAAATCTACCAGTGTCTATGACCAAGACATCAGTAGATT

GTACAATGTACATTTGTGGTGATTCAACTGAATGCAGCAATCTTTTGTGCAATATGGCAGTTTTT  
GTACACAATTAACCGTGCTTTAACTGGAATAGCTGTTGAACAAGACAAAAACACCAAGAAAGTTT  
TTGCACAAGTCAAACAAATTTACAAAACACCACCAATTAAGATTTTGGTGGTTTTAATTTTTCACA  
AATATTACCAGATCCATCAAAACCAAGCAAGAGGTCATTTATTGAAGATCTACTTTTCAACAAAGT  
5 GACACTTGCAGATGCTGGCTTCATCAAACAATATGGTGATTGCCTTGGTGATATTGCTGCTAGAG  
ACCTCATTTGTGCACAAAAGTTTAACGGCCTTACTGTTTTGCCACCTTGTCTACAGATGAAATGA  
TTGCTCAATACACTTCTGCACTGTTAGCGGGTACAATCACTTCTGGTTGGACCTTTGGTGCAGGT  
GCTGCATTACAAATACCATTTGCTATGCAAATGGCTTATAGGTTTAATGGTATTGGAGTTACACAG  
AATGTTCTCTATGAGAACCAAAAAATTGATTGCCAACCAATTTAATAGTGCTATTGGCAAAATTCAG  
10 ACTCACTTTCTTCCACAGCAAGTGCCTTGGAAAACCTCAAGATGTGGTCAACCAAAATGCACAA  
GCTTTAAACACGCTTGTTAAACAACCTTAGCTCCAATTTTGGTGCAATTTCAAGTGTTTTAAATGATA  
TCCTTTCACGCTTGACAAAAGTTGAGGCTGAAGTGCAAATTGATAGGTTGATCACAGGCAGACTT  
CAAAGTTTGCAGACATATGTGACTCAACAATTAATTAGAGCTGCAGAAATCAGAGCTTCTGCTAAT  
CTTGCTGCTACTAAAATGTCAGAGTGTGTACTTGGACAATCAAAAAGAGTTGATTTTTGTGGAAAG  
15 GGCTATCATCTTATGTCCTTCCCTCAGTCAGCACCTCATGGTGATGCTTCTTGCATGTGACTTAT  
GTCCCTGCACAAGAAAAGAACTTCACAACCTGCTCCTGCCATTTGTCATGATGGAAAAGCACACTT  
TCCTCGTGAAGGTGTCTTTGTTTCAAATGGCACACACTGGTTTGTAAACACAAAGGAATTTTTATGA  
ACCACAAATCATTACTACAGACAACACATTTGTGTCTGGTAACTGTGATGTTGTAATAGGAATTGT  
CAACAACACAGTTTATGATCCTTTGCAACCTGAATTAGACTCATTCAAGGAGGAGTTAGATAAATA  
20 TTTTAAGAATCATACATCACCAGATGTTGATTTAGGTGACATCTCTGGCATTAAATGCTTCAGTTGTA  
AACATTCAAAAAGAAATTGACCGCCTCAATGAGGTTGCCAAGAATTTAAATGAATCTCTCATCGAT  
CTCCAAGAACCTTGAAAGTATGAGCAGTATATAAAATGGCCATGGTACATTTGGCTAGGTTTTATA  
GCTGGCTTGATTGCCATAGTAATGGTGACAATTATGCTTTGCTGTATGACCAGTTGCTGTAGTTGT  
CTCAAGGGCTGTTGTTCTTGTGGATCCTGCTGCAAATTTGATGAAGACGACTCTGAGCCAGTGCT  
25 CAAAGGAGTCAAATTACATTACACATAAACGAACCTTATGGATTTGTTTATGAGAATCTTCACAATTG  
GAACTGTAACCTTTGAAGCAAGGTGAAATCAAGGATGCTACTCCTTCAGATTTTGTTCGCGCTACT  
GCAACGATACCGATACAAGCCTCACTCCCTTTGGGATGGCTTATTGTTGGCGTTGCACTTCTTGC  
TGTTTTTCAGAGCGCTTCCAAAATCATAACCCTCAAAAAGAGATGGCAACTAGCACTCTCCAAGG  
GTGTTCACTTTGTTTGCAACTTGCTGTTGTTGTTGTAACAGTTTACTCACACCTTTTGCTCGTTGC  
30 TGCTGGCCTTGAAGCCCCCTTTCTCTATCTTTATGCTTTAGTCTACTTCTTGCAGAGTATAAACTTT  
GTAAGAATAAATGAGGCTTTGGCTTTGCTGGAAATGCCGTTCCAAAACCCATTACTTTATGAT  
GCCAACTATTTCTTTGCTGGCATACTAATTGTTACGACTATTGTATACCTTACAATAGTGTAACCT  
CTTCAATTGTCATTACTTCAGGTGATGGCACAACAAGTCCTATTTCTGAACATGACTACCAGATTG  
GTGGTTATACTGAAAAATGGGAATCTGGAGTAAAAGACTGTGTTGTATTACACAGTTACTTCACTT  
35 CAGACTATTACCAGCTGTACTCAACTCAATTGAGTACAGACACTGGTGTTGAACATGTTACCTTCT  
TCATCTACAATAAAATTGTTGATGAGCCTGAAGAACATGTCCAAATTCACACAATCGACGGTTCAT  
CCGGAGTTGTTAATCCAGTAATGGAACCAATTTATGATGAACCGACGACGACTACTAGCGTGCCT  
TTGTAAGCACAAAGCTGATGAGTACGAACCTTATGTACTCATTCTGTTTCGGAAGAGACAGGTACGTT  
AATAGTTAATAGCGTACTTCTTTTTCTTGCTTTCTGTTGATTCTTGCTAGTTACACTAGCCATCCTT  
40 ACTGCGCTTCGATTGTGTGCGTACTGCTGCAATATTGTTAACGTGAGTCTTGTAACCTTCTTTT  
TACGTTTACTCTCGTGTTAAAAATCTGAATTCTTCTAGAGTTCCTGATCTTCTGGTCTAAACGAACT

AAATATTATATTAGTTTTCTGTTTGGAACTTTAATTTTAGCCATGGCAGATTCCAACGGTACTATT  
ACCGTTGAAGAGCTTAAAAAGCTCCTTGAACAATGGAACCTAGTAATAGGTTTCCTATTCTTACA  
TGGATTTGTCTTCTACAATTTGCCTATGCCAACAGGAATAGGTTTTGTATATAATTAAGTTAATTT  
TCCTCTGGCTGTTATGGCCAGTAACTTTAGCTTGTTTTGTGCTTGCTGCTGTTTACAGAATAAATT  
5 GGATCACCGGTGGAATTGCTATCGCAATGGCTTGCTTGATAGGCTTGATGTGGCTCAGCTACTTC  
ATTGCTTCTTTTCACTGTTTGCCTGACGCTTCCATGTGGTCATTCAATCCAGAACTAACATT  
CTTCTCAACGTGCCACTCCATGGCACTATTCTGACCAGACCGCTTCTAGAAAGTGAAGTCTGTAAT  
CGGAGCTGTGATCCTTCGTGGACATCTTCGTATTGCTGGACACCATCTAGGACGCTGTGACATCA  
AGGACCTGCCTAAAGAAATCACTGTTGCTACATCAGAACGCTTCTTATTACAAATTGGGAGCTT  
10 CGCAGCGTGTAGCAGGTGACTCAGTTTTGCTGCATACAGTCGCTACAGGATTGGCAACTATAAA  
TTAAACACAGACCATTCCAGTAGCAGTGACAATATTGCTTTGCTTGACAGTAAGTGACAACAGAT  
GTTTCATCTCGTTGACTTTTCAAGTTACTATAGCAGAGATATTACTAATTATTATGAGGACTTTTAAA  
GTTTCCATTTGGAATCTTGATTACATCATAAACCTCATAATTAATAATTTATCTAAGTCACTAACTGA  
GAATAAATATTCTCAATTAGATGAAGAGCAACCAATGGAGATTGATTAAACGAACATGAAAATTATT  
15 CTTTTCTTGGAAGTATAAAGTCTGCTACTTGTGAGCTTTATCACTACCAAGAGTGTGTTAGAGGT  
ACAACAGTACTTTTAAAGAACCTTGCTCTTCTGGAACATACGAGGGCAATTCACCATTTTATCCT  
CTAGCTGATAACAAATTTGCACTGACTTGCTTTAGCACTCAATTTGCTTTTGCTTGCTGACGGC  
GTAAACACGCTCTATCAGTTACGTGCCAGATCAGTTTACCTAACTGTTTATCAGACAAGAGGA  
AGTTCAAGAACTTTACTCTCCAATTTTCTTATTGTTGCGGCAATAGTGTATAACACTTTGCTTC  
20 AACTCAAAAGAAAGACAGAATGATTGAACCTTCATTAATTGACTTCTATTTGTGCTTTTTAGCCTT  
TCTGCTATTCTTGTGTTTAAATTATGCTTATTATCTTTGGTTCTCACTTGAAGTCAAGATCATAATG  
AACTTGTACGCTTAAACGAACATGAAATTTCTGTTTTCTTAGGAATCATCACAAGTGTAGCTG  
CATTTACCAAGAATGTAGTTTACAGTCATGTACTCAACATCAACCATATGTAGTTGATGACCCGT  
GTCCTATTCACTTCTATTCTAAATGGTATATTAGAGTAGGAGCTAGAAAATCAGCACCTTTAATTGA  
25 ATTGTGCGTGGATGAGGCTGGTTCTAAATCACCCATTCAGTACATCGATATCGGTAATTATACAGT  
TTCTGTTTACCTTTTACAATTAATTGCCAGGAACCTAAATTGGGTAGTCTTGATGCGTTGTTT  
GTTCTATGAAGACTTTTATAGATATCATGACGTTCTGTTGTTTATGATTTTATCTAAACGAACAAA  
CTAAATGTCTGATAATGGACCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCC  
TCAGATTCAACTGGCAGTAACCAAGATGGAGAACGCAAGTGGGGCGCGATCAAAACAACGTCGGC  
30 CCCAAGGTTTACCAATAATACTGCGTCTTGTTTACCGCTCTCACTCAACATGGCAAGGAAGAC  
CTTAAATTCCTCGAGGACAAGGCGTTCCAATTAACACCAATAGCAGTCCAGATGACCAAATTGG  
CTACTACCGAAGAGCTACCAGACGAATTCGTGGTGGTGACGGTAAATGAAAGATCTCAGTCCAA  
GATGGTATTTCTACTACCTAGGAAGTGGGCCAGAGCTGGACTTCCCTATGGTGCTAACAAGAGC  
GGCATCATATGGGTTGCAACTGAGGGAGCCTTGAATACACCAAAAGATCACATTGGCACCCGCA  
35 ATCCTGCTAACAATGCTGCAATCGTGCTACAACCTTCTCAAGGAACAACATTGCCAAAAGGCTTC  
TACGCAGAAGGGAGCAGAGGCGGCAGTCAAGCCTCTTCTCGTTCTCATCACGTAGTCGCAACA  
GTTCAAGAAATTCAACTCCAGGCAGCAGTAGGGGAACCTTCTCTGCTAGAATGGCTGGCAATGG  
CGGTGATGCTGCTCTTGCTTTGCTGCTGCTTGACAGATTGAACCAGCTTGAGAGCAAAATGTCTG  
GTAAAGGCCAACAACAACAAGGCCAACTGTCACTAAGAAATCTGCTGCTGAGGCTTCTAAGAAG  
40 CCTCGGCAAAAACGTAAGTCCACTAAAGCATACAATGTAAACACAAGCTTTCGGCAGACGTGGTCC  
AGAACAACCCCAAGGAAATTTGGGGACCAGGAATAATCAGACAAGGAAGTATTACAAACATT

GGCCGCAAATTGCACAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGCATTGGCAT  
 GGAAGTCACACCTTCGGGAACGTGGTTGACCTACACAGGTGCCATCAAATTGGATGACAAAGAT  
 CCAAATTTCAAAGATCAAGTCATTTTCTGCTGAATAAGCATATTGACGCATACAAAACATTCCCACCA  
 ACAGAGCCTAAAAAGGACAAAAAGAAGAAGGCTGATGAACTCAAGCCTTACCGCAGAGACAGA  
 5 AGAAACAGCAAAGTGTGACTCTTCTCCTGCTGCAGATTTGGATGATTTCTCCAAACAATTGCAAC  
 AATCCATGAGCAGTGCTGACTCAACTCAGGCCTAACTCATGCAGACCACACAAGGCAGATGGG  
 CTATATAAACGTTTTTCGCTTTTCCGTTTACGATATATAGTCTACTCTTGTGCAGAATGAATTCTCGT  
 AACTACATAGCACAAAGTAGATGTAGTTAACTTTAATCTCACATAGCAATCTTTAATCAGTGTGTAAC  
 ATTAGGGAGGACTTGAAAGAGCCACCACATTTTCACCGAGGCCACGCGGAGTACGATCGAGTGT  
 10 ACAGTGAACAATGCTAGGGAGAGCTGCCTATATGGAAGAGCCCTAATGTGTAAAATTAATTTTAGT  
 AGTGCTATCCCCATGTGATTTTAATAGCTTCTTAGGAGAATGACAAAAAAAAAAAAAAAAAAAAA  
 AAAAAAAAAA

**SEQ ID NO: 14, which is 30-44 of SEQ ID NO: 1 – only in sequence listing**  
 15 **SEQ ID NO: 15, which is 48-68 of SEQ ID NO: 1 – only in sequence listing**  
**SEQ ID NO: 16, which is 110-166 of SEQ ID NO: 1 – only in sequence listing**  
**SEQ ID NO: 17, which is 200-214 of SEQ ID NO: 1 – only in sequence listing**  
**SEQ ID NO: 18, which is 226-250 of SEQ ID NO: 1 – only in sequence listing**  
**SEQ ID NO: 19, which is 256-277 of SEQ ID NO: 1 – only in sequence listing**  
 20 **SEQ ID NO: 20, which is 328-342 of SEQ ID NO: 1 – only in sequence listing**  
**SEQ ID NO: 21, which is 399-414 of SEQ ID NO: 1 – only in sequence listing**  
**SEQ ID NO: 22, which is 434-448 of SEQ ID NO: 1 – only in sequence listing**  
**SEQ ID NO: 23, which is 550-572 of SEQ ID NO: 1 – only in sequence listing**  
**SEQ ID NO: 24, which is 590-604 of SEQ ID NO: 1 – only in sequence listing**  
 25 **SEQ ID NO: 25, which is 632-650 of SEQ ID NO: 1 – only in sequence listing**  
**SEQ ID NO: 26, which is 354-368 of SEQ ID NO: 1 – only in sequence listing**  
**SEQ ID NO: 27, which is 622-636 of SEQ ID NO: 1 – only in sequence listing**  
**SEQ ID NO: 28, which is 30-44 of SEQ ID NO: 1 – only in sequence listing**  
**SEQ ID NO: 29, which is 226-250 of SEQ ID NO: 1 – only in sequence listing**

30

**SEQ ID NO: 30: SARS-CoV-2 N protein**  
 MSDNGPQNQRNAPRITFGGPSDSTGSNQNNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDLK  
 FPRGQGVPIINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSRWWYFYLLGTGPEAGLPYGANKDGIW  
 VATEGALNTPKDHIGTRNPANNAIIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNSTP  
 35 GSSRGTSARMAGNGGDAALALLLDRLNQLESKMSGKGQQQQGQTVTKKSAEASKKPRQKRTA  
 TKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPIAQFAPSASAFFGMSRIGMEVTPSGTW  
 LTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTPKDKKKKADETQALPQRQKKQQTVLLPAAD  
 LDDFSKQLQQSMSSADSTQA

40 **SEQ ID NO: 31: RBD, a fragment from S1 domain from SARS-CoV-2 S protein**

RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNKRISNCVADYSVLYNSASFSTFKCYGVSPTK  
 LNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYR  
 LFRKSNLKPFRDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPA  
 TVCGPKKSTNLVKNKCVNF

5

**SEQ ID NO: 32: RBD, a fragment from S1 domain from SARS-CoV-2 S protein, with C-terminal His tag**

RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNKRISNCVADYSVLYNSASFSTFKCYGVSPTK  
 LNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYR  
 10 LFRKSNLKPFRDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPA  
 TVCGPKKSTNLVKNKCVNFLEHHHHHHHH

**SEQ ID NO: 33: S2 domain from SARS-CoV-2 S protein**

SVASQSIAYTMSLGAENSVAYSNNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECNLLLQY  
 15 GSFCTQLNRLTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKV  
 TLADAGFIKQYGDCLGDIAARDLCAQKFNGLTVPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQI  
 PFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVNQNAQALNTLVK  
 QLSSNFGAISSVLNDILSRDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVL  
 GQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHGDKAHFPREGVVFVSNGT  
 20 HWFVTQRNFYEPQIITDNTFVSGNCDVVIGVNNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDI  
 SGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMTIMLCCMTSC  
 CSCLKGCCSCGSCCKFDEDDSEPVLGKVKLHYT

**SEQ ID NO: 34: RBD as used in the examples**

25 MKHLWFFLLLVAAPRWLWLSGPMRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNKRISNCVA  
 DYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTG  
 CVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFRDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQ  
 PTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFLEHHHHHHHH

30 **SEQ ID NO: 35 (SEQ ID NO: 1-derived peptide reactive with SARS-CoV-2 antibodies)**

LTPGDSSSGWTAG

**SEQ ID NO: 36 (SEQ ID NO: 1-derived peptide reactive with SARS-CoV-2 antibodies)**

YQAGSTPCNGV

**SEQ ID NO: 37 (SEQ ID NO: 1-derived peptide reactive with SARS-CoV-2 antibodies)**

35 YGFQPTNGVGYQ

**SEQ ID NO: 38: His-tagged RBD**

MKHLWFFLLLVAAPRWLWLSGPMRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNKRISNCVA  
 DYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTG  
 40 CVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFRDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQ  
 PTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFLEHHHHHHHH

**SEQ ID NO: 39: human Angiotensin-converting enzyme 2 (ACE2)**

MSSSSWLLLSLVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNAGD  
 KWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQNGSSVLSSEKSKRLNTILNTMSTIYSTGKVCNP  
 5 DNPQECLLLEPGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLKNEMARANHYEDYGD  
 YWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGD  
 MWGRFWTNLYSLTVPFQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLT  
 DPGNVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAAHEMGGHIQYDMAYAAQPFLLRNGANEGF  
 HEAVGEIMSLSAATPKHLKSGILLSPDFQEDNETEINFLLKQALTIVGTLPTMYMLEKWRWVFKGEIP  
 10 KDQWMKKVWEMKREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTYLQFQFQEAALCQAACH  
 EGPLHKCDISNSTEAGQKLFNMLRLGKSEPWTALENVVGAKNMNVRLPNYFEPLFTWLKDQNKNS  
 FVGWSTDWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRRSSVAYAMRQYFLKVKNMILFGEED  
 VRVANLKPRISFNFFVTAPKNVSDIIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLGPNNQPPV  
 SIWLIVFGVVMGVVVGIVLIFTGIRDRKKKNKARSGENPYASIDISKGENNPGFQNTDDVQTSF

15

**SEQ ID NO: 40 (SEQ ID NO: 1-derived peptide reactive with SARS-CoV-2 antibodies)**

RTWLPPAYTNS

**SEQ ID NO: 41 (SEQ ID NO: 1-derived peptide reactive with SARS-CoV-2 antibodies)**

RTQLPPAYTNS

**SEQ ID NO: 42 (SEQ ID NO: 1-derived peptide reactive with SARS-CoV-2 antibodies)**

SGTNGTKRFDN

20

**SEQ ID NO: 43 (SEQ ID NO: 1-derived peptide reactive with SARS-CoV-2 antibodies)**

MSHHHHHHHHSPMYSIITPNILRLESEETMVLEAHDAQGDVPVTVVHDFPGKKLVLSSEKTVLTPAT  
 25 NHMGNVTFTIPANREFKSEKGRNKFVTVQATFGTQVVEKVVVSLQSGIEGRMRTQLPPAYTNSRTQ  
 LPPAYTNS

**SEQ ID NO: 44 (SEQ ID NO: 1-derived peptide reactive with SARS-CoV-2 antibodies with GST fusion)**

30 MSHHHHHHHHSPMYSIITPNILRLESEETMVLEAHDAQGDVPVTVVHDFPGKKLVLSSEKTVLTPAT  
 NHMGNVTFTIPANREFKSEKGRNKFVTVQATFGTQVVEKVVVSLQSGIEGRMMSSHHHHHHHHHSPM  
 YSIITPNILRLESEETMVLEAHDAQGDVPVTVVHDFPGKKLVLSSEKTVLTPATNHMGNVTFTIPANRE  
 FKSEKGRNKFVTVQATFGTQVVEKVVVSLQSGIEGRMSGTNGTKRFDNSGTNGTKRFDN

**SEQ ID NO: 45 (SEQ ID NO: 1-derived peptide reactive with SARS-CoV-2 antibodies with GST fusion)**

35 MSHHHHHHHHSPMYSIITPNILRLESEETMVLEAHDAQGDVPVTVVHDFPGKKLVLSSEKTVLTPAT  
 NHMGNVTFTIPANREFKSEKGRNKFVTVQATFGTQVVEKVVVSLQSGIEGRMLTPGDSSSGWTAGL  
 TPGDSSSGWTAG

40

**SEQ ID NO: 46 (SEQ ID NO: 1-derived peptide reactive with SARS-CoV-2 antibodies with GST fusion)**

MSHHHHHHHHSPMYSIITPNILRLESEETMVLEAHDAQGDVPVTVVHDFPGKKLVLSSEKTVLTPAT  
 NHMGNVTFTIPANREFKSEKGRNKFVTVQATFGTQVVEKVVLVSLQSGIEGRMNLDISKVGGNNLDS  
 5 KVG

**SEQ ID NO: 47 (SEQ ID NO: 1-derived peptide reactive with SARS-CoV-2 antibodies with GST fusion)**

MSHHHHHHHHSPMYSIITPNILRLESEETMVLEAHDAQGDVPVTVVHDFPGKKLVLSSEKTVLTPAT  
 10 NHMGNVTFTIPANREFKSEKGRNKFVTVQATFGTQVVEKVVLVSLQSGIEGRMYQAGSTPCNGVYQ  
 AGSTPCNGV

**SEQ ID NO: 48 (SEQ ID NO: 1-derived peptide reactive with SARS-CoV-2 antibodies with GST fusion)**

MSHHHHHHHHSPMYSIITPNILRLESEETMVLEAHDAQGDVPVTVVHDFPGKKLVLSSEKTVLTPAT  
 15 NHMGNVTFTIPANREFKSEKGRNKFVTVQATFGTQVVEKVVLVSLQSGIEGRMYGFQPTNGVGYQY  
 GFQPTNGVGYQ

**SEQ ID NO: 49 (SEQ ID NO: 1-derived peptide reactive with SARS-CoV-2 antibodies with GST fusion, P1-P6)**

MSHHHHHHHHSPMYSIITPNILRLESEETMVLEAHDAQGDVPVTVVHDFPGKKLVLSSEKTVLTPAT  
 20 NHMGNVTFTIPANREFKSEKGRNKFVTVQATFGTQVVEKVVLVSLQSGIEGRMRTLPPAYTNSSGT  
 NGTKRFDNLTPGDSSSGWTAGNNLDSKVGGYQAGSTPCNGVYGFQPTNGVGYQ

**SEQ ID NO: 50 (C-terminally His-tagged extracellular domain of human ACE2)**

MSSSSWLLLSLVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNAGD  
 KWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQQNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNP  
 DNPQECLLLEPGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLKNEMARANHYEDYGD  
 YWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGD  
 30 MWGRFWTNLYSLTVFPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLT  
 DPGNVQKAVCHPTAWDLGKGDFRILMCTKVMTDDFLTAAHEMIGHIQYDMAYAAQPFLLRNGANEGF  
 HEAVGEIMSLSAATPKHLKSIKLLSPDFQEDNETEINFLLKQALTIVGTLPTYMLEKWRWMVFKGEIP  
 KDQWMKKWWEMKREIVGVPEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQFQEALCQAAKH  
 EGPLHKCDISNSTEAGQKLFNMLRLGKSEPWTALENVGAKNMNVRPLLNYFEPLFTWLKDQNKNS  
 35 FVGWSTDWSPYADQSIKVRISLKSALGDKAYEWNENMYLFRSSVAYAMRQYFLKVKNQMILFGEED  
 VRVANLKPRIFFVTAPKNVSDIIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLGPPNQPPV  
 SLEGGSGSGSHHHHHHHHSGSLNDIFEAKIEWHE

**SEQ ID NO: 51 (SEQ ID NO1 with mutations of SARS-CoV-2 U.K. variant B.1.1.7– only in sequence listing)**

40

**SEQ ID NO: 52 (SEQ ID NO1 with mutations of SARS-CoV-2 South African variant B.1.351):** – only in sequence listing

**SEQ ID NO: 53 (SEQ ID NO1 with mutations of SARS-CoV-2 Brazilian variant P.1):** – only in sequence listing

5 **SEQ ID NO: 54 (SEQ ID NO1 with mutations of SARS-CoV-2 Mink Variant from Denmark):** – only in sequence listing

**SEQ ID NO55: (SEQ ID NO: 1-derived peptide reactive with SARS-CoV-2 antibodies)**

NNLDSKVGG

10

**SEQ ID NO: 56 (SEQ ID NO: 1-derived peptide reactive with SARS-CoV-2 antibodies with GST fusion, P1-P6)**

RTQLPPAYTNSSGTNGTKRFDNLTPGDSSSGWTAGNNLDSKVGGYQAGSTPCNGVYGFQPTNGVG  
YQ

15

The present invention is further illustrated by the following examples, sequences and figures from which further features, embodiments, aspects and advantages of the present invention may be taken. All methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, with suitable methods and materials being described herein. All  
20 publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

**Fig. 1** shows a time course of antibody levels to SEQ ID NO: 1 (IgA, IgG) and N protein (IgG, IgM) monitored in two patients (**Fig. 1**), as described in Example 3.

25 **Fig. 2** shows monitoring of another time course with a patient infected with SARS-CoV-2. IgA class antibodies to SEQ ID NO: 1 (squares), IgG class antibodies to SEQ ID NO: 1 (triangles) and IgG class antibodies to N protein (circles) were determined.

**Fig. 3** shows IFA using acetone-fixed S1-expressing or control plasmid-transfected HEK293 cells (**A**) incubated directly with anti-His tag (1:200) or patient serum (PS, 1:100) in the first step and anti-mouse-IgG-FITC or anti-human-IgG-FITC in the second step, (**B**) incubated 30 min with PBS prior to the two step incubation with PS3 as described in A, or (**C**) incubated 30 min with PBS, followed by an  
30 incubation with PS3 (1:100) in the first step, anti-human-IgG-Biotin (1:200) in the second step and ExtrAvidin-FITC (1:2000) in the third step. S1-expressing cells had been transfected with a pTriEx vector expressing SEQ ID NO: 2 using standard methods. Representative cells identified as reactive with patient antibody were marked using arrows.

35 **Fig. 4** shows the detection of IgG, IgA and IgM antibodies from the sample of a patient based on dot blot analysis using a fusion protein comprising RBD.

**Fig. 5** shows the detection of IgG, IgA and IgM antibodies from the sample of a patient based on dot blot analysis using a polypeptide comprising SEQ ID NO: 1.

40 **Fig. 6** shows the detection of IgM antibodies from the sample of a patient based on dot blot analysis using fragments of SEQ ID NO: 1 and fusion proteins thereof.

**Fig. 7** shows the detection of IgA antibodies from the sample of a patient based on dot blot analysis using fragments of SEQ ID NO: 1 and fusion proteins thereof.

**Fig. 8** shows the detection of IgG antibodies from the sample of a patient based on dot blot analysis using fragments of SEQ ID NO: 1 and fusion proteins thereof.

5 **Fig. 9** shows the time-dependent detection of IgM antibodies from samples from patients based on dot blot analysis using fragments of SEQ ID NO: 1 and fusion proteins thereof.

**Fig. 10** shows the time-dependent detection of IgG antibodies from samples from patients based on dot blot analysis using fragments of SEQ ID NO: 1 and fusion proteins thereof.

**Fig. 11** shows the time-dependent detection of IgA antibodies from samples from patients based on dot blot analysis using fragments of SEQ ID NO: 1 and fusion proteins thereof.

**Fig. 12** shows the detection of IgA antibodies from the sample of a patient based on Western analysis using a polypeptide comprising RBD.

**Fig. 13** shows the detection of IgM antibodies from the sample of a patient based on Western blot analysis using a polypeptide comprising RBD.

15 **Fig. 14** shows the detection of IgG antibodies from the sample of a patient based on Western blot analysis using a polypeptide comprising RBD.

**Fig. 15** shows the time-dependent detection of IgA antibodies from samples of patients based on Western blot analysis using a polypeptide comprising SEQ ID NO: 1.

**Fig. 16** shows the time-dependent detection of IgM antibodies from samples of patients based on Western blot analysis using polypeptide comprising SEQ ID NO: 1.

**Fig. 17** shows the time-dependent detection of IgG antibodies from samples of patients based on Western blot analysis using a polypeptide comprising SEQ ID NO: 1.

#### **Example 1: Detection of antibodies to SEQ ID NO: 1 using an ELISA immunoassay**

##### **Samples**

25 Eight samples from patients tested SARS-CoV-2 positive by PCR as described by Corman *et al.* (Corman et al. (2020) Diagnostic detection of 2019-nCoV by real-time RT-PCR, [https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c\\_2](https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2)) were obtained 6 to 14 days after the infection and 14 samples from such patients obtained at an earlier time point after the infection were available.

In addition, a range of samples containing various coronaviruses was available, including 18 samples from patients infected with MERS, three samples from patients infected with SARS-CoV-1, four patients with NL63, three patients with 229E, six patients with OC43 and three patients with HKU1.

35 **Preparation of microtiter plates coated with antigen:** SEQ ID NO: 2 was expressed in HEK293T cells using standard cloning of SEQ ID NO: 4 into the pTriEx-1 plasmid with an artificial signal sequence and a C-terminal His tag, resulting in the expression of SEQ ID NO: 2 and, after removal of the signal peptide, SEQ ID NO: 3. Transfected cells were cultured at 37°C and 8.5% CO<sub>2</sub> in Dulbecco's modified eagle's medium with 10% fetal calf serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin for three to five days. Cells were harvested, resuspended in 20 mM Tris-HCl pH 7.4, 10% (w/v) sucrose, 5 mM EDTA, 1 mM PMSF and stored at -80°C until further use.

To prepare SEQ ID NO: 3, cell culture supernatant was adjusted to 5 mmol/l tris chloride pH 8.0, 164 mmol/l sodium chloride, 50 mmol/l magnesium chloride, 20 mmol/l imidazole, 0,1% Triton X-100, cleared by centrifugation for 30 minutes at 17,600xg, 4°C, applied to Nickel Rapid Run (Agarose Bead Technologies, Miami, FL, USA) equilibrated with 5 mmol/l tris chloride pH 8.0, 300 mmol/l sodium chloride, 20 mmol/l imidazole and eluted by increasing the imidazole concentration to 150 mmol/l. All fractions containing SEQ ID NO: 3 were pooled and concentrated by ultrafiltration (VivaSpin, Sartorius, Göttingen, Germany). The final preparation was stored at -80°C until further use.

The final protein preparation of SEQ ID NO: 3 was treated with or without 16 mmol/l dithiothreitol and incubated at 70°C or at room temperature for 10 minutes, followed by SDS gel electrophoresis and Coomassie staining. Protein identity was verified by mass spectrometry.

For use in microtiter ELISA the purified protein was diluted in PBS to final concentrations of approximately 1.5 µg/ml and used to coat ELISA microtiter plates (Nunc, Roskilde, Denmark) overnight.

**Experimental procedure:** Samples were diluted 1:101 in IgG sample buffer, applied to microtiter plates and incubated as described for commercial EUROIMMUN ELISA Test-Kits, using reagents commercially available (e.g. EI 2260-9601 G/A, which is a buffer having essentially physiological conditions regarding salt concentration and pH). The manual of EI 2260-9601 G/A was followed. In brief: 60 min at 37 °C; 3 washing steps using washing buffer; addition of 100 µl of peroxidase-labelled anti-human IgG conjugate (rabbit) or anti-human IgA conjugate (rabbit) per well; incubation for 30 min at 37 °C; 3 washing steps using EUROIMMUN washing buffer; addition of 100 µl of chromogen/substrate solution (TMB/H<sub>2</sub>O<sub>2</sub>) per well; incubation for 30 min at room temperature; addition of 100 µl stop-solution (0.5 M sulfuric acid); measurement of optical density at 450 nm against 630 nm as a reference. Calibration was carried out using commercially available calibrators (product number EI 2606-9601 A, EUROIMMUN Medizinische Labordiagnostika AG). A ratio was calculated by dividing extinction of the control or patient sample by the extinction of the calibrator. Results below 0.8 were considered negative, results between 0.8 and 1.1 borderline, and results of more than 1.1 positive.

**Results:** The primary data are shown in Table 1:

|   |            | raw data |         | Cut-Off OD  | raw data |         | Cut-Off OD  |
|---|------------|----------|---------|-------------|----------|---------|-------------|
|   |            |          |         | 0,100       |          |         | 0,200       |
|   |            | IgG (OD) | dil. 1: | IgG (Ratio) | IgA (OD) | dil. 1: | IgA (Ratio) |
|   |            |          |         | pos: ≥ 1,1  |          |         | pos: ≥ 1,1  |
|   |            |          |         | bl: 0,8-1,0 |          |         | bl: 0,8-1,0 |
| 1 | Calibrator | 1,911    | 300     | 19,1        | 3,055    | 600     | 15,3        |
| 2 | Calibrator | 1,593    | 600     | 15,9        | 2,204    | 1200    | 11,0        |
| 3 | Calibrator | 1,077    | 1200    | 10,8        | 1,068    | 2400    | 5,3         |
| 4 | Calibrator | 0,697    | 2400    | 7,0         | 0,529    | 4800    | 2,6         |
| 5 | Calibrator | 0,441    | 4800    | 4,4         | 0,314    | 9600    | 1,6         |
| 6 | Calibrator | 0,248    | 9600    | 2,5         | 0,155    | 19200   | 0,8         |
| 7 | Calibrator | 0,132    | 19200   | 1,3         | 0,078    | 38400   | 0,4         |

|    |              |       |       |            |       |       |             |
|----|--------------|-------|-------|------------|-------|-------|-------------|
| 8  | Calibrator   | 0,066 | 38400 | 0,7        | 0,051 | 76800 | 0,3         |
| 9  | SARS-CoV-2*  | 0,157 |       | <b>1,6</b> | 1,402 |       | <b>7,0</b>  |
| 10 | SARS-CoV-2*  | 0,075 |       | <u>0,8</u> | 0,377 |       | <b>1,9</b>  |
| 11 | SARS-CoV-2*  | 0,276 |       | <b>2,8</b> | 9,999 |       | <b>50,0</b> |
| 12 | SARS-CoV-2*  | 0,027 |       | 0,3        | 0,027 |       | 0,1         |
| 13 | SARS-CoV-2*  | 0,023 |       | 0,2        | 0,064 |       | 0,3         |
| 14 | SARS-CoV-2*  | 0,119 |       | <b>1,2</b> | 1,142 |       | <b>5,7</b>  |
| 15 | SARS-CoV-2*  | 0,031 |       | 0,3        | 0,203 |       | <b>1,0</b>  |
| 16 | SARS-CoV-2*  | 0,079 |       | <u>0,8</u> | 0,385 |       | <b>1,9</b>  |
| 17 | SARS-COV-2** | 0,021 |       | 0,2        | 0,055 |       | 0,3         |
| 18 | SARS-COV-2** | 0,019 |       | 0,2        | 0,084 |       | 0,4         |
| 19 | SARS-COV-2** | 0,021 |       | 0,2        | 0,225 |       | <b>1,1</b>  |
| 20 | SARS-COV-2** | 0,018 |       | 0,2        | 0,192 |       | <b>1,0</b>  |
| 21 | SARS-COV-2** | 0,033 |       | 0,3        | 0,599 |       | <b>3,0</b>  |
| 22 | SARS-COV-2** | 0,029 |       | 0,3        | 0,331 |       | <b>1,7</b>  |
| 23 | SARS-COV-2** | 0,013 |       | 0,1        | 0,030 |       | 0,2         |
| 24 | SARS-COV-2** | 0,017 |       | 0,2        | 0,097 |       | 0,5         |
| 25 | SARS-COV-2** | 0,027 |       | 0,3        | 0,214 |       | <b>1,1</b>  |
| 26 | SARS-COV-2** | 0,016 |       | 0,2        | 0,021 |       | 0,1         |
| 27 | SARS-COV-2** | 0,014 |       | 0,1        | 0,073 |       | 0,4         |
| 28 | SARS-COV-2** | 0,014 |       | 0,1        | 0,042 |       | 0,2         |
| 29 | SARS-COV-2** | 0,015 |       | 0,2        | 0,030 |       | 0,2         |
| 30 | SARS-COV-2** | 0,015 |       | 0,2        | 0,079 |       | 0,4         |
| 31 | MERS 1       | 0,013 |       | 0,1        | 0,042 |       | 0,2         |
| 32 | MERS 2       | 0,008 |       | 0,1        | 0,017 |       | 0,1         |
| 33 | MERS 3       | 0,011 |       | 0,1        | 0,038 |       | 0,2         |
| 34 | MERS 4       | 0,008 |       | 0,1        | 0,011 |       | 0,1         |
| 35 | MERS 5       | 0,008 |       | 0,1        | 0,027 |       | 0,1         |
| 36 | MERS 6       | 0,008 |       | 0,1        | 0,035 |       | 0,2         |
| 37 | MERS 7       | 0,009 |       | 0,1        | 0,013 |       | 0,1         |
| 38 | MERS 8       | 0,017 |       | 0,2        | 0,036 |       | 0,2         |
| 39 | MERS 9       | 0,010 |       | 0,1        | 0,024 |       | 0,1         |
| 40 | MERS 10      | 0,007 |       | 0,1        | 0,012 |       | 0,1         |
| 41 | MERS 11      | 0,020 |       | 0,2        | 0,026 |       | 0,1         |
| 42 | MERS 12      | 0,008 |       | 0,1        | 0,026 |       | 0,1         |
| 43 | MERS 13      | 0,015 |       | 0,2        | 0,021 |       | 0,1         |
| 44 | MERS 14      | 0,012 |       | 0,1        | 0,036 |       | 0,2         |
| 45 | MERS 15      | 0,024 |       | 0,2        | 0,066 |       | 0,3         |
| 46 | MERS 16      | 0,021 |       | 0,2        | 0,080 |       | 0,4         |
| 47 | MERS 17      | 0,008 |       | 0,1        | 0,039 |       | 0,2         |
| 48 | MERS 18      | 0,029 |       | 0,3        | 0,104 |       | 0,5         |
| 49 | SARS-1       | 0,214 |       | <b>2,1</b> | 0,285 |       | <b>1,4</b>  |
| 50 | SARS-1       | 0,596 |       | <b>6,0</b> | 0,227 |       | <b>1,1</b>  |
| 51 | SARS-1       | 0,128 |       | <b>1,3</b> | 0,260 |       | <b>1,3</b>  |
| 52 | OC43         | 0,035 |       | 0,4        | 0,126 |       | 0,6         |
| 53 | OC43         | 0,029 |       | 0,3        | 0,098 |       | 0,5         |
| 54 | OC43         | 0,016 |       | 0,2        | 0,041 |       | 0,2         |

|    |      |       |  |     |       |  |     |
|----|------|-------|--|-----|-------|--|-----|
| 55 | OC43 | 0,011 |  | 0,1 | 0,048 |  | 0,2 |
| 68 | NL63 | 0,013 |  | 0,1 | 0,023 |  | 0,1 |
| 69 | NL63 | 0,027 |  | 0,3 | 0,039 |  | 0,2 |
| 70 | NL63 | 0,036 |  | 0,4 | 0,057 |  | 0,3 |
| 71 | NL63 | 0,019 |  | 0,2 | 0,030 |  | 0,2 |
| 72 | 229E | 0,041 |  | 0,4 | 0,045 |  | 0,2 |
| 73 | 229E | 0,022 |  | 0,2 | 0,024 |  | 0,1 |
| 74 | 229E | 0,030 |  | 0,3 | 0,034 |  | 0,2 |
| 75 | OC43 | 0,050 |  | 0,5 | 0,100 |  | 0,5 |
| 76 | OC43 | 0,079 |  | 0,8 | 0,201 |  | 1,0 |
| 77 | HKU1 | 0,023 |  | 0,2 | 0,054 |  | 0,3 |
| 78 | HKU1 | 0,019 |  | 0,2 | 0,055 |  | 0,3 |
| 79 | HKU1 | 0,010 |  | 0,1 | 0,029 |  | 0,1 |

(\*late stage; \*\* < day 6)

### Conclusions

The results show that antibodies to SEQ ID NO: 1 may be used to diagnose a SARS-CoV-2 infection in samples from human patients.

- 5 Comparison of the data obtained with secondary antibodies recognizing IgG and IgA class antibodies shows that the detection of IgA antibodies is more sensitive, at least at an early stage of the disease: 4/14 patient samples taken at an earlier stage of the infection, before six days post onset of illness, could be correctly identified as positive when IgA class antibodies were detected, while the detection of IgG in the same samples gave negative results.
- 10 Both assays showed cross-reactivity with samples from SARS-CoV-1 patients, but virtually none of the samples from patients infected with MERS, NL63, 229E, OC43 and HKU1. Distinction between SARS-CoV-1 and SARS-CoV-2 is possible based on the different time-resolved Ig class signature, in particular the later emergence of IgA class antibodies in SARS-CoV-1 (Hsue, P. R., Huang, L. M., Chen, P. J., Kao, C. L., and Yang P. C. (2004) Chronological evolution of IgM, IgA, IgG and neutralization antibodies after infection with SARS-associated coronavirus, *Clinical Microbiology and Infection*, 10(12), 1062-1066). Not in the least, hardly any cases of SARS-CoV have been reported since the outbreak of SARS-CoV-2.

Various post published publications by independent researchers confirmed the inventors' findings:

- Jääskeläinen *et al.* concluded from a comparative study with six commercially available serological assays for detection of SARS-CoV-2 IgG, IgA and/or IgM antibodies that, among the six assays tested, the EUROIMMUN assay based on the detection of IgA to S1 provided the highest sensitivity (Jääskeläinen, A. J., Kuivanen, S., Kekäläinen, E., Ahava, M.J., Loginov, R., Kallio-Kokko, H., Vapalahti, O., Jarva, H., Kurkela, and Lappalainen, M. (2020), *J. Clin. Virology* 129, 104512). Okba *et al.* obtained similar results. (Okba *et al.*, Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease Patients. *Emerg Infect Dis.* 2020 Jul;26(7):1478-1488, prepublished on medRxiv 2020.03.18.20038059). Beavis *et al.* confirmed that the sensitivity of the IgA-based assay according to the present invention is superior at an early stage of the disease, while the IgG assay is superior at a later stage (Beavis, K. G., Mathushek, S. M., Abeleda, A. P. F., Bethel, C., Hunt, C., Gillen, S., Moran, A., and Tesic, V. (2020) Evaluation of the EUROIMMUN Anti-SARS-CoV-2 ELISA Assay for detection of IgA and IgG antibodies, *J. Clin. Virology* 129, 104468).

In summary, the assay according to the present invention may be used for the early detection of diagnostically relevant antibodies to SEQ ID NO: 1. In some patients, true positive results can be found before six days have passed since the onset of symptoms. Therefore, the assay helps close or at least narrow down the diagnostic gap between the period when PCR-based assays and immuno-  
 5 assays may be used.

**Example 2: An extended ELISA study aiming to further characterize the diagnostic reliability and relevance of tests for the detection of IgA antibodies to SEQ ID NO: 1**

For the purpose of determining the sensitivity of the instant assay, the presence or absence of IgA antibodies was detected in 166 samples from 152 European patients using EUROIMMUN product no. EI 2606-9601 A, based on an assay similar as described in Example 1. In each of the subject  
 10 samples, a SARS-CoV-2 infection had been confirmed using RT-PCR according to Corman VM, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 25(3): pii=2000045 (2020-01-23) based on a sample from an early stage of the infection.

Briefly, this assay is based on an ELISA using a microtiter plate coated with an antigen comprising purified SEQ ID NO: 1. Following incubation with samples and extensive washing steps using physiological buffers, a secondary antibody to IgA labeled with an enzymatically active label is used to specifically decorate IgA antibodies to SEQ ID NO: 1, followed by incubation of a chromogenic  
 15 substrate. Further details can be found in the manual supplied with the product (EUROIMMUN product no. EI 2606-9601 A), which product manual is herewith incorporated by reference.

The serological characterization was carried out based on samples obtained in the following course of the infection.

A sensitivity of 60.2% regarding IgA to SEQ ID NO: 1 was determined with samples obtained until day 10 post onset of symptoms (or obtained until day 10 after direct detection of infection by positive RT-PCR). The sensitivity was 98.6% for samples obtained after day 10.  
 25

The results and immunoresponses are highly individual though. For example, a minority of patients may not show IgA, IgG or IgM responses at all.

Taken together, this further study also consistently demonstrates that the specific detection of IgA class antibodies directed against SEQ ID NO: 1 provides particularly high sensitivity for detecting  
 30 SARS-CoV-2 infection already at an early stage of disease.

**Example 3: Persistence of various diagnostically relevant antibodies over time in SARS-CoV-2 patients as shown by ELISA**

The time course of antibody levels to SEQ ID NO: 1 (IgA, IgG) and N protein (IgG, IgM) was monitored in two patients (**Fig. 1**). Both presented with a mild course of the disease, but the infection had been confirmed by RT-PCR. Typical specific symptoms such as temporary loss of smell were observed. EUROIMMUN products EI 2606-9601 A and EI 2606-9601 G (S1 protein as antigen, IgA or IgG detection, respectively) and EI 2606-9601-2 G and EI 2606-9601-2 M (N protein as antigen, IgG or IgM detection, respectively) were used according to the manufacturer's instructions, which are  
 35 incorporated here by reference. The principle and major components of the tests are outlined in Example 2; however, different antigens (N protein as antigen instead of a polypeptide comprising SEQ  
 40

ID NO: 1) as well as different secondary antibodies (binding to IgA, IgG or IgM) were used depending on which antibody was to be detected. Additional information is available in the manufacturer's instructions of EI 2606-9601 A, EI 2606-9601 G, EI 2606-9601-2 G and EI 2606-9601-2 M (EUROIMMUN).

- 5 The course of the disease was monitored over more than four months. IgA and IgG to SEQ ID NO: 1 and IgG, but no IgM to N protein were detectable initially. In both patients, at least one of IgA or IgG to SEQ ID NO: 1 was detectable after four months, whereas IgG to N protein was absent or only weak as early as two months after the first positive PCR.

10 These results show that antibodies to SEQ ID NO: 1 persist at least in some patients longer than those to N protein do. The level of IgA antibodies decreases more rapidly than the IgG antibody level, but IgA antibodies may still be predominant as they disappear, owing to their initially higher signal.

The monitoring of another time course with a third patient is shown in **Fig. 2**. Essentially, IgA class antibodies to SEQ ID NO: 1 (squares), IgG class antibodies to SEQ ID NO: 1 (triangles) and IgG class antibodies to N protein (circles) were determined using the same methods. As evident from Fig. 2, IgA class antibodies to S1 [SEQ ID NO: 1] were detectable over the entire time period monitored, *i.e.* even after 40 days after RT-PCR-confirmed SARS-CoV-2 infection, whereas levels of IgG class antibodies to either SEQ ID NO: 1 or to SARS-CoV-2 N protein remained under (or close to) the cut-off. Thus, these data additionally confirm the superior sensitivity of the instant assay which is based on the specific detection of the IgA class antibodies bound to the SEQ ID NO: 1 antigen.

20

**Example 4: Chemiluminescence-based assay for detection of antibodies to SEQ ID NO: 1 used to monitor various antibodies over time**

A EUROIMMUN Random Access RA 10 Analyzer (YG 0710-0101) was used according to the manufacturer's instructions and default settings, including a reagent cartridge (LS 1254-10010 G).

- 25 Tosylactivated paramagnetic beads (M-280, Invitrogen) were coated using the manufacturer's instructions. Briefly, beads were washed in coating buffer (0.1 M sodium phosphate pH 7.4) for 10 minutes at 37 °C on an IKA Roller 10 (supplied by VWR), followed by magnetic concentration of beads and removal of supernatant. Recombinant polypeptide (SEQ ID NO: 3) purified according to Example 1 was added in the same buffer, followed by addition of 3 M ammonium sulphate and incubation under the same conditions for 19 h, followed by two washing steps using washing buffer (PBS pH 7.4 0.1% BSA 0.2% Tween-20), blocking in the same buffer for 4 h at 37 °C (PBS pH 7.4 0.1% BSA 0.2% Tween-20) and two additional wash steps. Beads were stored in the same buffer for at least 16 h at 4 °C.

- 35 The assay was carried out by mixing paramagnetic beads with sample buffer (BSA/Tween-20 in Tris-HCl EDTA pH 7. 20 µl suspension of beads (1 mg/ml) was contacted with 5 µl of sample (*i.e.* a blood sample of a patient) in a total of 200 µl in sample buffer. After incubation for ten minutes, beads were washed thrice in sample buffer, followed by addition of 160 µl IgG / IgA conjugate (EUROIMMUN Medizinische Labordiagnostik AG, LK 0711-10010, essentially an IgG or IgA-specific secondary antibody labeled with acridinium ester) and incubation for 10 minutes at 37 °C. After three washing steps, alkaline hydrogen peroxide was rapidly added and mixed to trigger the emission of light, followed by immediate luminescence detection for 10 seconds. Results are shown in Table 2:

40

| Nr. |                                  | ELISA                                       | ELISA                                       |
|-----|----------------------------------|---------------------------------------------|---------------------------------------------|
|     |                                  | IgA                                         | IgG                                         |
|     |                                  | Ratio<br>pos: $\geq 1,1$<br>bl: $0,8 < 1,1$ | Ratio<br>pos: $\geq 1,1$<br>bl: $0,8 < 1,1$ |
| 1   | IgG / IgA<br>positive<br>samples | 8,2                                         | 2,2                                         |
| 2   |                                  | 12,7                                        | 6,3                                         |
| 3   |                                  | 13,1                                        | 5,4                                         |
| 4   | Negative<br>samples              | 0,3                                         | 0,3                                         |
| 5   |                                  | 0,3                                         | 0,1                                         |
| 6   |                                  | 0,3                                         | 0,0                                         |
| 7   |                                  | 0,4                                         | 0,1                                         |
| 8   |                                  | 0,5                                         | 0,3                                         |
| 15  | IgM<br>positive<br>samples       | 2,3                                         | 2, 1                                        |
| 16  |                                  | 1,1                                         | 5, 6                                        |
| 17  |                                  | 0,9                                         | 6,5                                         |
| 20  | blank                            |                                             |                                             |

|    | Chemiluminescence            |                                           | Chemiluminescence            |                                           | Chemiluminescence            |                                           |
|----|------------------------------|-------------------------------------------|------------------------------|-------------------------------------------|------------------------------|-------------------------------------------|
|    | IgG                          |                                           | IgA                          |                                           | IgM                          |                                           |
|    | MW (RLU)<br>Cutoff<br>40.000 | Ratio<br>pos: $\geq 1,1$<br>bl: $0,8-1,1$ | MW (RLU)<br>Cutoff<br>50.000 | Ratio<br>pos: $\geq 1,1$<br>bl: $0,8-1,1$ | MW (RLU)<br>Cutoff<br>50.000 | Ratio<br>pos: $\geq 1,1$<br>bl: $0,8-1,1$ |
| 1  | 36.051                       | 0,9                                       | 79.014                       | 1,6                                       | 9.535                        | 0,2                                       |
| 2  | 128.217                      | 3,2                                       | 244.029                      | 4,9                                       | 24.354                       | 0,5                                       |
| 3  | 223.597                      | 5,6                                       | 307.108                      | 6,1                                       | 21.478                       | 0,4                                       |
| 4  | 3.222                        | 0,1                                       | 6.861                        | 0,1                                       | 4.495                        | 0,1                                       |
| 5  | 4.152                        | 0,1                                       | 4.707                        | 0,1                                       | 2.095                        | 0,0                                       |
| 6  | 2.005                        | 0,1                                       | 3.943                        | 0,1                                       | 2.133                        | 0,0                                       |
| 7  | 15.280                       | 0,4                                       | 9.498                        | 0,2                                       | 4.929                        | 0,1                                       |
| 8  | 11.401                       | 0,3                                       | 10.970                       | 0,2                                       | 2.745                        | 0,1                                       |
| 15 |                              |                                           |                              |                                           | 9.292                        | 1,0                                       |
| 16 |                              |                                           |                              |                                           | 50.715                       | 1,3                                       |
| 17 |                              |                                           |                              |                                           | 62.684                       | 0,0                                       |
| 20 |                              | 576                                       |                              |                                           | 1.826                        | 0,0                                       |

These results show that antibodies to SEQ ID NO:1 can be detected using chemiluminescence. There is a good correlation with results obtained by ELISA. Therefore, chemiluminescence can be used to practice the present invention.

#### Example 5: Indirect immunofluorescence assay (IFA) with HEK-S1 cells expressing SEQ ID NO: 2

IFA was conducted using slides with a biochip array of recombinant HEK293 cells expressing SARS-CoV-2 S1 protein or HEK293 control cells to demonstrate that this method may be used for the detection of antibodies to SEQ ID NO: 1.

Each biochip mosaic was incubated with 35  $\mu$ L of 1:100 PBS-diluted serum samples at room temperature for 30 min, washed with PBS-Tween and immersed in PBS-Tween for 5 min. In the second step, fluorescein isothiocyanate (FITC)-labelled goat anti-human IgG (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck) was applied and incubated at room temperature for 30 min. Slides were washed again with a flush of PBS-Tween and then immersed in PBS-Tween for 5 min. Slides were embedded in PBS-buffered, DABCO containing glycerol (approximately 10  $\mu$ L per field) and examined by fluorescence microscopy. Alternatively, slides were incubated 30 min with PBS prior to serum incubation and bound human IgGs were detected with by 30 min incubation with anti-human-IgG-Biotin (1:200, 109-065-098, Dianova), followed by a washing step as described above and 30 min incubation with ExtrAvidin-FITC (1:2000, E2761, Sigma-Aldrich), followed by another washing step. Samples were classified as positive or negative based on the fluorescence intensity of the transfected cells in direct comparison with control-transfected cells and control samples. Results were evaluated by two independent observers using a EUROStar II microscope (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany). Reagents were obtained from Merck, Darmstadt, Germany or Sigma-Aldrich, Heidelberg, Germany if not specified otherwise.

The sera of a S1 IgG ELISA-positive patient serum (PS) showed a positive reaction with S1 (SEQ ID NO: 2) but not control transfected HEK cells (**Fig. 3**), whereas none of the 49 S1 IgG ELISA-negative control sera reacted. Signal intensity of the patient serum was improved by incubating the HEK-S1 cells with PBS prior to serum incubation and detection of human IgG antibodies with anti-human-IgG-Biotin/ExtrAvidin-FITC. Altogether 15/24 S1 IgG ELISA-positive patient sera showed a positive reaction in HEK-S1 IFA with anti-human-IgG-Biotin/ExtrAvidin-FITC.

Therefore, IFT is another method which can be used to practice the invention.

#### Example 6: Vaccination studies

A healthy subject received on day 1 an injection into the musculus quadriceps of 12.86  $\mu$ g recombinant S1 protein in physiological PBS (SEQ ID NO: 3). Alum adjuvans (Twinrix for adults, EMRA-MED Arzneimittel GmbH) was applied according to the manufacturer's instructions. On days 9, 21 and 28, the subject received an injection of another 12.86  $\mu$ g S1 protein in physiological PBS buffer and 10  $\mu$ L Imject alu adjuvans (Thermo Scientific Imject Alum Adjuvant Alaun) in 500  $\mu$ L sodium chloride solution. Blood samples were obtained on days 10, 23 and 29.

Presence of IgG and IgA antibodies to SEQ ID NO1 (S1) and N protein was determined in serum samples from the healthy subject using serological kits (EUROIMMUN Medizinische Labordiagnostika AG, EI 2606-9601 A, EI 2606-9601 G, EI 2606-9601-2 G and EI 2606-9601-2 M, as described in Examples 2 and 3) according to the manufacturer's instructions. Determined antibody titers are shown in Table 3.

**Table 3: Antibodies to SARS-CoV-2 antigens in a subject vaccinated using S1 protein**

| Day | IgA (S1)<br>(Cut off: <0.8) | IgG (S1)<br>(Cut off: <0.8) | IgG (NCP)<br>(Cut off: <0.8) | IgM (NCP)<br>(Cut off: <0.8) |
|-----|-----------------------------|-----------------------------|------------------------------|------------------------------|
| 10  | 0.5                         | 0.5                         | -                            | -                            |
| 23  | 1.0                         | 0.2                         | -                            | -                            |

|    |     |     |     |     |
|----|-----|-----|-----|-----|
| 29 | 3.5 | 6.2 | 0.6 | 0.7 |
|----|-----|-----|-----|-----|

As positive and negative controls, samples from patients suffering from SARS-CoV-2 infection (*i.e.*, expected presence of antibodies to SARs-CoV-2) and samples from healthy blood donors (*i.e.*, expected absence of antibodies to SARS-CoV-2) were used; see Tables 4 and 5, respectively.

- 5 In two patients suffering from SARS-CoV-2 infection (positive controls), samples were obtained 17 and 19 days after the onset of symptoms (usually 5-6 days after the infection). Determined antibody titers are shown in Table 4.

**Table 4: Antibodies to SARS-CoV-2 antigens in two patients suffering from SARS-CoV-2 infection**

| Day       | IgA (S1)<br>(Cut off: <0.8) | IgG (S1)<br>(Cut off: <0.8) | IgG (N)<br>(Cut off: <0.8) | IgM (N)<br>(Cut off: <0.8) |
|-----------|-----------------------------|-----------------------------|----------------------------|----------------------------|
| Patient 1 | 2,6                         | 1,8                         | 1,7                        | 1,3                        |
| Patient 2 | 1.3                         | 6.3                         | 3.5                        | 11.4                       |

In four healthy subjects who did not receive any vaccination (negative controls), samples were obtained. Antibody titers are shown in Table 5.

**Table 5: Antibodies to SARS-CoV-2 antigens in healthy subjects**

| Day       | IgA (S1)<br>(Cut off: <0.8) | IgG (S1)<br>(Cut off: <0.8) | IgG (NCP)<br>(Cut off: <0.8) | IgM (NCP)<br>(Cut off: <0.8) |
|-----------|-----------------------------|-----------------------------|------------------------------|------------------------------|
| Patient 1 | 0,0                         | 0,1                         | 0,0                          | 0,5                          |
| Patient 2 | 0,2                         | 0,3                         | 0,1                          | 0,4                          |
| Patient 3 | 0,1                         | 0,1                         | 0,1                          | 0,2                          |
| Patient 4 | 0,1                         | 0,1                         | 0,1                          | 0,1                          |

These results show that subjects vaccinated with SEQ ID NO: 1 or a variant thereof can be distinguished from infected subjects or subjects treated with a vaccine which does not comprise SEQ ID NO: 1 or a variant thereof by using an assay based on the detection of an antibody to SEQ ID NO: 1.

- 20 While antibodies to S1 comprised in the vaccine can be detected in both, antibodies to the N protein are only detected in infected patients, but not in the vaccinated subjects.

#### **Example 7: Detection of antibodies to RBD and S1 using a dot blot**

**Reagents:** RBD (SEQ ID NO: 34) and S1 (SEQ ID NO: 3) antigens were obtained as described in Example 1. Dilutions of the samples from patients suffering from SARS-CoV-2 as shown by a positive PCR test (1075, 1076, 1078, 1079, 1080, 1084, 1085, 1098, 1099, 1100) in dilution buffer (3% Bovine Serum Albumin in 1x Universal buffer (10x concentrate, product number 20125896) were prepared. Samples from healthy blood donors (BS01, BS10, BS25, BS32, BS43) were used as additional negative controls.

Monoclonal antibodies AK78, AK76 and AK80, used as positive controls, were each monoclonal anti-His tag antibodies (Lindner P, Bauer K, Krebber A, Nieba L, Kremmer E, Krebber C, Honegger A, Klinger B, Mocikat R, Plückthun A. Specific detection of his-tagged proteins with recombinant anti-His tag scFv-phosphatase or scFv-phage fusions. *Biotechniques*. 1997 Jan;22(1):140-9).

- 5 Blot strips were made by transferring 1  $\mu$ l of the antigen solution (2.69 mg ml<sup>-1</sup>) on the strip. The membrane was a 0.22  $\mu$ m cellulose nitrate membrane (Sartorius).

Secondary antibodies to IgA, IgG and IgM („Anti-human-IgA-AP“, „Anti-human-IgG-AP“ and „Anti-human-IgM-AP“, respectively) were from EUROIMMUN (Alkaline phosphatase-labelled anti-human IgA/G/M (goat), product no. ZD 1129 A / G / M) as was NBT/BCIP, product no. 10 123964)

- 10 **Method:** Blot strips were blocked by incubation for 15 minutes in washing buffer (3% Bovine Serum Albumin in 1x Universal buffer (10x concentrate product number 20125896)), followed by incubation of the strip in appropriately diluted sample for 3 hours at room temperature, followed by three washing steps using washing buffer, followed by incubation of the strip in appropriately diluted sample for another 30 minutes, followed again by the three washing steps, followed by staining by incubation in
- 15 NBT/BCIP solution for ten minutes, which was stopped by washing the strips thrice thoroughly in demineralized water.

**Results:** Figs. 4 and 5 show the results of dot blot analysis of the samples and controls. The assay could be established successfully as judged by positive results using the monoclonal antibodies instead of samples and no signals if negative controls were used.

- 20 While both S1 and RBD can be used to detect IgM, IgA and IgG antibodies, reactions are generally slightly stronger if S1 is used, suggesting that both the RBD and sequences flanking it contain epitopes or parts thereof. Moreover, part of the antigen-antibody interaction may be affected by the fold of the antigen.

- Interestingly, IgA and IgM, but no IgG antibodies could be detected in patient 1085, confirming the
- 25 ELISA-based results that detection of IgA antibodies enhances the sensitivity, particularly in addition to IgG. The patient may have been examined at an early stage of the disease when no IgG antibodies were detectable yet. Vice versa, only IgG antibodies were detectable in patient 1100, whose sample may have been obtained at a later stage of the disease, following disappearance of IgA antibodies.

IgA and IgG antibodies gave generally stronger signals than IgM antibodies.

30

#### **Example 8: Detection of antibodies to peptides derived from RBD and S1, using a dot blot**

- Peptides P1 (RTQLPPAYTNS, SEQ ID NO: 41), P2 (SGTNGTKRFDN, SEQ ID NO: 42), P3 (LTPGDSSSGWTAG, SEQ ID NO: 35), P4 (NNLDSKVGG, SEQ ID NO: 55), P5 (YQAGSTPCNGV, SEQ ID NO: 36), P6 (YGFQPTNGVGYYQ, SEQ ID NO: 37) and a fusion comprising P1-P6 (SEQ ID
- 35 NO: 56) were expressed as N-terminal His tag fusions comprising a protease cleavage site (sequence of fusion comprising P1: SEQ ID NO: 43; P2: SEQ ID NO: 44; P3: SEQ ID NO: 45; P4: SEQ ID NO: 46; P5: SEQ ID NO: 47; P6: SEQ ID NO: 48; fusion comprising P1-P6: SEQ ID NO: 49 by *E. coli* Rosetta(DE3)pLacI cells using standard methods based on the pET24d plasmid, at 37°C for 3 h in LB Medium, containing Kanamycin and Chloramphenicol, using IPTG induction. Cells were harvested,
- 40 resuspended in Phosphate-Buffered Saline and stored at -20°C until further use.

**Figs. 6, 7 and 8** show the results of the dot blot assays using the P1 to P6 constructs. P6, which is part of the RBD, showed the strongest reaction no matter whether IgA, IgM or IgG were detected, but some reactivity could also be demonstrated with P1, P2 (except for IgM), P3 and P4 (except for IgM) and P5. Reactions with IgG and IgA were generally stronger than IgM.

5 Samples representing time courses with more than one samples from several patients demonstrated that the dot blot may be used to monitor patients. For example, patient SK1586 (three time points) has the strongest IgM reaction with the first sample (1.3i), while the signal is weaker if the second (1.11.i) and the third sample (1.19) are used, in particular with regard to P3 (**Fig. 9**). The same samples showed a stronger reaction if peptides P1 and P6 were used to detect IgA (**Fig. 10**). The reaction was  
10 generally stronger if IgG antibodies were detected, but a time-dependent reaction can be monitored using the same samples, especially peptides P6, P1 and the fusion protein comprising P1 to P6 (**Fig. 11**). Examples 7 and 8 show that antibodies to SEQ ID NO:1 can be detected using dot blot and various fragments of SEQ ID NO: 1.

#### 15 **Example 9: Detection of antibodies to RBD and S1 using a Western Blot**

A non-reducing SDS PAGE was run using 2 µg of RBD (SEQ ID NO: 34) or S1 (SEQ ID NO: 3). A sample comprising 10 µl of protein was mixed with 4 µl NuPAGE-PP (NuPage LDS Sample Buffer (4x), Firma Fisher Scientific GmbH) and 1 µl of EU-PBS (Phosphate-Buffered Saline), incubated for ten minutes at 70 °C. 12 µl of the resulting solution were applied per lane to a non-reducing 2D gel  
20 (NuPAGE 4-12% Bis-Tris Gel 1.0 mm x 2 D, Fisher Scientific GmbH) and subjected to electrophoresis in running buffer (NuPage MOPS SDS Running Buffer, Fisher Scientific GmbH). Separated Protein bands were transferred to a nitrocellulose membrane for 60 minutes at 400 mA (PowerPac HC Power Supply, Firma Bio-Rad). Membrane strips could be stained using PonceauS, followed by washing in 50 mM Tris, but were in any event subjected to blocking for 15 minutes in washing buffer (3% Bovine  
25 Serum Albumin in 1x Universal buffer (10x concentrate order no.: 20125896), followed by incubation of the strip in appropriately diluted sample for 3 hours at room temperature, followed by three washing steps using washing buffer, followed by incubation of the strip in appropriately diluted sample for another 30 minutes, followed again by the three washing steps, followed by staining by incubation in NBT/BCIP solution for ten minutes, which was stopped by washing the strips thrice thoroughly in  
30 demineralized water.

**Results:** Positive control (anti-His antibodies as before) and positive samples from SARS-CoV-2 infected patients show that dimers, trimers and tetramers were detected in addition to RBD monomers. A weak reaction was detected using secondary antibodies detecting IgA class antibodies with six sera, a weak reaction with another three sera and a strong reaction with one serum. If two or more  
35 samples from different time points were available and reactive, a decrease of the concentration of IgA antibodies was observed, for example with samples SK159822.1i to 2.4i, as well as with samples 6i and 61i and 7i and 7.1i (**Fig. 12**).

As for the detection of IgG class antibodies, all samples which were positive, as judged by ELISA, showed a reaction (**Fig. 13**). Sample SK1606 169i was negative based on the results of both meth-  
40 ods.

As for the detection of IgM class antibodies, a decreasing concentration of antibodies was detected in some cases, notably SK15862.2 to SK15862.16 as well as SK159986i to SK1599861i, if two or more samples from different time points were available (Fig. 14). Results were highly comparable if S1 was used rather than RBD as antigen (Figs. 15, 16 and 17).

- 5 It is concluded that Western blotting is another method that may be used to practice the present invention.

**Example 10: Detection of IgA, IgM and IgG antibodies to RBD using a competitive test format**

A EUROIMMUN SARS-CoV-2 NeutralISA kit (EI 2606-9601-4) was used for the following experiment according to manufacturer's instructions unless specified to the contrary.

10 Briefly, a microtiter plate coated with the S1 domain of the spike protein of SARS-CoV-2 expressed recombinantly in the human cell line HEK293 (SEQ ID NO: 3) was used. In the first step of the analysis, the controls and samples (blood samples of a subject) were diluted with a sample buffer containing soluble biotinylated human ACE2 (SEQ ID NO: 50) and incubated in the reagent wells of a microtiter plate. Neutralising antibodies present in the sample compete with the ACE2 for the binding sites of the coated SARS-CoV-2 S1 spike protein. Unbound ACE2 and unbound sample was removed in a subsequent washing step. To detect bound ACE2, a second incubation was performed using peroxidase-labelled streptavidin, which binds to the biotinylated ACE2 immobilized on the antigen on the microtiter plate and catalyses a colour reaction in the third step. The intensity of the produced colour is inversely proportional to the concentration of neutralising antibodies in the sample as shown in Table 6:

| ACE2-Concen-tration<br>6,0 µg/ml |                 |                 |                 |
|----------------------------------|-----------------|-----------------|-----------------|
| Probe                            | Positive sample | Positive sample | Negative sample |
| 1:2,5                            | 0,248           | 0,166           | 1,177           |
| 1:5                              | 0,342           | 0,294           | 1,208           |
| 1:10                             | 0,521           | 0,446           | 1,213           |
| 1:20                             | 0,697           | 0,605           | 1,190           |
| 1:40                             | 0,870           | 0,738           | 1,231           |
| 1:80                             | 0,910           | 0,829           | 1,172           |
| 1:160                            | 0,932           | 0,877           | 1,187           |
| Blank                            | 0,878           | 0,911           | 1,107           |

The results show that an increasing dilution of a positive sample, *i.e.* a decreasing concentration of neutralizing antibodies, leads to an increase in the produced colour. By contrast, the signal is high and does not correlate with the dilution of the sample if a negative sample is used.

This confirms that the assay can be used to detect and to quantify the neutralizing antibodies against the RBD in a sample.

**Example 11: Detection of antibodies to SARS-CoV-2 antigens S1 and N proteins by ELISA**

30 A panel comprising several samples taken at different time points after the infection from each of 43 German COVID-19 patients was used to detect the presence or absence of antibodies over time. All

sera were tested for antibodies against the S1 domain (SEQ ID NO: 3) of the SARS-CoV-2 spike domain (IgA and IgG ELISA kits, EUROIMMUN, products as in Examples 2 and 3) and antibodies against the N protein (IgG and IgM ELISA kits, EUROIMMUN, as in Examples 2 and 3).

Between >10 to <21 days after the onset of illness IgG and IgA antibodies against S1 of SARS-CoV-2 were detected in 70.4% and 88.9% of the samples, while IgG and IgM against N protein were detected in 86.2% and 50%, respectively. In six patients, IgA antibody to S1 was the first antibody that could be detected, while only in two cases an antibody *other than* IgA to S1 was the first antibody to be detected. In 30 patients no IgM antibody to N protein could be detected at any time.

More than 60 days after the onset of illness) IgG and IgA antibodies against S1 of SARS-CoV-2 were detected in 85.1% and 80.5% of the samples, while IgG and IgM against N protein were detected in 81.4% and 0%, respectively. In four patients, IgG to S1 was detectable, but not IgG to N protein. By contrast, only in one patient IgG to N protein was detectable, but not IgG to S1.

These results confirm that the detection of IgA to S1 is the most sensitive assay for the early detection of an antibody response against SARS-CoV-2, more specifically its S1 protein. By contrast, the detection of IgG to S1 is particularly sensitive at a later stage of the infection. Both assays may be combined by detection of IgA to S1 and IgG to S1 in one reaction or in separate reactions for increased sensitivity, optionally in combination with additional assays for increased sensitivity over an extended period of time.

#### Example 12: Detection of antibodies to SARS-CoV-2 antigens S1 and N proteins by ELISA during the late phase of a SARS-CoV-2 infection

Using the same methodology as in Example 11, samples from another cohort of 15 patients suffering from a SARS-CoV-2 infection taken 21 days or later after the onset of illness were obtained and tested for the presence of IgG and IgM antibodies to N protein and IgA and IgG antibodies to the S1 protein.

The results are shown in **Table 7**:

| Assay         | Positive | Borderline | negative | Sensitivity |
|---------------|----------|------------|----------|-------------|
| NCP ELISA IgG | 13       | 0          | 2        | 86,7%       |
| NCP ELISA IgM | 5        | 2          | 8        | 46,7%       |
| S1 ELISA IgG  | 14       | 0          | 1        | 93,3%       |
| S1 ELISA IgA  | 12       | 3          | 0        | 100%        |

The study on this additional cohort confirms that the sensitivity of diagnosis is highest at the late phase of a SARS-CoV-2 infection if antibodies to the S1 protein are detected.

#### Example 13: The specificity of the detection of antibodies to SARS-CoV-2 antigens S1 and N proteins

The specificity of the Anti-SARS-CoV-2 ELISA (IgA, EI 2606-9601 A, carried out according to manufacturer's instructions, more details in Examples 2 and 3) was determined by analyzing 210 patient samples that were positive, for instance, for antibodies against other human pathogenic coronaviruses, other pathogens or for rheumatoid factors. Additionally, 1052 samples from blood

donors, children and pregnant women obtained before the occurrence of SARS-CoV-2 (before January 2020) were analysed. Results in the borderline range (n=9) were not included in the calculation of the specificity. This resulted in a specificity of 98.3% as shown in **Table 8**.

- 5 The specificity of the Anti-SARS-CoV-2 ELISA (IgG, EI 2606-9601 G, carried out according to manufacturer's instructions, more details in Examples 2 and 3) was determined in the same manner, based on 222 positive patient samples and 1052 samples from blood donors, children and pregnant. This resulted in a specificity of 98.3% as shown in **Table 8**:

10

| Panel                                                | n    | Specificity IgA<br>ELISA in % | Panel                                                | n    | Specificity IgG<br>ELISA in % |
|------------------------------------------------------|------|-------------------------------|------------------------------------------------------|------|-------------------------------|
| Blood donors                                         | 849  | 98.2                          | Blood donors                                         | 849  | 99.5                          |
| Pregnant women                                       | 99   | 97                            | Pregnant women                                       | 199  | 99.5                          |
| Children                                             | 104  | 100                           | Children                                             | 74   | 100                           |
| Elderly people                                       | 97   | 99                            | Elderly people                                       | 97   | 100                           |
| Infections with other human pathogenic coronaviruses | 11   | 100                           | Infections with other human pathogenic coronaviruses | 23   | 100                           |
| Influenza (freshly vaccinated)                       | 40   | 100                           | Influenza (freshly vaccinated)                       | 40   | 100                           |
| Acute EBV infections & heterophile antibodies        | 22   | 90.5                          | Acute EBV infections & heterophile antibodies        | 22   | 100                           |
| Rheumatoid factors                                   | 40   | 100                           | Rheumatoid factors                                   | 40   |                               |
| Total                                                | 1262 | 98.3                          | Total                                                | 1344 | 99.6                          |

---

Claims

---

- 5           1.    A method for diagnosing a SARS-CoV-2 infection comprising the step of detecting the presence or absence of an antibody to SEQ ID NO1, preferably IgA class antibody, in a sample from a subject.
- 10           2.    A method for the differential diagnosis of a coronavirus infection, preferably for distinguishing between a SARS-CoV; preferably SARS-CoV-2; MERS, NL63, 229E, OC43 and HKU1 infection, comprising the step of detecting the presence or absence of an antibody to SEQ ID NO1 in a sample from a subject, preferably an IgA and/or IgG class antibody, more preferably an IgA class antibody.
- 15           3.    The method according to any of claims 1 or 2, wherein the presence of an IgG and/or IgM class antibody to SEQ ID NO1 is detected in addition to an IgA class antibody to SEQ ID NO1.
- 20           4.    The method according to any of claims 1 to 3, wherein the sample is a blood sample, preferably selected from the group comprising whole blood, serum or plasma.
5.    The method according to any of claims 1 to 4, wherein an IgA class antibody to SEQ ID NO1 is detected using a labeled secondary antibody, preferably binding to IgA class antibodies.
- 25           6.    The method according to any of claims 1 to 5, wherein the IgA antibody is detected using a method selected from the group comprising colorimetry, immunofluorescence, detection of enzymatic activity, chemiluminescence and radioactivity.
- 30           7.    The method according to any of claims 1 to 6, wherein the infection is detected at an early stage.
- 35           8.    A use of an antibody to SEQ ID NO1, preferably IgA class antibody, for diagnosing a SARS-CoV-2 infection or for the differential diagnosis of a coronavirus infection, preferably for distinguishing between a SARS-CoV, preferably SARS-CoV-2, MERS and NL63, 229E, OC43 and HKU1 infection.
9.    The use according to claim 8, wherein the use is for the early diagnosis of a SARS-CoV-2 infection.

- 5 10. A kit comprising a polypeptide comprising SEQ ID NO1 or a variant thereof, preferably coated to a diagnostically useful carrier and one or more, preferably all reagents from the group comprising an antibody to SEQ ID NO1, a washing buffer, a means for detecting the presence of antibody to SEQ ID NO1, preferably an IgA class antibody, preferably a secondary antibody binding specifically to IgA class antibodies, preferably comprising a detectable label, and a dilution buffer.
- 10 11. The kit according to claim 10, wherein the diagnostically useful carrier is selected from the group comprising a bead, preferably a paramagnetic bead, a test strip, a microtiter plate, a membrane, preferably from the group comprising western blot, line blot and dot blot, a lateral flow device, a glass surface, a slide, a microarray and a biochip and is preferably a microtiter plate.
- 15 12. The kit according to claim 10 or 11, wherein the kit comprises two or more, preferably three or more calibrators.
- 20 13. The kit according to claim 12, wherein each calibrator is a recombinant antibody binding to SEQ ID NO1 which is preferably recognized by a secondary antibody binding to IgA class antibodies.
- 25 14. A use of a polypeptide comprising SEQ ID NO1 or a variant thereof or an antibody to SEQ ID NO1, preferably an IgA class antibody, for the manufacture of a diagnostic kit.
- 15 15. A use of a recombinant antibody binding to SEQ ID NO1 which is preferably recognized by a secondary antibody binding to IgA class antibodies as a calibrator for the early diagnosis of a SARS-CoV-2 infection.