



(51) International Patent Classification:

G01N 33/569 (2006.01)

(21) International Application Number:

PCT/US2021/065581

(22) International Filing Date:

29 December 2021 (29.12.2021)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/132,155 30 December 2020 (30.12.2020) US

63/180,209 27 April 2021 (27.04.2021) US

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(81) Designated States (unless otherwise indicated, for every

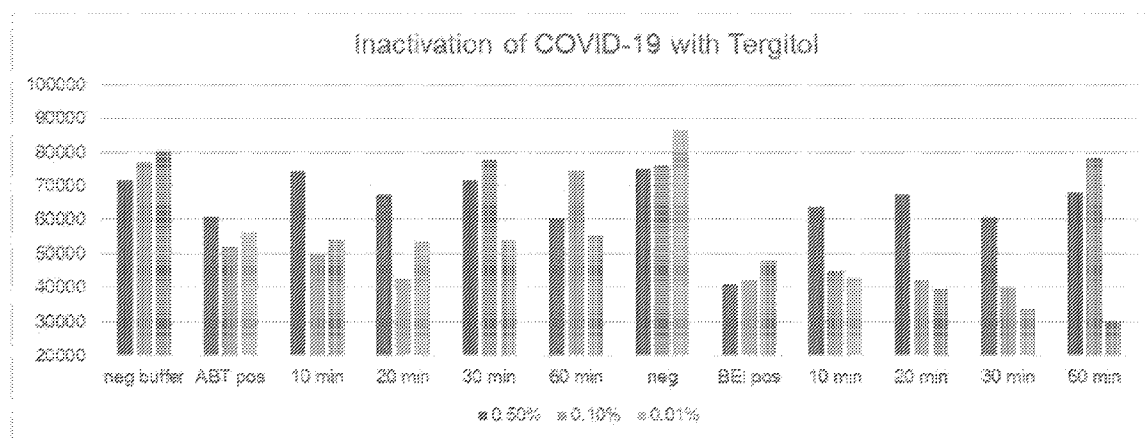
kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,

(54) Title: IMPROVED METHODS, REAGENTS AND KITS FOR DETERGENT-BASED INACTIVATION OF BETACORONAVIRUS PRIOR TO AND/OR WHILE ASSESSING A BIOLOGICAL SAMPLE FOR SARS-COV-2 ANTIGEN OR ANTIBODY

FIG. 1



(57) Abstract: Disclosed herein are methods of inactivating any β -coronaviruses (e.g., SARS-CoV or SARS-CoV-2) in a biological sample prior to testing the sample for the presence of a β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) antigen or antibody, which involves maintaining the sample in a medium comprising at least one non-ionic surfactant. The at least one non-ionic surfactant inactivates the β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) as determined by an inability of the β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) to replicate in culture.

MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

**IMPROVED METHODS, REAGENTS AND KITS FOR DETERGENT-BASED
INACTIVATION OF BETACORONAVIRUS PRIOR TO AND/OR WHILE ASSESSING
A BIOLOGICAL SAMPLE FOR SARS-COV-2 ANTIGEN OR ANTIBODY**

RELATED APPLICATION INFORMATION

[0001] This application claims priority to U.S. Application No. 63/132,155 filed on December 30, 2020, and U.S. Application No. 63/180,209 filed on April 27, 2021, the contents of each of which is herein incorporated by reference.

TECHNICAL FIELD

[0002] The present disclosure relates to methods of inactivating β -coronaviruses (e.g., SARS-CoV or SARS-CoV-2) in a biological sample prior to, while (i.e., concurrent with), or both prior to and while testing the sample for the presence of a β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) antigen or antibody.

BACKGROUND

[0003] Viruses of the family *Coronaviridae* possess a single-strand, positive-sense RNA genome ranging from 26 to 32 kilobases in length (reviewed by Lu et al., *The Lancet*, 395:565-574 (February 22, 2020)). The *Coronaviridae* are further subdivided (initially based on serology but now based on phylogenetic clustering) into four groups, the alpha, beta, gamma and delta coronaviruses. Coronaviruses have been identified in several avian hosts, as well as in various mammals, including camels, bats, masked palm civets, mice, dogs, and cats.

[0004] Among the several coronaviruses that are pathogenic to humans, most are associated with mild clinical symptoms, with three exceptions. Severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) is a novel betacoronavirus (β -coronavirus) that emerged in Guangdong, southern China, in November 2002 and resulted in more than 8000 human infections and 774 deaths in 37 countries in 2002-03. Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) was first detected in Saudi Arabia in 2012 and was responsible for 2494 laboratory-confirmed cases of infection and 858 deaths from 2012-20. In December 2019, a cluster of pneumonia cases caused by a newly identified β -coronavirus was found to be epidemiologically-associated with the Huanan seafood market in Wuhan, China, where a number of animals, such as birds and rabbits, were on sale before the outbreak. This

coronavirus was named January 2020 by the World Health Organization (WHO) as the 2019-novel coronavirus (2019-nCov or COVID-19), and February 2020 by the International Committee as SARS-CoV-2. SARS-CoV-2 was declared a pandemic due to its rapid, uncontrolled, and vast worldwide spread.

[0005] Coronavirus virions are spherical with diameters of approximately 125 nanometers, as demonstrated by cryo-electron tomography and cryo-electron microscopy. A prominent feature of coronaviruses is the club-shape spike projections emanating from the surface of the virion, giving the virion the appearance of a solar corona and resulting in the name, coronaviruses. Within the envelope of the coronavirus virion is the helically symmetrical nucleocapsid, which binds to and creates a shell around the coronavirus RNA genome. The spike (S) and nucleocapsid (N) proteins are the main immunogens of the coronavirus. The other two main structural proteins of the coronavirus particles are the membrane (M) and envelope (E) proteins. All four proteins are encoded within the 3' end of the viral genome.

[0006] The S protein (~150 kDa) is heavily N-linked glycosylated and utilizes an N-terminal signal sequence to gain access to the endoplasmic reticulum (ER). Homotrimers of the virus-encoding S protein make up the distinctive spike structure on the surface of the virus. In many, but not all, coronaviruses, the S protein is cleaved by a host cell furin-like protease into two separate polypeptides known as S1 and S2. S1 makes up the large receptor-binding domain of the S protein while S2 forms the stalk of the spike molecule. The trimeric S glycoprotein mediates attachment of the coronavirus virion to the host cell by interactions between the S protein and its receptor. In humans, angiotensin-converting enzyme 2 (ACE2) is the receptor for SARS-CoV. The sites of receptor binding domains (RBD) within the S1 region of a coronavirus S protein vary depending on the virus. Some viruses have the RBD at the N-terminus of S1 (e.g., murine hepatitis virus) and others have the RBD at the C-terminus of S1 (e.g., SARS-CoV). The S-protein/receptor interaction is the primary determinant for the coronavirus to infect a host species and also governs the tissue tropism of the virus.

[0007] The M protein is the most abundant structural protein in the virion. It is a small (~25–30 kDa) protein with 3 transmembrane domains and is believed to give the virion its shape. It has a small N-terminal glycosylated ectodomain and a much larger C-terminal endodomain that extends 6-8 nm into the viral particle.

[0008] The E protein (~8-12 kDa) is found in small quantities within the virion. E proteins in coronaviruses are highly divergent but have a common architecture. Data suggests that the E protein is a transmembrane protein with an N-terminal ectodomain and a C-terminal endodomain that has ion channel activity. Recombinant viruses lacking the E protein are not always lethal - although this is virus-type dependent. The E protein facilitates assembly and release of the virus, but also has other functions (e.g., ion channel activity in SARS-CoV E protein is not required for viral replication but is required for pathogenesis).

[0009] The N protein is the only protein present in the nucleocapsid. It is composed of two separate domains, an N-terminal domain (NTD) and a C-terminal domain (CTD), both capable of binding RNA *in vitro* using different mechanisms, which may suggest that optimal RNA binding requires contributions from both domains. The N protein is heavily phosphorylated, and phosphorylation has been suggested to trigger a structural change enhancing the affinity for viral versus non-viral RNA. The N protein binds the viral genome in a beads-on-a-string type conformation. Two specific RNA substrates have been identified for N protein; the transcriptional regulatory sequences and the genomic packaging signal. The genomic packaging signal has been found to bind specifically to the second, or C-terminal RNA binding domain. The N protein also binds nsp3, a key component of the replicase complex, and the M protein. These protein interactions likely help tether the viral genome to the replicase-transcriptase complex, and subsequently package the encapsidated genome into viral particles.

[0010] In February 2020, Lu et al. reported obtaining complete and partial SARS-CoV-2 genome sequences using next-generation sequencing of bronchoalveolar lavage fluid samples and cultured isolates from nine patients from Wuhan diagnosed with viral pneumonia but negative for common respiratory pathogens. Lu et al., *The Lancet*, 395: 565-574 (February 22, 2020). Based on their analysis, Lu et al. further reported that SARS-CoV-2 was closely related (with 88% identity) to two bat-derived severe acute respiratory syndrome (SARS)-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21, collected in eastern China in 2018, but was more distant from SARS-CoV (about 79%) and MERS-CoV (about 50%). Additionally, Zhou et al. confirmed that SARS-CoV-2 uses the same cellular entry receptor, ACE2, as SARS-CoV. Zhou et al., *Nature*, 579:270-273 (March 2020).

[0011] SARS-CoV-2 primarily spreads through the respiratory tract, by droplets, respiratory secretions, and direct contact. Additionally, SARS-CoV-2 has been found in fecal swabs and

blood, indicating the possibility of multiple routes of transmission. Zhang et al., *Microbes* 9(1):386-9 (2020). SARS-CoV-2 is highly transmissible in humans, especially in the elderly and people with underlying diseases. Symptoms can appear 2 to 14 days after exposure. Patients present with symptoms such as fever, malaise, cough, and/or shortness of breath. Most adults or children with SARS-CoV-2 infection present with mild flu-like symptoms, however, critical patients rapidly develop acute respiratory distress syndrome, respiratory failure, multiple organ failure and even death.

[0012] Because of the health risks imposed by SARS-CoV-2 transmission, there is a need for methods and kits to assess coronavirus transmission in humans in one or more samples obtained from a subject. Provided herein are such improved methods, reagents, and kits.

SUMMARY

[0013] In one aspect, the present disclosure relates to methods of inactivating any SARS-CoV-2 in a biological sample prior to or while (i.e., concurrent with), or both prior to and while, testing the sample for the presence of a SARS-CoV-2 antigen or antibody. The method involves the steps of maintaining the sample in a medium comprising at least about 0.1% (v/v) of at least one non-ionic surfactant under conditions appropriate for and for an amount of time sufficient for the at least one non-ionic surfactant to inactivate the SARS-CoV-2 in the sample as determined by an inability of the SARS-CoV-2 to replicate in culture, without substantially interfering with testing the sample for the presence of a SARS-CoV-2 antigen or antibody.

[0014] In some aspects of the above method, the at least one non-ionic surfactant is a secondary alcohol ethoxylate, such as tergitol. In other aspects, the at least one non-ionic surfactant is tergitol and polyethylene glycol sorbitan monolaurate.

[0015] In other aspects of the above method, the medium comprises from about 0.1% to about 1.0% (v/v) of the at least one non-ionic surfactant. In some aspects, the medium comprises from about 0.1% to about 0.75% (v/v) of the at least one non-ionic surfactant. In further aspects, the medium comprises from about 0.1% to about 0.5% (v/v) of the at least one non-ionic surfactant. In further aspects, the medium comprises about 0.2% (v/v) of at least one non-ionic surfactant. Specifically, in some aspects, the medium comprises about 0.2% (v/v) of tergitol. In other aspects, the medium comprises about 0.1% (v/v) of tergitol and about 0.1% (v/v) of polyethylene glycol sorbitan monolaurate.

[0016] In further aspects of the above method, the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or a universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0017] In other aspects of the above method, the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0018] In other aspects of the above method, testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0019] In other aspects of the above method, testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or point-of-care assay.

[0020] In further aspects of the above method, testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

[0021] The present disclosure also relates to an improvement of a method of testing a biological sample for the presence of a SARS-CoV-2 antigen or antibody. The improvement comprises maintaining the sample in a medium comprising at least about 0.1% v/v of at least one non-ionic surfactant prior to or while (i.e., concurrent with), or both prior to and while, testing the sample for the presence of a SARS-CoV-2 antigen or antibody, wherein the sample is maintained under conditions and for an amount of time sufficient for the at least one non-ionic surfactant to inactivate any SARS-CoV-2 present in the sample as determined by an inability of the SARS-CoV-2 to replicate in culture, without substantially interfering with testing the sample for the presence of a SARS-CoV-2 antigen or antibody.

[0022] In some aspects of the above improvement, the at least one non-ionic surfactant is a secondary alcohol ethoxylate, such as tergitol. In other aspects, the at least one non-ionic surfactant is tergitol and polyethylene glycol sorbitan monolaurate.

[0023] In other aspects of the above improvement, the medium comprises from about 0.1% to about 1.0% (v/v) of the at least one non-ionic surfactant. In some aspects, the medium comprises from about 0.1% to about 0.75% (v/v) of the at least one non-ionic surfactant. In further aspects, the medium comprises from about 0.1% to about 0.5% (v/v) of the at least one non-ionic surfactant. In further aspects, the medium comprises about 0.2% (v/v) of at least one non-ionic

surfactant. Specifically, in some aspects, the medium comprises about 0.2% (v/v) of tergitol. In other aspects, the medium comprises about 0.1% (v/v) of tergitol and about 0.1% (v/v) of polyethylene glycol sorbitan monolaurate.

[0024] In further aspects of the above improvement, the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or a universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0025] In other aspects of the above improvement, the sample saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0026] In other aspects of the above improvement, testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0027] In other aspects of the above improvement, testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0028] In further aspects of the above improvement, testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] FIG. 1 is graph showing inactivation of SARS-CoV-2 (the causative virus of COVID-19) in transport culture media to which has been added 0.5%, 0.1%, or 0.01% of the non-ionic surfactant tergitol.

DETAILED DESCRIPTION

The present disclosure is predicated, at least in part, on the discovery that incubating a biological sample with a non-ionic surfactant (e.g., tergitol) inactivates a β -coronavirus such as SARS-CoV or SARS-CoV-2, if present in the sample. Generally, inactivation is achieved by lysis of the virus particles. For safety reasons, a β -coronavirus, such as SARS-CoV or SARS-CoV-2, typically is inactivated before any diagnostic testing on a patient sample is initiated (e.g., an

antigen assay, a molecular assay, or an antibody assay). Typically, heat inactivation is used, but heat treatment could decrease assay signal. The present disclosure demonstrates that, surprisingly, incubating the biological sample with a non-ionic surfactant such as tergitol can be employed instead of heat inactivation.

[0030] Thus, in some aspects, the disclosure provides a method of inactivating any β -coronavirus, such as SARS-CoV or SARS-CoV-2, in a biological sample prior to or while (i.e., concurrent with), or both prior to and while, testing the sample for the presence of a β -coronavirus, such as SARS-CoV or SARS-CoV-2, antigen or antibody, the method comprising maintaining biological sample in a medium containing at least about 0.1% (v/v) of at least one non-ionic surfactant, wherein the at least one non-ionic surfactant inactivates the β -coronavirus, such as SARS-CoV or SARS-CoV-2, in the biological sample as determined by an inability of the β -coronavirus, such as SARS-CoV or SARS-CoV-2, to replicate in culture, without substantially interfering with testing the sample for the presence of a β -coronavirus, such as SARS-CoV or SARS-CoV-2, antigen or antibody. The biological sample used in the methods of the present disclosure may be obtained from an asymptomatic subject or from a subject exhibiting one or more symptoms of infection with a β -coronavirus (e.g., SARS-CoV or SARS-CoV-2). However, the invention is not limited to any particular mechanism of action and an understanding of the mechanism is not necessary to practice the invention.

[0031] Section headings as used in the entire disclosure herein are merely for organizational purposes and are not intended to be limiting.

1. Definitions

[0032] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present disclosure. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

[0033] The terms “comprise(s),” “include(s),” “having,” “has,” “can,” “contain(s),” and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms “a,” “an” and “the” include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments “comprising,” “consisting of” and “consisting essentially of,” the embodiments or elements presented herein, whether explicitly set forth or not.

[0034] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

[0035] “Antibody” and “antibodies” as used herein refers to monoclonal antibodies, monospecific antibodies (e.g., which can either be monoclonal, or may also be produced by other means than producing them from a common germ cell), multispecific antibodies, human antibodies, humanized antibodies (fully or partially humanized), animal antibodies such as, but not limited to, a bird (for example, a duck or a goose), a shark, a whale, and a mammal, including a non-primate (for example, a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, etc.) or a non-human primate (for example, a monkey, a chimpanzee, etc.), recombinant antibodies, chimeric antibodies, single-chain Fvs (“scFv”), single chain antibodies, single domain antibodies, Fab fragments, F(ab’) fragments, F(ab’)₂ fragments, disulfide-linked Fvs (“sdFv”), and anti-idiotypic (“anti-Id”) antibodies, dual-domain antibodies, dual variable domain (DVD) or triple variable domain (TVD) antibodies (dual-variable domain immunoglobulins and methods for making them are described in Wu, C., et al., *Nature Biotechnology*, 25(11):1290-1297 (2007) and PCT International Application WO 2001/058956, the contents of each of which are herein incorporated by reference), or domain antibodies (dAbs) (e.g., such as described in Holt et al., *Trends in Biotechnology* 21:484-490 (2014)), and including single domain antibodies (sdAbs) that are naturally occurring, e.g., as in cartilaginous fishes and camelid, or which are synthetic, e.g., nanobodies, VHH, or other domain structure), and functionally active epitope-binding fragments of any of the above. In particular, antibodies include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, namely, molecules that contain

an analyte-binding site. Immunoglobulin molecules can be of any type (for example, IgG, IgE, IgM, IgD, IgA, and IgY), class (for example, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2), or subclass. For simplicity sake, an antibody against an analyte is frequently referred to herein as being either an “anti-analyte antibody” or merely an “analyte antibody”.

[0036] “Antibody fragment,” as used herein, refers to a portion of an intact antibody comprising the antigen-binding site or variable region. The portion does not include the constant heavy chain domains (i.e., CH2, CH3, or CH4, depending on the antibody isotype) of the Fc region of the intact antibody. Examples of antibody fragments include, but are not limited to, Fab fragments, Fab’ fragments, Fab’-SH fragments, F(ab’)2 fragments, Fd fragments, Fv fragments, diabodies, single-chain Fv (scFv) molecules, single-chain polypeptides containing only one light chain variable domain, single-chain polypeptides containing the three CDRs of the light-chain variable domain, single-chain polypeptides containing only one heavy chain variable region, and single-chain polypeptides containing the three CDRs of the heavy chain variable region.

[0037] “Anti-species antibodies” as used herein refers to an antibody, such as an IgG and/or IgM antibody, that recognize antibodies of another species of interest. For example, anti-human antibodies, e.g., anti-human IgG or IgM antibodies, recognize, respectively, other human IgG or IgM antibodies.

[0038] “Binding protein” is used herein to refer to a monomeric or multimeric protein that binds to and forms a complex with a binding partner, such as, for example, a polypeptide, an antigen, a chemical compound or other molecule, or a substrate of any kind. A binding protein specifically binds a binding partner. Binding proteins include antibodies, as well as antigen-binding fragments thereof and other various forms and derivatives thereof as are known in the art and described herein below, and other molecules comprising one or more antigen-binding domains that bind to an antigen molecule or a particular site (epitope) on the antigen molecule. Accordingly, a binding protein includes, but is not limited to, an antibody a tetrameric immunoglobulin, an IgG molecule, an IgG1 molecule, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, an affinity matured antibody, and fragments of any such antibodies that retain the ability to bind to an antigen.

[0039] As used herein, the term “coronavirus” refers to viruses that belonging to the family *Coronaviridae* that have a positive-sense, RNA genome ranging from 26 to 32 kilobases in

length. Coronaviruses having four main structural proteins: the spike glycoprotein (S protein), the membrane protein (M protein), the envelope protein (E protein) and the nucleocapsid protein (N protein). Coronavirus can be further subdivided into four groups, alpha, beta, gamma and delta coronaviruses. Examples of alpha coronaviruses include HCoV-229E and HCoV-NL63. Examples of beta coronaviruses are HCoV-OC43, HCoV-HKU1, Middle East Respiratory Syndrome (MERS-CoV), severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) and SARS-CoV-2 (also known as 2019-nCoV, COVID-19, coronavirus disease, and Coronavirus Disease 2019).

[0040] In one aspect, the present disclosure relates to β -coronaviruses. In another aspect, the β -coronaviruses are MERS-CoV, SARS-CoV and SARS-CoV-2. In still yet another aspect, the β -coronaviruses are SARS-CoV and SARS-CoV-2. In still yet another aspect, the β -coronavirus is SARS-CoV-2. The sequence of SARS-CoV-2 has been described in a variety of publications, such as, for example, Lu et al., *Lancet*, 395:565-574 (February 2020); NCBI Reference Sequence: NC_045512.2, and ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/, the contents of each of which are herein incorporated by reference.

[0041] “Component,” “components,” or “at least one component,” refer generally to elements such as, for example, a calibrator, a control, a sensitivity panel, a container, a buffer, a diluent (including an assay specific diluent), a salt, an enzyme, a co-factor for an enzyme, a detection reagent, a pretreatment reagent/solution, a substrate (e.g., as a solution), a stop solution, and the like that can be included in a kit for assay of a test sample, such as a patient saliva, oropharyngeal specimen, nasopharyngeal specimen, nasal mucus specimen, urine, whole blood, serum or plasma sample (e.g., as per “Sample” below), in accordance with the methods described herein and other methods known in the art. Some components can be in solution or lyophilized for reconstitution for use in an assay.

[0042] “Controls” as used herein generally refers to a reagent whose purpose is to evaluate the performance of a measurement system in order to assure that it continues to produce results within permissible boundaries (e.g., boundaries ranging from measures appropriate for a research use assay on one end to analytic boundaries established by quality specifications for a commercial assay on the other end). To accomplish this, a control should be indicative of patient results and optionally should somehow assess the impact of error on the measurement (e.g., error due to reagent stability, calibrator variability, instrument variability, and the like). As used

herein, a “control subject” relates to a subject or subjects that has not been infected with a coronavirus, such as a β -coronavirus, or been exposed to any subject that has had a coronavirus, such as a β -coronavirus.

[0043] “Determined by an assay” is used herein to refer to the determination of a reference level by any appropriate assay. The determination of a reference level may, in some embodiments, be achieved by an assay of the same type as the assay that is to be applied to the sample from the subject (for example, by an immunoassay, clinical chemistry assay, a single molecule detection assay, protein immunoprecipitation, immunoelectrophoresis, a point-of-care assay, chemical analysis, SDS-PAGE and Western blot analysis, or protein immunostaining, electrophoresis analysis, a protein assay, a competitive binding assay, or a functional protein assay). The determination of a reference level may, in some embodiments, be achieved by an assay of the same type and under the same assay conditions as the assay that is to be applied to the sample from the subject. As noted herein, this disclosure provides exemplary reference levels (e.g., calculated by comparing reference levels at different time points). It is well within the ordinary skill of one in the art to adapt the disclosure herein for other assays to obtain assay-specific reference levels for those other assays based on the description provided by this disclosure. For example, a set of training samples comprising samples obtained from subjects known to have been infected by a coronavirus, such as a β -coronavirus (such as SARS-CoV or SARS-CoV-2), and samples obtained from human subjects known not to have been infected with a coronavirus, such as a β -coronavirus (such as SARS-CoV or SARS-CoV-2), or been exposed to a subject that has been infected with a coronavirus, such as a β -coronavirus (such as SARS-CoV or SARS-CoV-2), may be used to obtain assay-specific reference levels. It will be understood that a reference level “determined by an assay” and having a recited level of “sensitivity” and/or “specificity” is used herein to refer to a reference level which has been determined to provide a method of the recited sensitivity and/or specificity when said reference level is adopted in the methods of the disclosure. It is well within the ordinary skill of one in the art to determine the sensitivity and specificity associated with a given reference level in the methods of the disclosure, for example by repeated statistical analysis of assay data using a plurality of different possible reference levels.

[0044] Practically, when discriminating between a subject as having been infected by a coronavirus, such as a β -coronavirus (such as SARS-CoV or SARS-CoV-2), or not having been

infected by a coronavirus, such as a β -coronavirus (such as SARS-CoV or SARS-CoV-2), the skilled person will balance the effect of raising a cutoff on sensitivity and specificity. Raising or lowering a cutoff will have a well-defined and predictable impact on sensitivity and specificity, and other standard statistical measures. It is well known that raising a cutoff will improve specificity but is likely to worsen sensitivity (proportion of those with disease who test positive). In contrast, lowering a cutoff will improve sensitivity but will worsen specificity (proportion of those without disease who test negative). The ramifications for detecting or measuring a coronavirus, such as a β -coronavirus (such as SARS-CoV or SARS-CoV-2), will be readily apparent to those skilled in the art. In discriminating whether a subject has or has not been infected by a coronavirus, such as a β -coronavirus (such as SARS-CoV or SARS-CoV-2), the higher the cutoff, specificity improves as more true negatives (i.e., subjects not having been infected by a coronavirus, such as β -coronavirus (such as SARS-CoV or SARS-CoV-2)) are distinguished from those having been infected by a coronavirus, such as a β -coronavirus (such as SARS-CoV or SARS-CoV-2). But at the same time, raising the cutoff decreases the number of cases identified as positive overall, as well as the number of true positives, so the sensitivity must decrease. Conversely, the lower the cutoff, sensitivity improves as more true positives (i.e., subjects having been infected with a coronavirus, such as a β -coronavirus (such as SARS-CoV or SARS-CoV-2)) are distinguished from those who have not been infected (e.g., do not have) with a coronavirus, such as a β -coronavirus (such as SARS-CoV or SARS-CoV-2). But at the same time, lowering the cutoff increases the number of cases identified as positive overall, as well as the number of false positives, so the specificity must decrease.

[0045] Generally, a high sensitivity value helps one of ordinary skill rule out disease or condition (such as infection with a coronavirus, such as a β -coronavirus (such as SARS-CoV or SARS-CoV-2)), and a high specificity value helps one of skill rule in disease or condition. Whether one of ordinary skill desires to rule out or rule in disease depends on what the consequences are for the patient for each type of error. Accordingly, one cannot know or predict the precise balancing employed to derive a test cutoff without full disclosure of the underlying information on how the value was selected. The balancing of sensitivity against specificity and other factors will differ on a case-by-case basis. This is why it is sometimes preferable to provide alternate cutoff (e.g., reference) values so a physician or practitioner can choose.

[0046] “Epitope,” or “epitopes,” or “epitopes of interest” refer to a site(s) on any molecule that is recognized and can bind to a complementary site(s) on its specific binding partner. The molecule and specific binding partner are part of a specific binding pair. For example, an epitope can be on a polypeptide, a protein, a hapten, a carbohydrate antigen (such as, but not limited to, glycolipids, glycoproteins or lipopolysaccharides), or a polysaccharide. Its specific binding partner can be, but is not limited to, an antibody.

[0047] “Identical” or “identity,” as used herein in the context of two or more polypeptide or polynucleotide sequences, can mean that the sequences have a specified percentage of residues that are the same over a specified region. The percentage can be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of the single sequence are included in the denominator but not the numerator of the calculation.

[0048] “Monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. Monoclonal antibodies may include “chimeric” antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological properties.

[0049] “Nucleocapsid protein” or “N” protein may be used interchangeably herein to refer to one of four main structural proteins of a coronavirus. The N protein is the only protein present in the nucleocapsid. It is composed of two separate domains, an N-terminal domain (NTD) and a C-terminal domain (CTD), both capable of binding RNA *in vitro* using different mechanisms, which may suggest that optimal RNA binding requires contributions from both domains.

[0050] “Point-of-care device” refers to a device used to provide medical diagnostic testing at or near the point-of-care (namely, outside of a laboratory), at the time and place of patient care (such as in a hospital, physician’s office, urgent or other medical care facility, a patient’s home, a nursing home and/or a long-term care and/or hospice facility). Examples of point-of-care devices include those produced by Abbott Laboratories (Abbott Park, IL) (e.g., i-STAT and i-STAT Alinity, Universal Biosensors (Rowville, Australia); see US 2006/0134713), Axis-Shield PoC AS (Oslo, Norway) and Clinical Lab Products (Los Angeles, USA).

[0051] “Quality control reagents” in the context of immunoassays and kits described herein, include, but are not limited to, calibrators, controls, and sensitivity panels. A “calibrator” or “standard” typically is used (e.g., one or more, such as a plurality) in order to establish calibration (standard) curves for interpolation of the concentration of an analyte, such as an antibody or an analyte. Alternatively, a single calibrator, which is near a reference level or control level (e.g., “low”, “medium”, or “high” levels), can be used. Multiple calibrators (i.e., more than one calibrator or a varying amount of calibrator(s)) can be used in conjunction to comprise a “sensitivity panel.”

[0052] “Reference level” as used herein refers to an assay cutoff value (or level) that is used to assess diagnostic, prognostic, or therapeutic efficacy and that has been linked or is associated herein with various clinical parameters (e.g., presence of disease, stage of disease, severity of disease, progression, non-progression, or improvement of disease, etc.). As used herein, the term “cutoff” refers to a limit (e.g., such as a number) above which there is a certain or specific clinical outcome and below which there is a different certain or specific clinical outcome.

[0053] “Sample,” “test sample,” “specimen,” “sample from a subject,” “biological sample,” and “patient sample” as used interchangeably herein may be a sample of blood, such as whole blood (including for example, capillary blood, venous blood, dried blood spot, etc.), tissue, urine, serum, plasma, nasal mucus, amniotic fluid, lower respiratory specimens such as, but not limited to, sputum, saliva, endotracheal aspirate or bronchoalveolar lavage, cerebrospinal fluid, placental

cells or tissue, endothelial cells, leukocytes, or monocytes. The sample can be used directly as obtained from a patient or can be pre-treated, such as by filtration, distillation, extraction, concentration, centrifugation, inactivation of interfering components, addition of reagents, and the like, to modify the character of the sample in some manner as discussed herein or otherwise as is known in the art. Additionally, the sample can be a nasopharyngeal or oropharyngeal sample obtained using one or more swabs that, once obtained, is placed in a sterile tube containing a virus transport media (VTM) or universal transport media (UTM), for testing. In other embodiments, the sample can be obtained using a dry swab, in which case a VTM or UTM is not required. In other embodiments, the sample may comprise saliva or a nasal mucus specimen.

[0054] A variety of cell types, tissue, or bodily fluid may be utilized to obtain a sample. Such cell types, tissues, and fluid may include sections of tissues such as biopsy and autopsy samples, oropharyngeal specimens, nasopharyngeal specimens, an anal swab specimen, frozen sections taken for histologic purposes, blood (such as whole blood, dried blood spots, etc.), plasma, serum, red blood cells, platelets, interstitial fluid, cerebral spinal fluid, etc. Cell types and tissues may also include lymph fluid, cerebrospinal fluid, or any fluid collected by aspiration. A tissue or cell type may be provided by removing a sample of cells from a human and a non-human animal, but can also be accomplished by using previously isolated cells (e.g., isolated by another person, at another time, and/or for another purpose). Archival tissues, such as those having treatment or outcome history, may also be used. Protein or nucleotide isolation and/or purification may not be necessary. In some embodiments, the sample is a whole blood sample. In some embodiments, the sample is a capillary blood sample. In some embodiments, the sample is a dried blood spot. In some embodiments, the sample is a serum sample. In yet other embodiments, the sample is a plasma sample. In some embodiments, the sample is an oropharyngeal specimen. In other embodiments, the sample is a nasopharyngeal specimen. In other embodiments, the sample is sputum. In other embodiments, the sample is endotracheal aspirate. In still yet other embodiments, the sample is bronchoalveolar lavage. In still yet other embodiments, the sample is an anal swab specimen.

[0055] In some embodiments, a biological sample may be diluted or undiluted either prior to or during the performance of the method. In some aspects, the dilution occurs prior to performing the method. In other aspects, the dilution occurs during the performance of the

method. In one aspect, the sample can be from about 1 to about 25 microliters, about 1 to about 24 microliters, about 1 to about 23 microliters, about 1 to about 22 microliters, about 1 to about 21 microliters, about 1 to about 20 microliters, about 1 to about 18 microliters, about 1 to about 17 microliters, about 1 to about 16 microliters, about 15 microliters or about 1 microliter, about 2 microliters, about 3 microliters, about 4 microliters, about 5 microliters, about 6 microliters, about 7 microliters, about 8 microliters, about 9 microliters, about 10 microliters, about 11 microliters, about 12 microliters, about 13 microliters, about 14 microliters, about 15 microliters, about 16 microliters, about 17 microliters, about 18 microliters, about 19 microliters, about 20 microliters, about 21 microliters, about 22 microliters, about 23 microliters, about 24 microliters or about 25 microliters. In some embodiments, the sample is from about 1 to about 150 microliters or less or from about 1 to about 25 microliters or less. Alternatively, in another aspect, the biological sample can be diluted about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 10-fold, about 11-fold, about 12-fold, about 13-fold, about 15-fold, about 16-fold, about 17-fold, about 18-fold, about 19-fold, about 20-fold, about 21-fold, about 22-fold, about 23-fold, about 24-fold, about 25-fold, about 26-fold, about 27-fold, about 28-fold, about 29-fold, about 30-fold, about 31-fold, about 32-fold, about 33-fold, about 34-fold, about 35-fold, about 36-fold, about 37-fold, about 38-fold, about 39-fold, about 40-fold, about 41-fold, about 42-fold, about 43-fold, about 44-fold, about 45-fold, about 46-fold, about 47-fold, about 48-fold, about 49-fold, about 50-fold, about 100-fold, or greater prior to use (either before performing the method or during the performance of the method).

[0056] “Sensitivity” of an assay as used herein refers to the proportion of subjects for whom the outcome is positive that are correctly identified as positive (e.g., correctly identifying those subjects with a disease or medical condition for which they are being tested). For example, this might include correctly identifying subjects as having been infected with a coronavirus, such as a β -coronavirus, from those who have not been infected with a coronavirus, such as a β -coronavirus.

[0057] “Specificity” of an assay as used herein refers to the proportion of subjects for whom the outcome is negative that are correctly identified as negative (e.g., correctly identifying those subjects who do not have a disease or medical condition for which they are being tested). For example, this might include correctly identifying subjects not infected with a coronavirus, such

as a β -coronavirus, from those who have not been infected with a coronavirus, such as a β -coronavirus.

[0058] As used herein the term “single molecule detection” refers to the detection and/or measurement of a single molecule of an analyte in a test sample at very low levels of concentration (such as pg/mL or femtogram/mL levels). A number of different single molecule analyzers or devices are known in the art and include nanopore and nanowell devices. Examples of nanopore devices are described in PCT International Application WO 2016/161402, which is hereby incorporated by reference in its entirety. Examples of nanowell device are described in PCT International Application WO 2016/161400, which is hereby incorporated by reference in its entirety.

[0059] “Specific binding” or “specifically binding” as used herein may refer to the interaction of an antibody, a protein, or a peptide with a second chemical species, wherein the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope “A”, the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled “A” and the antibody, will reduce the amount of labeled A bound to the antibody.

[0060] “Specific binding partner” or “Specific binding member,” may be used interchangeable herein to refer to a member of a specific binding pair. A specific binding pair comprises two different molecules, which specifically bind to each other through chemical or physical means. Therefore, in addition to antigen and antibody specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin (or streptavidin), carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzymes and enzyme inhibitors, and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, and antibodies, including monoclonal and polyclonal antibodies as well as complexes and fragments thereof, whether isolated or recombinantly produced.

[0061] “Spike protein” or “S” protein may be used interchangeably herein to refer to one of four main structural proteins of a coronavirus. The spike protein is heavily N-linked glycosylated and utilizes an N-terminal signal sequence to gain access to the endoplasmic

reticulum (ER). Homotrimers of the virus-encoding S protein make up the distinctive spike structure on the surface of the virus. In many coronaviruses, the S protein is cleaved by a host cell furin-like protease into two separate polypeptides noted S1 and S2. S1 makes up the large receptor-binding domain (RBD) of the S protein while S2 forms the stalk of the spike molecule. For example, the SARS-CoV-2 RBD is disclosed in M. Yuan et al., *Science* 10.1126/science.abb7269 (2020). The trimeric S glycoprotein mediates attachment of the coronavirus virion to the host cell by interactions between the S protein and its receptor. In humans, angiotensin-converting enzyme 2 (ACE2) is the receptor for SARS-CoV and SARS-CoV-2.

[0062] “Statistically significant” as used herein refers to the likelihood that a relationship between two or more variables is caused by something other than random chance. Statistical hypothesis testing is used to determine whether the result of a data set is statistically significant. In statistical hypothesis testing, a statistically significant result is attained whenever the observed *p*-value of a test statistic is less than the significance level defined of the study. The *p*-value is the probability of obtaining results at least as extreme as those observed, given that the null hypothesis is true. Examples of statistical hypothesis analysis include Wilcoxon signed-rank test, t-test, Chi-Square or Fisher’s exact test. “Significant” as used herein refers to a change that has not been determined to be statistically significant (e.g., it may not have been subject to statistical hypothesis testing).

[0063] “Subject” and “patient” as used herein interchangeably refers to any vertebrate, including, but not limited to, a mammal (e.g., a bear, cow, cattle, pig, camel, llama, horse, goat, rabbit, sheep, hamster, guinea pig, cat, tiger, lion, cheetah, jaguar, bobcat, mountain lion, dog, wolf, coyote, rat, mouse, and a non-human primate (for example, a monkey, such as a cynomolgous or rhesus monkey, chimpanzee, etc.) and a human). In some embodiments, the subject may be a human, a non-human primate or a cat. In some embodiments, the subject is a human. The subject or patient may be undergoing other forms of treatment. In some embodiments, the subject is a human that may be undergoing other forms of treatment. In some embodiments, the subject is suspected to have, have had or has been exposed to a subject that has had or tested positive for infection with a coronavirus, such as a β -coronavirus (such as SARS-CoV or SARS-CoV-2). In other embodiments, the subject is completely asymptomatic and does not exhibit any symptoms of a coronavirus, such as a β -coronavirus (such as SARS-CoV or

SARS-CoV-2), and may or may not have been exposed to a subject that has or has been exposed or infected with a coronavirus, such as a β -coronavirus.

[0064] The terms “surface active agent,” “surfactant,” and “detergent” are used interchangeably herein and refer to amphipathic molecules that consist of a non-polar hydrophobic portion, usually a straight or branched hydrocarbon or fluorocarbon chain containing 8-18 carbon atoms, attached to a polar or ionic hydrophilic portion. The hydrophilic portion can be nonionic, ionic, or zwitterionic. The hydrocarbon chain interacts weakly with the water molecules in an aqueous environment, whereas the polar or ionic head group interacts strongly with water molecules via dipole or ion-dipole interactions. Based on the nature of the hydrophilic group, surfactants are classified into anionic, cationic, zwitterionic, non-ionic, and polymeric surfactants.

[0065] As used herein, a “system” refers to a plurality of real and/or abstract elements operating together for a common purpose. In some embodiments, a “system” is an integrated assemblage of hardware and/or software elements. In some embodiments, each element of the system interacts with one or more other elements and/or is related to one or more other elements. In some embodiments, a system refers to a combination of elements and software for controlling and directing methods.

[0066] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. For example, any nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those that are well known and commonly used in the art. The meaning and scope of the terms should be clear; in the event, however of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

2. Medium and methods for inactivating β -coronavirus (such as SARS-CoV or SARS-CoV-2) in a biological sample

[0067] The present disclosure relates to methods for inactivating any β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) in a biological sample (e.g., a sample obtained from a subject who may or may not exhibit signs and/or symptoms of infection and suspected of having a β -

coronavirus (e.g., SARS-CoV or SARS-CoV-2)) prior to and/or while testing the sample for the presence of a β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) antigen or antibody. In certain embodiments, the method comprises maintaining the biological sample in a medium containing at least one non-ionic surfactant. The term “medium,” as used herein (“media” being the plural form), refers to any solution (e.g., buffer or diluent) that is capable of inactivating a β -coronavirus (e.g., SARS-CoV or SARS-CoV-2). In some embodiments, the medium is particularly useful as an assay component and/or as a diluent of assay components (as defined above).

[0068] The medium desirably comprises a non-ionic surfactant that can inactivate β -coronavirus (e.g., SARS-CoV or SARS-CoV-2). Non-ionic surfactants contain uncharged, hydrophilic head groups that consist of either polyoxyethylene moieties, or glycosidic groups, as in octyl glucoside and dodecyl maltoside. Since non-ionic surfactants break lipid-lipid and lipid-protein, but not protein-protein interactions, they are considered non-denaturing. Thus, they are widely used in the isolation of membrane proteins in their biologically active form. Unlike ionic detergents, salts have minimal effect on the micellar size of non-ionic detergents (le Maire et al., *Biochim Biophys Acta*, 1508(1-2): 86-111(2000)).

[0069] Nonionic surfactants include, but are not limited to, an ethoxylated surfactant, an alcohol ethoxylated, a secondary alcohol ethoxylate, an alkyl phenol ethoxylated, a fatty acid ethoxylated, a monoalkanolamide ethoxylated, a sorbitan ester ethoxylated, a fatty amino ethoxylated, an ethylene oxide-propylene oxide copolymer, Bis(polyethylene glycol bis(imidazolyl carbonyl)), nonoxynol-9, Bis(polyethylene glycol bis(imidazolyl carbonyl)), BRIJ 35, BRIJ 56, BRIJ 72, BRIJ 76, BRIJ 92V, BRIJ 97, BRIJ 58P, CREMOPHOR, EL, decaethylene glycol monododecyl ether, N-decanoyl-N-methylglucamine, n-decyl α -D-glucopyranoside, decyl β -D-maltopyranoside, n-dodecanoyl-N-methylglucamide, n-dodecyl α -D-maltoside, n-dodecyl β -D-maltoside, n-dodecyl β -D-maltoside, heptaethylene glycol monododecyl ether, heptaethylene glycol monododecyl ether, heptaethylene glycol monotetradecyl ether, n-hexadecyl β -D-maltoside, hexaethylene glycol monododecyl ether, hexaethylene glycol monohexadecyl ether, hexaethylene glycol monooctadecyl ether, hexaethylene glycol monotetradecyl ether, igepal CA-630, igepal CA-630, Methyl-6-O-(N-heptylcarbamoyl)- α -D-glucopyranoside, nonaethylene glycol monododecyl ether, N-nonanoyl-N-methylglucamine, N-nonanoyl-N-methylglucamine, octaethylene glycol monododecyl

ether, octaethylene glycol monododecyl ether, octaethylene glycol monohexadecyl ether, octaethylene glycol monooctadecyl ether, octaethylene glycol monotetradecyl ether, octyl-beta-D-glucopyranoside, pentaethylene glycol monodecyl ether, pentaethylene glycol monododecyl ether, pentaethylene glycol monohexadecyl ether, pentaethylene glycol monoheptyl ether, pentaethylene glycol monooctadecyl ether, pentaethylene glycol monoethyl ether, polyethylene glycol diglycidyl ether, polyethylene glycol ether W-1, polyoxyethylene 10 tridecyl ether, polyoxyethylene 100 stearate, polyoxyethylene 20 isohexadecyl ether, polyoxyethylene 20 oleyl ether, polyoxyethylene 40 stearate, polyoxyethylene 50 stearate, polyoxyethylene 8 stearate, polyoxyethylene bis(imidazolyl carbonyl), polyoxyethylene 25 propylene glycol stearate, Saponin from Quillaja bark, SPAN 20, SPAN 40, SPAN 60, SPAN 65, SPAN 80, SPAN 85, tergitol type 15-S-12, tergitol type 15-S-30, tergitol type 15-S-5, tergitol type 15-S-7, tergitol Type 15-S-9, tergitol type NP-10, tergitol type NP-4, tergitol type NP-40, tergitol type NP-7, tergitol type NP-9, tergitol type TMN-10, tergitol type TMN-6, tetradecyl-beta-D-maltoside, tetraethylene glycol monodecyl ether, tetraethylene glycol monododecyl ether, tetraethylene glycol monotetradecyl ether, triethylene glycol monodecyl ether, triethylene glycol monododecyl ether, triethylene glycol monohexadecyl ether, triethylene glycol monoethyl ether, triethylene glycol monotetradecyl ether, triton CF-21, triton CF-32, triton DF-12, triton DF-16, triton GR-5M, triton QS-15, triton QS-44, triton X-100 (Polyethylene glycol *tert*-octylphenyl ether), triton X-102, triton X-15, triton X-151, triton X-200, triton X-207, TRITON X-100, TRITON X-114, TRITON X-165, TRITON X-305, TRITON X-405, TRITON X-45, TRITON X-705-70, TWEEN 20 (polyethylene glycol sorbitan monolaurate), TWEEN 21 (polyoxyethylenesorbitan monolaurate), TWEEN 40 (polyoxyethylenesorbitan monopalmitate), TWEEN 60 polyoxyethylene sorbitan monostearate), TWEEN 61 (polyoxyethylene (4) sorbitan monostearate), TWEEN 65 (polyoxyethylenesorbitan Tristearate), TWEEN 81 (polyoxyethylenesorbitan monooleate), TWEEN 85 (polyoxyethylenesorbitan Trioleate), tyloxapol, n-undecyl beta-D-glucopyranoside, semi-synthetic derivatives thereof, or combinations thereof.

[0070] In some embodiments, the non-ionic surfactant is a secondary alcohol ethoxylate, such as tergitol. Tergitol surfactants, like Tergitol-type NP-40 and other varieties, are nonionic and nonylphenol-ethoxylate based. They have a pH value of six (pH ~ 6.0) and are commercially available as detergent coupling agents. Tergitol is an emulsifier and stabilizer, and several

varieties having slightly different properties and uses are commercially available. Exemplary tergitol varieties include, but are not limited to, tergitol 15-S-12, tergitol 15-S-30, tergitol 15-S-5, tergitol type 15-S-7, tergitol 15-S-9, tergitol 15-S-40, tergitol 15-S-40 (70%), tergitol NP-10, tergitol NP-4, tergitol NP-40, tergitol NP-40 (70%), tergitol NP-7, tergitol NP-9, tergitol TMN-10, tergitol TMN-6, and tergitol TMN-100X. Tergitol surfactants can be purchased from commercial sources such as Sigma-Aldrich (St. Louis, MO) and Dow Chemical Co. (Midland, MI). In other embodiments, the non-ionic surfactant is a secondary alcohol ethoxylate, such as tergitol, and polyethylene glycol sorbitan monolaurate, polyoxyethylenesorbitan monolaurate, polyoxyethylenesorbitan monopalmitate, polyoxyethylene sorbitan monostearate, polyoxyethylene (4) sorbitan monostearate, polyoxyethylenesorbitan tristearate, polyoxyethylenesorbitan monooleate, polyoxyethylenesorbitan trioleate and any combinations thereof. In still other embodiments, the non-ionic surfactant is a secondary alcohol ethoxylate, such as tergitol, and polyethylene glycol sorbitan monolaurate (Tween 20).

[0071] The medium may contain any suitable amount of non-ionic surfactant, so long as the amount of non-ionic surfactant is sufficient to inactivate any β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) present in the sample without substantially interfering with testing the sample for the presence of a β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) antigen or antibody. In this regard, for example, the medium comprises from about 0.1% to about 1.0% (v/v) of the at least one non-ionic surfactant (e.g., about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, or about 0.9% (v/v)). In one embodiment, the medium comprises from about 0.1% to about 0.75% (v/v) of the at least one non-ionic surfactant (e.g., about 0.15%, about 0.25%, about 0.35%, about 0.45%, about 0.55%, or about 0.65% (v/v)). In other embodiments, the medium comprises from about 0.1% to about 0.5% (v/v) of the at least one non-ionic surfactant (e.g., about 0.125%, about 0.225%, about 0.325%, about 0.425%, about 0.475%, or about 0.499% (v/v)). In another embodiment, the medium comprises about 0.2% (v/v) of at least one non-ionic surfactant. In yet other embodiments, the medium comprises about 0.2 (v/v) of at least tergitol. In yet still other aspects, the medium comprises about 0.1% (v/v) of tergitol and about 0.1% (v/v) of: polyethylene glycol sorbitan monolaurate, polyoxyethylenesorbitan monolaurate, polyoxyethylenesorbitan monopalmitate, polyoxyethylene sorbitan monostearate, polyoxyethylene (4) sorbitan monostearate, polyoxyethylenesorbitan tristearate, polyoxyethylenesorbitan monooleate, or polyoxyethylenesorbitan trioleate. In yet still other

aspects, the medium comprises about 0.1% (v/v) of tergitol and about 0.1% (v/v) of polyethylene glycol sorbitan monolaurate.

[0072] Contacting and/or maintaining the sample in a medium comprising at least one non-ionic surfactant desirably is performed under conditions appropriate for, and for an amount of time sufficient for, the at least one non-ionic surfactant to inactivate any β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) in the sample. The “appropriate conditions” and “amount of time” sufficient to inactivate a virus are well understood by one of ordinary skill in the art. For example, an amount of time sufficient for virus inactivation may be at least about 10 seconds (e.g., where the medium is changed or transferred), up to years (e.g., where the medium is used for transport or storage of a sample). In this regard, an amount of time sufficient for virus inactivation may be at least about 1 minute, at least about 30 minutes, at least about one hour, at least about two hours, at least about 5 hours, at least about 10 hours, at least about 12 hours, or at least about 24 hours. In other embodiments, the amount of time sufficient for virus inactivation may be 1, 2, 3, 4, 5, or more days (e.g., 7 days, 14 days, 30 days, etc.), about 6 months, or a year or more (e.g., 2, 3, 4, or 5 years). Appropriate conditions for virus inactivation typically include standard laboratory or hospital conditions for testing a sample, and also include conventional storage conditions (e.g., where the medium is used for transport or storage of a sample). In one embodiment, appropriate conditions include room temperature. In another embodiment, appropriate conditions include incubation at 37 °C with 5% CO₂.

[0073] A virus is “inactivated” when infectivity of the virus is destroyed but immunogenicity is maintained. β -coronavirus infectivity may be destroyed or disrupted at any state of the life cycle of the virus. In this respect, the coronavirus lifecycle includes (1) virus binding and entry into cells, (2) release of viral genome, (3) translation of viral polymerase protein, (4) RNA genome replication, (5) translation of viral structural proteins, (6) viral particle (virion) assembly, and (7) exocytosis. In some embodiments, the amount of non-ionic surfactant that is added to or present in the medium desirably inactivates β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) without denaturing viral proteins or secondary structures that bind to assay reagents (e.g., antibodies) for detecting β -coronavirus (e.g., SARS-CoV or SARS-CoV-2). Inactivation of β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) may be determined by an inability of the β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) to replicate in culture. Any suitable method for measuring virus replication or infectivity known in the art may be used in the

context of the invention. Many of such methods assess viral-induced cytopathic effects (CPE) in host cells caused by lytic virions. The terms “cytopathic effect” and “cytopathogenic effect” may be used interchangeably herein to refer to structural changes in host cells that are caused by viral infection. For example, an infecting virus may cause lysis of a host cell or cell death without lysis due to an inability to reproduce. CPE may also cause changes in cell morphology, cell physiology, and biosynthetic events. CPE may be detected using a variety of techniques, including, but not limited to, qualitative methods such as light microscopy, electron microscopy, and fluorescence microscopy. CPE may also be detected using quantitative methods such as real-time cell analysis (see, e.g., Teng et al., *J. Virol. Methods*, 193(2): 364-370 (2013)), plaque assays (e.g., focus forming assays (FFAs)), and cell viability assays (e.g., colorimetric tetrazolium reduction assays, resazurin reduction assays, protease viability marker assays, and the luminogenic ATP assay). Methods for measuring virus infectivity and replication are further described in, for example, Storch, G.A., *Clinical Infectious Diseases*, Volume 31, Issue 3, pp. 739-751, September 2000; Diagnostic Virology Protocols (Stephenson, J.R., and Warnes, A. (eds.), *Methods in Molecular Biology*, 665, 2nd ed (2011)).

[0074] The medium provided by the invention may be used to collect, transport and/or dilute the biological sample prior to performing the assay itself, or, it can be used to dilute assay components when performing the assay, such as when serial dilutions of test sample or other assay components are performed according to a particular assay method. When the medium is used to collect and/or transport the biological sample, the medium desirably is designed to preserve the viability of microorganisms (e.g., virus or bacteria) during transport without allowing their multiplication. It will be appreciated that the success of detecting and diagnosing virus infections, such as β -coronavirus (e.g., SARS-CoV or SARS-CoV-2), depends largely on the quality of the specimen and the conditions under which the specimen is transported and stored before being processed in a laboratory. Thus, in some aspects, the at least one non-ionic surfactant is present in or added to a viral transport medium (VTM) or a universal transport medium (UTM). The term “viral transport medium,” as used herein, refers to a liquid medium for the transport of virus specimens to a laboratory for analysis. VTM allows for the safe transfer of viruses, chlamydia, and mycoplasma for further research, including conventional cell culture methods, diagnostic tests, and molecular biology techniques. VTM typically contain components designed to provide an isotonic solution (e.g., protective protein), antibiotics to

control microbial contamination, and one or more buffers to control the pH. In some embodiments, viral transport media may be used for transporting collection swabs or materials released into the medium from a collection swab. Liquid media may be added to other specimens when inactivation of the viral agent is likely and when the resultant dilution is acceptable.

[0075] The U.S. Centers for Disease Control and Prevention (CDC) has published a standard operating procedure (SOP) for producing VTM, and recommends that VTM contain the following components: Hanks Balanced Salt Solution (HBSS) 1X with calcium and magnesium ions, no phenol red, 500mL bottle; sterile, heat-inactivated fetal bovine serum (FBS); gentamicin sulfate (50mg/mL) (or similar antibiotic at an appropriate concentration to prevent bacterial contamination and growth); amphotericin B (250µg/mL) (Fungizone) (or similar antifungal at an appropriate concentration to prevent fungal contamination and growth); blood agar plate; and disinfectant (e.g., 70% ethanol) (Centers for Disease Control and Prevention, Preparation of Viral Transport Medium, SOP DSR-052-05; [cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf](https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf)). Commercially available transport culture media and systems also may be employed. In one embodiment, for example the medium may be a universal transport media (UTM), which is a room temperature stable viral transport medium for collection, transport, maintenance, and long-term freeze storage of viruses, such as Influenza H1N1, *Chlamydia*, *Mycoplasma* and *Ureaplasma* specimens. UTM is used in the art for rapid antigen testing, direct fluorescent antibody (DFA) testing, viral culture, and for molecular-based assays. Commercially available UTMs that may be used in connection with the inventive methods include, for example, UTM® (COPAN Diagnostics, Murrieta, CA), UNITRANZ-RT™ (Puritan Medical Products, Guilford, ME), BD universal viral transport (UVT) system (Becton, Dickinson and Company, Franklin Lakes, NJ), and Hardy Diagnostics Universal Transport Medium™ (Hardy Diagnostics, Santa Maria, CA). Other commercially available virus transport media that may be used in the methods described herein include, but are not limited to, VIROCULT® and Σ -VIROCULT® (Medical Wire & Equipment, Wiltshire, England), Eagle Minimum Essential Medium (E-MEM) (Sigma-Aldrich, St. Louis, MO), and VTM marketed by Rhino Diagnostics (see also, e.g., Hubbard et al., *International Journal of Microbiology*, Volume 2011, Article ID 46309 (2011) and Johnson, F.B., *Clinical Microbiology Reviews*, 3(2): 120-131 (1990)). Methods for producing viral transport media are described in, e.g., Smith et al., *J. Clinical Microbiology*,

58(8): e00913-20 (2020); DOI: 10.1128/JCM.00913-20. The VTM used or generated desirably conforms to the *Enforcement Policy for Viral Transport Media During the Coronavirus Disease 2019 (COVID19) Public Health Emergency*: Guidance for Commercial Manufacturers, Clinical Laboratories, and Food and Drug Administration Staff (FDA-2020-D-1138).

[0076] In other embodiments, the at least one non-ionic surfactant is present in or added to a pretreatment solution or reagent (i.e., the medium is a pretreatment solution). The terms “pretreatment solution” and “pretreatment regimen,” may be used interchangeably to refer to a solution that may lyse, solubilize, and/or precipitate an analyte present in a sample prior to testing the sample for the presence of the analyte. Pretreatment is not necessary for all samples, however. Among other things, solubilizing the analyte (e.g., β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) antigen or antibody) entails release of the analyte from any endogenous binding proteins present in the sample. A pretreatment solution may be homogeneous (not requiring a separation step) or heterogeneous (requiring a separation step). With use of a heterogeneous pretreatment solution there is removal of any precipitated analyte binding proteins from the test sample prior to proceeding to the next step of the assay. The pretreatment solution or reagent optionally may comprise: (a) one or more solvents and salt, (b) one or more solvents, salt, and detergent, (c) detergent, (d) detergent and salt, or (e) any reagent or combination of reagents appropriate for cell lysis and/or solubilization of analyte.

[0077] In some embodiments, the at least one non-ionic surfactant is present in or added to an assay-specific reagent (i.e., the medium is an assay-specific reagent). The term “assay-specific reagent” and “analyte-specific reagent,” may be used interchangeably herein to refer to a class of biological molecules which can be used to identify and measure the amount of an individual chemical substance in biological specimens. For example, assay-specific reagents may include monoclonal or polyclonal antibodies, receptors, target ligands, peptides, and other molecules or compounds that have the potential to recognize specific sequences or structural features that are unique to the analyte. Assay-specific reagents contribute to the sensitivity and selectivity of the assay.

[0078] The at least one non-ionic surfactant may be added to the medium prior to addition of a biological sample to the medium. Alternatively, the at least one non-ionic surfactant may be added to the medium after the biological sample is added to the medium. In some embodiments, the medium may comprise a plurality of different non-ionic surfactants. For example, the

medium may comprise 2, 3, 4, 5, or more different non-ionic surfactants, such as any of those described above or known in the art. In some embodiments, at least one of the plurality of non-ionic surfactants is tergitol.

3. Applications of the Method of Inactivating β -coronavirus (e.g., SARS-CoV or SARS-CoV-2)

[0079] The method of inactivating β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) in a biological sample may be used in conjunction with one or more methods for diagnosing a β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) infection, such as testing the biological sample for the presence of a β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) antigen or antibody. In some embodiments, for example, the methods described herein can be used in conjunction with clinical presentation and other laboratory tests to aid in the diagnosis of β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) infection in a subject (e.g., who may or may not exhibit signs and/or symptoms of infection and suspected of having β -coronavirus (e.g., SARS-CoV or SARS-CoV-2)). Such laboratory tests include, for example, PCR or other nucleic acid amplification-based assays (also referred to as “molecular” tests), serology or antibody assays, and antigen assays. Molecular and antigen tests are used to diagnose an active coronavirus infection, while antibody tests are used to assess a potential past coronavirus infection. Molecular tests detect the genetic material or nucleic acid present inside a virus particle. Most molecular tests employ PCR-based methods (e.g., RT-PCR), which are also referred to as nucleic acid amplification tests (NAAT). Antigen tests detect one or more specific proteins from a virus particle. Antigen tests tend to be highly specific but are typically less sensitive than molecular tests. Serology/antibody tests detect antibodies produced by a subject in response to a viral infection. With respect to SARS-CoV-2, serology/antibody tests typically detect antibodies (e.g., IgM and/or IgG antibodies) to the spike (S) protein or nucleocapsid (N) protein.

[0080] The FDA has authorized molecular tests for SARS-CoV-2 for use in clinical laboratory settings and authorized some for use in a point-of-care (POC) setting, including, for example, ID NOW™ COVID-19, REALTIME™ SARS-CoV-2 EUA, and ALINITY™ m SARS-CoV-2 assay (all marketed by Abbott Laboratories, Abbott Park, IL). All antigen tests currently authorized by the FDA are POC tests and provide results in less than an hour. An exemplary SARS-CoV-2 antigen test is BINAXNOW™ (Abbott Laboratories, Abbott Park, IL). The format of most FDA-authorized serology tests are lateral flow assays, ELISA (enzyme-

linked immunosorbent assays), or chemiluminescent immunoassays (CLIA). For example, SARS-CoV-2 antibody tests may be performed on the ARCHITECT® and/or ALINITY automated analyzers (Abbott Laboratories, Abbott Park, IL).

[0081] Other methods of detection include the use of or can be adapted for use on a nanopore device or nanowell device, e.g., for single molecule detection. Examples of nanopore devices are described in PCT International Application WO 2016/161402 and examples of nanowell devices are described in PCT International Application WO 2016/161400, both of which are hereby incorporated by reference herein. Other devices and methods appropriate for single molecule detection also can be employed.

4. Kit

[0082] A medium comprising at least about 0.1% (v/v) of at least one non-ionic surfactant as described herein may be provided in a kit, along with components which may be used in methods for assaying or assessing a biological sample for β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) infection. The kit comprises at least one component for assaying the biological sample for the presence of β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) (e.g., components for a molecular assay, an antigen assay, or an antibody assay as described above), and instructions for assaying the sample. Instructions included in a kit can be affixed to packaging material, can be included as a package insert, or can be viewed or downloaded from a particular website that is recited as part of the kit packaging or inserted materials. While the instructions are typically written or printed materials, they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this disclosure. Such media include, but are not limited to, electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. As used herein, the term “instructions” can include the address of an internet site that provides the instructions.

[0083] Alternatively or additionally, the kit can comprise a calibrator or control, e.g., purified, and optionally frozen or lyophilized, and/or at least one container (e.g., tube, microtiter plates or strips) for conducting the assay, and/or a buffer, such as an assay buffer or a wash buffer, either one of which can be provided as a concentrated solution, a substrate solution for the detectable label (e.g., an enzymatic label), or a stop solution. Preferably, the kit comprises all components, i.e., reagents, standards, buffers, diluents, etc., which are necessary to perform the assay. The instructions also can include instructions for generating a standard curve.

[0084] The kit may further comprise reference standards for quantifying β -coronavirus (e.g., SARS-CoV or SARS-CoV-2). The reference standards may be employed to establish standard curves for interpolation and/or extrapolation of β -coronavirus (e.g., SARS-CoV or SARS-CoV-2) concentration.

[0085] Optionally, the kit includes quality control components (for example, sensitivity panels, calibrators, and positive controls). Preparation of quality control reagents is well-known in the art and is described on insert sheets for a variety of immunodiagnostic products. Sensitivity panel members optionally are used to establish assay performance characteristics, and further optionally are useful indicators of the integrity of the immunoassay kit reagents, and the standardization of assays.

[0086] The kit can also optionally include other reagents required to conduct a diagnostic assay or facilitate quality control evaluations, such as buffers, salts, enzymes, enzyme co-factors, substrates, detection reagents, and the like. Other components, such as buffers and solutions for the isolation and/or treatment of a test sample (e.g., pretreatment reagents), also can be included in the kit. One or more of the components of the kit can be lyophilized, in which case the kit can further comprise reagents suitable for the reconstitution of the lyophilized components.

[0087] The various components of the kit optionally are provided in suitable containers as necessary, e.g., a microtiter plate. The kit can further include containers for holding or storing a sample (e.g., a container or cartridge for a urine, whole blood, plasma, or serum sample). Where appropriate, the kit optionally also can contain reaction vessels, mixing vessels, and other components that facilitate the preparation of reagents or the test sample. The kit can also include one or more instruments for assisting with obtaining a test sample, such as a syringe, pipette, forceps, measured spoon, or the like.

[0088] If desired, the kit can contain a solid phase, such as a magnetic particle, bead, test tube, microtiter plate, cuvette, membrane, scaffolding molecule, film, filter paper, disc, or chip.

[0089] The kit (or components thereof) and methods for detecting the presence of SARS-CoV-2 in a test sample as described herein, can be adapted for use in a variety of automated and semi-automated systems or platforms (including those wherein the solid phase comprises a microparticle), as described, e.g., U.S. Patent No. 5,063,081, U.S. Patent Application Publication Nos. 2003/0170881, 2004/0018577, 2005/0054078, and 2006/0160164 and as commercially marketed e.g., by Abbott Laboratories (Abbott Park, IL) as Abbott Point of Care (i-STAT® or i-

STAT Alinity, Abbott Laboratories) as well as those described in U.S. Patent Nos. 5,089,424 and 5,006,309, and as commercially marketed, e.g., by Abbott Laboratories (Abbott Park, IL) as ARCHITECT® or the series of Abbott Alinity devices.

[0090] Other platforms available from Abbott Laboratories include, but are not limited to, AXSYM®, IMX® (see, e.g., U.S. Patent No. 5,294,404, which is hereby incorporated by reference in its entirety), PRISM®, EIA (bead), and QUANTUM™ II, as well as other platforms. Additionally, the assays, kits, and kit components can be employed in other formats, for example, on electrochemical or other hand-held or point-of-care assay systems. As mentioned previously, the present disclosure is, for example, applicable to the commercial Abbott Point of Care (i-STAT®, Abbott Laboratories) electrochemical immunoassay system that performs sandwich immunoassays. Immunosensors and their methods of manufacture and operation in single-use test devices are described, for example in, U.S. Patent No. 5,063,081, U.S. Patent App. Publication Nos. 2003/0170881, 2004/0018577, 2005/0054078, and 2006/0160164, which are incorporated in their entireties by reference for their teachings regarding same.

[0091] The present disclosure has multiple aspects, which are illustrated by the following non-limiting examples.

5. Examples

[0092] It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the present disclosure described herein are readily applicable and appreciable, and may be made using suitable equivalents without departing from the scope of the present disclosure or the aspects and embodiments disclosed herein. Having now described the present disclosure in detail, the same will be more clearly understood by reference to the following examples, which are merely intended only to illustrate some aspects and embodiments of the disclosure and should not be viewed as limiting to the scope of the disclosure. The disclosures of all journal references, U.S. patents, and publications referred to herein are hereby incorporated by reference in their entireties.

Example 1

[0093] This example demonstrates the addition of tergitol to a transport culture medium to inactivate SARS-CoV-2.

[0094] Experiments were conducted to determine if tergitol inactivates SARS-CoV-2 (especially high titer stocks) by assessing infectivity on Vero cells and to determine the optimal tergitol exposure time for virus inactivation. On day 1, Vero cells (ATCC CCL-81) were plated overnight. A 96-well white-walled plate with clear bottom was labeled for luminescence detection. 200 µl of cells were dispensed at 1.5×10^5 cells/mL concentration into each well using a trough and multichannel pipette. On day 2, virus infection and inactivation assessment were performed. To this end, SARS-CoV-2 virus stocks maintained by Abbott Laboratories (Abbott Laboratories, Abbott Park, IL) were diluted in 2 mL screw cap vials using media lacking fetal bovine serum ('no fetal bovine serum (FBS)' media) as diluent. A separate SARS-CoV-2 lysate purchased from BEI Resources (Isolate USA-WA1/2020; Catalog No. NR-52281; Lot: 70036318, referred to in tables below as "BEI") was diluted in the same manner. 100 µl of VTM+2% (v/v) tergitol was dispensed to a 96-well dilution plate in all wells except columns 1 and 7 for non-inactivated controls. 100 µl of diluted virus was transferred to a 96-well dilution/inactivation plate, beginning with a 2-hour timepoint working backwards to 10 minutes. Virus was incubated at room temperature until infection.

[0095] Media was aspirated from cells in the luminescence plate. PBS (20 mL) was poured into the trough and 150 µl of PBS was dispensed into each well with a multichannel pipette gently down the right side. PBS was removed from the 96-well plate by aspiration using a non-filter tip on a Pasteur pipette to lessen the force of suction on cells. 200 µl of 1:1 'no FBS':VTM dilution was dispensed to each well in the cell culture plate with a multichannel pipette, gently down the right side. Once all wells were infected, the plate was incubated at 37 °C with 5% CO₂ for 2 hours. Inoculum was removed from one column at a time by aspiration, starting with the lowest concentration of virus. The non-filter tip on the Pasteur pipette was changed after each sample. 20 mL PBS was poured into the trough and 150 µl PBS was dispensed into each well with a multichannel pipette, gently down the right side. PBS was then aspirated. About 20 mL +10% FBS media was poured into the trough and 200 µl of complete media were dispensed to each well with a multichannel pipette, gently down the left side. There was no need to change tips. Once addition of media to all wells was complete, plates were placed back into an incubator at 37 °C with 5% CO₂ for 3-4 days.

[0096] Any well containing 1% (v/v) tergitol had cells that were essentially dead immediately after the 2-hour infection. Thus, detergent at this amount was toxic to cells, as the cells infected

with VTM lacking tergitol remained viable. Accordingly, the above experiments were repeated with higher dilutions of tergitol to lower the total concentration applied to cells. Using serial dilutions of VTM containing tergitol (starting concentration 2% tergitol), concentrations of 1%-0.25% tergitol immediately killed cells, but cells tolerated levels at or below 0.0156% tergitol. Using one of the Abbott SARS-CoV-2 virus stocks (“#13”) and BEI SARS-CoV-2 virus stock (both diluted to 56 μ l /1600 μ l), four different concentrations of VTM + tergitol (“inactivation formulation” or “IF”) were then tested but only plated at the 0.01% concentration on Vero cells. The formats in Tables 1 and 2 below were used:

Table 1. Dilution plate 1

	Buffer	#13 NYB	IF	IF	IF	IF	Buffer	BEI	IF	IF	IF	IF
1	No virus 1%	10 ⁸ virus 0%	10 min 1%	20 min 1%	30 min 1%	60 min 1%	No virus 1%	10 ⁸ virus 0%	10 min 1%	20 min 1%	30 min 1%	60 min 1%
2	0.1%	10 ⁷ 0%	0.1%	0.1%	0.1%	0.1%	0.1%	10 ⁷ 0%	0.1%	0.1%	0.1%	0.1%
3	0.01%	10 ⁶ 0%	0.01%	0.01%	0.01%	0.01%	0.01%	10 ⁶ 0%	0.01%	0.01%	0.01%	0.01%
4	No virus 0.5%	10 ⁸ virus 0%	10 min 0.5%	20 min 0.5%	30 min 0.5%	60 min 0.5%	No virus 0.5%	10 ⁸ virus 0%	10 min 0.5%	20 min 0.5%	30 min 0.5%	60 min 0.5%
5	0.1%	2x10 ⁷ 0%	0.1%	0.1%	0.1%	0.1%	0.1%	2x10 ⁷ 0%	0.1%	0.1%	0.1%	0.1%
6	0.01%	2x10 ⁶ 0%	0.01%	0.01%	0.01%	0.01%	0.01%	2x10 ⁶ 0%	0.01%	0.01%	0.01%	0.01%

Table 2. Dilution plate 2

	buffer	#13 NYB	IF	IF	IF	IF	Buffer	BEI	IF	IF	IF	IF
7	No virus 0.1%	10 ⁸ virus 0%	10 min 0.1%	20 min 0.1%	30 min 0.1%	60 min 0.1%	No virus 0.1%	10 ⁸ virus 0%	10 min 0.1%	20 min 0.1%	30 min 0.1%	60 min 0.1%
8	0.01%	10 ⁷ 0%	0.01%	0.01%	0.01%	0.01%	0.01%	10 ⁷ 0%	0.01%	0.01%	0.01%	0.01%
9	No virus 0.01%	10 ⁸ virus 0%	10 min 0.01%	20 min 0.01%	30 min 0.01%	60 min 0.01%	No virus 0.01%	10 ⁸ virus 0%	10 min 0.01%	20 min 0.01%	30 min 0.01%	60 min 0.01%

[0097] Three concentrations of VTM+tergitol were made (2%, 1% 0.2%, and 0.02%) and 100 µl was dispensed to four rows (rows 1, 4, 7, 9). When diluted 1:1 with virus, final inactivation concentrations were 1%, 0.5% 0.1%, and 0.01%. 1.2 mL of each buffer dilution was needed (140 µl +1.26 mL of no tergitol VTM). To be able to dilute the tergitol prior to plating, 180 µl of media (+10% FBS) was dispensed to rows 2, 3, 6, 7. Row 5 had 160 µl of media.

[0098] Virus (10⁸/100 µl) was added at the appropriate time to each of the four concentrations, starting with a 60-minute inactivation time period. For no virus, media was added.

[0099] At time zero ("t0"), 20 µl was transferred from rows 1 to 2, then from 2 to 3. At t0, 40 µl was transferred from rows 4 to 5, then 20 µl from 5 to 6. At t0, 20 µl was transferred from rows 7 to 8. Row 9 was at the correct concentration already (0.01%). Rows 3, 6, 8, 9 above were at a final concentration of 0.01% tergitol plated on Vero cells. Row 2 was plated at 0.1% to demonstrate toxicity. The 200 µl of 1:1 VTM+0.01% tergitol with media+virus was incubated with cells for 1-2 hours. Virus and detergent were removed by aspiration, washed with 150 µl of PBS, and fresh MEM+10% FBS was added to cells. Cytopathic effects (CPE) were determined microscopically. CPE was determined quantitatively using the Viral TOXGLO™ assay (Promega Corp., Madison, WI), which measures cellular ATP as a surrogate measure of host cell viability. The amount of ATP detected is directly proportional to the number of viable host cells in culture and can be used to quantify viral-induced CPE. Results are shown in **Tables 3 and 4**.

Table 3. Microscopy (-): no CPE, (+): CPE, (tox): toxicity

	buffer	#13 NYB	IF	IF	IF	IF	IF	buffer	BEI	IF	IF	IF	IF
1.0%	tox	-	tox	tox	tox	tox	tox	tox	+	tox	tox	tox	tox
0.5%	-	+	-	-	-	-	-	-	+	-	-	-	-
0.1%	-	+	+	+	-	-	-	-	+	+	+	+	-
0.01%	-	+	+	+	+	+	+	-	+	+	+	+	+

Table 4. TOXGLO™ Assay

	neg buffer	ABT pos	10 min	20 min	30 min	60 min	neg	BEI pos	10 min	20 min	30 min	60 min
1.00%	31084	82871	29481	26204	26466	32664	34019	38848	30244	28227	33557	31030
0.50%	71567	60908	74508	67383	71812	60385	75084	40801	63707	67449	60587	67965
0.10%	76982	51897	49639	42598	77728	74541	75964	42096	44954	41979	40182	78052
0.01%	80345	56530	54219	53605	53761	55255	86770	48062	42777	39444	33705	30215
Toxic	2493	52513	2365	1952	1976	2001	2531	44637	2229	1786	1607	1409
Healthy	97885	91456	94387	89798	88311	90420	87288	91212	89469	94265	88290	91558

The addition of 0.1% tergitol directly to cells resulted in very low (~2000) relative light units (RLUs) (e.g. toxic, row 5). Untreated wells (e.g. healthy, row 6) showed high RLUs (~90,000).

[0100] Dilution of the 1% tergitol for plating on cells at 0.01% final was unsuccessful, as the cells were distressed. Eventually they recovered, but they did not fill the monolayers in sufficiently, and thus RLUs were low. All wells with buffer only resulted in ~30,000 RLUs, except the wells with Abbott virus stock (ABT positive), in which 10^6 copies was insufficient to cause CPE. The BEI virus stock, diluted to the same level as ABT, exhibited a higher titer: the drop to 38,848 RLUs was caused by CPE in this case.

[0101] For 0.5% tergitol, the decrease in signal for the ABT positive virus control due to CPE was not as pronounced as in 0.1% and 0.01%; this was due to less virus present after dilution (2×10^6 genome copies (cp)/100 μ l), but this drop in RLU was much clearer with the more concentrated BEI stock. The main observation was that the addition of 0.5% tergitol for any amount of time (≥ 10 minutes) blocked CPE. In other words, there was no CPE and RLU levels for cells with virus added were the same as the negative control buffer alone, so 0.5% tergitol inactivated the virus.

[0102] For the 0.1% tergitol, the delta for the ABT and BEI positive virus controls at 10^7 cp/100 μ l compared to the negative controls was $>33\%$. The addition of 0.1% tergitol was required for at least 30 minutes for the ABT stock and at least 60 minutes for the BEI stock to inactivate COVID-19. Virus was not inactivated with shorter treatment times, and therefore CPE developed and killed cells, leading to a drop in RLUs.

[0103] For the 0.01% tergitol, the delta for the ABT and BEI positive virus controls at 10^8 cp/100 μ l compared to the negative controls was clear. The addition of 0.01% for any amount of time failed to inactivate COVID-19. All wells where virus was added produced CPE and a drop in RLUs. Data for the 0.5%, 0.1%, and 0.01% treatments are plotted in FIG. 1.

[0104] It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope, which is defined solely by the appended claims and their equivalents.

[0105] Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, compositions, formulations, or methods of use, may be made without departing from the spirit and scope thereof.

[0106] For reasons of completeness, various aspects are set out in the following numbered clauses:

[0107] Clause 1. A method of inactivating any SARS-CoV-2 in a biological sample prior to testing the sample for the presence of a SARS-CoV-2 antigen or antibody, the method comprising maintaining the sample in a medium comprising at least about 0.1% (v/v) of at least one non-ionic surfactant under conditions appropriate for and for an amount of time sufficient for the at least one non-ionic surfactant to inactivate the SARS-CoV-2 in the sample as determined by an inability of the SARS-CoV-2 to replicate in culture, without substantially interfering with testing the sample for the presence of a SARS-CoV-2 antigen or antibody.

[0108] Clause 2. The method of clause 1, wherein the non-ionic surfactant is a secondary alcohol ethoxylate.

[0109] Clause 3. The method of clause 1 or clause 2, wherein the non-ionic surfactant is tergitol.

[0110] Clause 4. The method of any one of clauses 1-3, wherein the at least one non-ionic surfactant is tergitol and polyethylene glycol sorbitan monolaurate.

[0111] Clause 5. The method of any one of clauses 1-4, wherein the medium comprises from about 0.1% to about 1.0% (v/v) non-ionic surfactant.

[0112] Clause 6. The method of clause 5, wherein the medium comprises from about 0.1% to about 0.75% (v/v) non-ionic surfactant.

[0113] Clause 7. The method of clause 6, wherein the medium comprises from about 0.1% to about 0.5% (v/v) non-ionic surfactant.

[0114] Clause 8. The method of clause 7, wherein the medium comprises about 0.2% (v/v) of the at least one non-ionic surfactant.

[0115] Clause 9. The method of clause 8, wherein the medium comprises about 0.1% (v/v) of tergitol and about 0.1% (v/v) polyethylene glycol sorbitan monolaurate.

[0116] Clause 10. The method of any one of clauses 1-9, wherein the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0117] Clause 11. The method of any one of clauses 1-10, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0118] Clause 12. The method of any one of clauses 1-11, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0119] Clause 13. The method of any one of clauses 1-12, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0120] Clause 14. The method of any one of clauses 1-13, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

[0121] Clause 15. In an improvement of a method of assaying a biological sample for the presence of a SARS-CoV-2 antigen or antibody, the improvement comprising incubating the biological sample in a medium containing at least 0.1% (v/v) of at least one non-ionic surfactant prior to assaying the biological sample for the presence of a SARS-CoV-2 antigen or antibody, wherein the at least one non-ionic surfactant inactivates the SARS-CoV-2 present in the biological sample as determined by an inability of the SARS-CoV-2 to replicate in culture.

[0122] Clause 16. In the improvement of clause 15, wherein the non-ionic surfactant is a secondary alcohol ethoxylate.

[0123] Clause 17. In the improvement of clause 15 or clause 16, wherein the non-ionic surfactant is tergitol.

[0124] Clause 18. In the improvement of any of clauses 15-17, wherein the at least one non-ionic surfactant is tergitol and polyethylene glycol sorbitan monolaurate.

[0125] Clause 19. In the improvement of any one of clauses 15-18, wherein the medium comprises from about 0.1% to about 1.0% (v/v) non-ionic surfactant.

[0126] Clause 20. In the improvement of clause 19, wherein the medium comprises from about 0.1% to about 0.75% (v/v) non-ionic surfactant.

[0127] Clause 21. In the improvement of clause 20, wherein the medium comprises from about 0.1% to about 0.5% (v/v) non-ionic surfactant.

[0128] Clause 22. In the improvement of clause 21, wherein the medium comprises about 0.2% (v/v) of the at least one non-ionic surfactant.

[0129] Clause 23. In the improvement of clause 22, wherein the medium comprises about 0.1% (v/v) tergitol and about 0.1% (v/v) polyethylene glycol sorbitan monolaurate.

[0130] Clause 24. In the improvement of any one of clauses 15-23, wherein the at least one non-ionic surfactant is: (a) present in or added to a VTM or UTM; (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0131] Clause 25. In the improvement of any one of clauses 15-24, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0132] Clause 26. In the improvement of any one of clauses 15-25, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0133] Clause 27. In the improvement of any of clauses 15-26, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0134] Clause 28. In the improvement of any of clauses 15-27, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

[0135] Clause 29. A method of inactivating any SARS-CoV-2 in a biological sample prior to testing the sample for the presence of a SARS-CoV-2 antigen or antibody, the method comprising maintaining the sample in a medium comprising at least about 0.1% (v/v) of at least one non-ionic surfactant under conditions appropriate for and for an amount of time sufficient for the at least one non-ionic surfactant to inactivate the SARS-CoV-2 in the sample as determined by an inability of the SARS-CoV-2 to replicate in culture, without substantially interfering with testing the sample for the presence of a SARS-CoV-2 antigen or antibody, wherein the non-ionic surfactant is a secondary alcohol ethoxylate.

[0136] Clause 30. The method of clause 29, wherein the secondary alcohol ethoxylate is tergitol.

[0137] Clause 31. The method of clause 29 or clause 30, wherein the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0138] Clause 32. The method of any one of clauses 29-31, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0139] Clause 33. The method of any one of clauses 29-32, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0140] Clause 34. The method of any one of clauses 29-33, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0141] Clause 35. The method of any one of clauses 29-34, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

[0142] Clause 36. A method of inactivating any SARS-CoV-2 in a biological sample prior to testing the sample for the presence of a SARS-CoV-2 antigen or antibody, the method comprising maintaining the sample in a medium comprising at least about 0.1% (v/v) of at least one non-ionic surfactant under conditions appropriate for and for an amount of time sufficient for the at least one non-ionic surfactant to inactivate the SARS-CoV-2 in the sample as determined by an inability of the SARS-CoV-2 to replicate in culture, without substantially interfering with testing the sample for the presence of a SARS-CoV-2 antigen or antibody, wherein the non-ionic surfactant is tergitol.

[0143] Clause 37. The method of clause 36, wherein the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0144] Clause 38. The method of clause 36 or clause 37, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0145] Clause 39. The method of any one of clauses 36-38, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0146] Clause 40. The method of any one of clauses 36-39, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0147] Clause 41. The method of any one of clauses 36-40, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

[0148] Clause 42. A method of inactivating any SARS-CoV-2 in a biological sample prior to testing the sample for the presence of a SARS-CoV-2 antigen or antibody, the method comprising maintaining the sample in a medium comprising at least about 0.1% (v/v) of at least one non-ionic surfactant under conditions appropriate for and for an amount of time sufficient for the at least one non-ionic surfactant to inactivate the SARS-CoV-2 in the sample as determined by an inability of the SARS-CoV-2 to replicate in culture, without substantially interfering with testing the sample for the presence of a SARS-CoV-2 antigen or antibody, wherein the non-ionic surfactant is tergitol and polyethylene glycol sorbitan monolaurate.

[0149] Clause 43. The method of clause 43, wherein the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0150] Clause 44. The method of clause 42 or clause 43, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0151] Clause 45. The method of any one of clauses 42-44, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0152] Clause 46. The method of any one of clauses 42-45, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0153] Clause 47. The method of any one of clauses 42-46, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

[0154] Clause 48. A method of inactivating any SARS-CoV-2 in a biological sample prior to testing the sample for the presence of a SARS-CoV-2 antigen or antibody, the method comprising maintaining the sample in a medium comprising from about 0.1% (v/v) to about 1.0% (v/v) of at least one non-ionic surfactant under conditions appropriate for and for an amount of time sufficient for the at least one non-ionic surfactant to inactivate the SARS-CoV-2 in the sample as determined by an inability of the SARS-CoV-2 to replicate in culture, without substantially interfering with testing the sample for the presence of a SARS-CoV-2 antigen or antibody, wherein the non-ionic surfactant is a secondary alcohol ethoxylate.

[0155] Clause 49. The method of clause 48, wherein the secondary alcohol ethoxylate is tergitol.

[0156] Clause 50. The method of clause 48 or clause 49, wherein the medium comprises from about 0.1% to about 0.75% (v/v) non-ionic surfactant.

[0157] Clause 51. The method of clause 50, wherein the medium comprises from about 0.1% to about 0.5% (v/v) non-ionic surfactant.

[0158] Clause 52. The method of clause 51, wherein the medium comprises about 0.2% (v/v) of the at least one non-ionic surfactant.

[0159] Clause 53. The method of any one of clauses 48-52, wherein the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0160] Clause 54. The method of any one of clauses 48-53, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0161] Clause 55. The method of any one of clauses 48-54, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0162] Clause 56. The method of any one of clauses 48-55, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0163] Clause 57. The method of any one of clauses 48-56, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

[0164] Clause 58. A method of inactivating any SARS-CoV-2 in a biological sample prior to testing the sample for the presence of a SARS-CoV-2 antigen or antibody, the method comprising maintaining the sample in a medium comprising from about 0.1% (v/v) to about 1.0% (v/v) of at least one non-ionic surfactant under conditions appropriate for and for an amount of time sufficient for the at least one non-ionic surfactant to inactivate the SARS-CoV-2 in the sample as determined by an inability of the SARS-CoV-2 to replicate in culture, without substantially interfering with testing the sample for the presence of a SARS-CoV-2 antigen or antibody, wherein the non-ionic surfactant is tergitol.

[0165] Clause 59. The method of clause 58, wherein the medium comprises from about 0.1% to about 0.75% (v/v) of tergitol.

[0166] Clause 60. The method of clause 59, wherein the medium comprises from about 0.1% to about 0.5% (v/v) of tergitol.

[0167] Clause 61. The method of clause 60, wherein the medium comprises about 0.2% (v/v) of tergitol.

[0168] Clause 62. The method of any one of clauses 58-61, wherein the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0169] Clause 63. The method of any one of clauses 58-62, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0170] Clause 64. The method of any one of clauses 58-63, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0171] Clause 65. The method of any one of clauses 58-64, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0172] Clause 66. The method of any one of clauses 58-65, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

[0173] Clause 67. A method of inactivating any SARS-CoV-2 in a biological sample prior to testing the sample for the presence of a SARS-CoV-2 antigen or antibody, the method comprising maintaining the sample in a medium comprising at least one non-ionic surfactant under conditions appropriate for and for an amount of time sufficient for the at least one non-ionic surfactant to inactivate the SARS-CoV-2 in the sample as determined by an inability of the SARS-CoV-2 to replicate in culture, without substantially interfering with testing the sample for the presence of a SARS-CoV-2 antigen or antibody, wherein the non-ionic surfactant is tergitol and polyethylene glycol sorbitan monolaurate and the medium comprises about 0.1% (v/v) tergitol and about 0.1% (v/v) polyethylene glycol sorbitan monolaurate.

[0174] Clause 68. The method of clause 67, wherein the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0175] Clause 69. The method of clause 67 or clause 68, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0176] Clause 70. The method of any one of clauses 67-69, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0177] Clause 71. The method of any one of clauses 67-70, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0178] Clause 72. The method of any one of clauses 67-71, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

[0179] Clause 73. In an improvement of a method of assaying a biological sample for the presence of a SARS-CoV-2 antigen or antibody, the improvement comprising incubating the biological sample in a medium containing at least 0.1% (v/v) of at least one non-ionic surfactant prior to assaying the biological sample for the presence of a SARS-CoV-2 antigen or antibody, wherein the at least one non-ionic surfactant inactivates the SARS-CoV-2 present in the biological sample as determined by an inability of the SARS-CoV-2 to replicate in culture, wherein the non-ionic surfactant is a secondary alcohol ethoxylate.

[0180] Clause 74. The improvement of clause 73, wherein the secondary alcohol ethoxylate is tergitol.

[0181] Clause 75. The improvement of clause 73 or clause 74, wherein the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0182] Clause 76. The improvement of any one of clauses 73-75, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0183] Clause 77. The improvement of any one of clauses 73-76, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0184] Clause 78. The improvement of any one of clauses 73-77, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0185] Clause 79. The improvement of any one of clauses 73-78, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

[0186] Clause 80. In an improvement of a method of assaying a biological sample for the presence of a SARS-CoV-2 antigen or antibody, the improvement comprising incubating the biological sample in a medium containing at least 0.1% (v/v) of at least one non-ionic surfactant prior to assaying the biological sample for the presence of a SARS-CoV-2 antigen or antibody, wherein the at least one non-ionic surfactant inactivates the SARS-CoV-2 present in the

biological sample as determined by an inability of the SARS-CoV-2 to replicate in culture, wherein the non-ionic surfactant is tergitol.

[0187] Clause 81. The improvement of clause 80, wherein the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0188] Clause 82. The improvement of clause 80 or clause 81, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0189] Clause 83. The improvement of any of clauses 80-82, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0190] Clause 84. The improvement of any of clauses 80-83, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0191] Clause 85. The improvement of any of clauses 80-84, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

[0192] Clause 86. In an improvement of a method of assaying a biological sample for the presence of a SARS-CoV-2 antigen or antibody, the improvement comprising incubating the biological sample in a medium containing at least 0.1% (v/v) of at least one non-ionic surfactant prior to assaying the biological sample for the presence of a SARS-CoV-2 antigen or antibody, wherein the at least one non-ionic surfactant inactivates the SARS-CoV-2 present in the biological sample as determined by an inability of the SARS-CoV-2 to replicate in culture, wherein the non-ionic surfactant is tergitol and polyethylene glycol sorbitan monolaurate

[0193] Clause 87. The improvement of clause 86, wherein the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0194] Clause 88. The improvement of clause 86 or clause 87, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0195] Clause 89. The improvement of any of clauses 86-88, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0196] Clause 90. The improvement of any of clauses 86-89, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0197] Clause 91. The improvement of any of clauses 86-90, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

[0198] Clause 92. In an improvement of a method of assaying a biological sample for the presence of a SARS-CoV-2 antigen or antibody, the improvement comprising incubating the biological sample in a medium containing from about 0.1% (v/v) to about 1.0% (v/v) of at least one non-ionic surfactant prior to assaying the biological sample for the presence of a SARS-CoV-2 antigen or antibody, wherein the at least one non-ionic surfactant inactivates the SARS-CoV-2 present in the biological sample as determined by an inability of the SARS-CoV-2 to replicate in culture, wherein the non-ionic surfactant is a secondary alcohol ethoxylate.

[0199] Clause 93. The improvement of clause 92, wherein the secondary alcohol ethoxylate is tergitol.

[0200] Clause 94. The improvement of clause 91 or clause 92, wherein the medium comprises from about 0.1% to about 0.75% (v/v) non-ionic surfactant.

[0201] Clause 95. The improvement of clause 94, wherein the medium comprises from about 0.1% to about 0.5% (v/v) non-ionic surfactant.

[0202] Clause 96. The improvement of clause 95, wherein the medium comprises about 0.2% (v/v) of the at least one non-ionic surfactant.

[0203] Clause 97. The improvement of any one of clauses 91-96, wherein the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0204] Clause 98. The improvement of any one of clauses 91-97, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0205] Clause 99. The improvement of any one of clauses 91-98, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0206] Clause 100. The improvement of any one of clauses 91-99, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0207] Clause 101. The improvement of any one of clauses 91-100, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

[0208] Clause 102. In an improvement of a method of assaying a biological sample for the presence of a SARS-CoV-2 antigen or antibody, the improvement comprising incubating the biological sample in a medium containing from about 0.1% (v/v) to about 1.0% (v/v) of at least one non-ionic surfactant prior to assaying the biological sample for the presence of a SARS-CoV-2 antigen or antibody, wherein the at least one non-ionic surfactant inactivates the SARS-CoV-2 present in the biological sample as determined by an inability of the SARS-CoV-2 to replicate in culture, wherein the non-ionic surfactant is tergitol.

[0209] Clause 103. The improvement of clause 102, wherein the medium comprises from about 0.1% to about 0.75% (v/v) of tergitol.

[0210] Clause 104. The improvement of clause 103, wherein the medium comprises from about 0.1% to about 0.5% (v/v) of tergitol.

[0211] Clause 105. The improvement of clause 104, wherein the medium comprises about 0.2% (v/v) of tergitol.

[0212] Clause 106. In the improvement of any one of clauses 102-105, wherein the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0213] Clause 107. The improvement of any one of clauses 102-106, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0214] Clause 108. The improvement of any one of clauses 102-107, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0215] Clause 109. The improvement of any one of clauses 102-108, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0216] Clause 110. The improvement of any one of clauses 102-109, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

[0217] Clause 111. In an improvement of a method of assaying a biological sample for the presence of a SARS-CoV-2 antigen or antibody, the improvement comprising incubating the biological sample in a medium containing at least one non-ionic surfactant prior to assaying the biological sample for the presence of a SARS-CoV-2 antigen or antibody, wherein the at least one non-ionic surfactant inactivates the SARS-CoV-2 present in the biological sample as determined by an inability of the SARS-CoV-2 to replicate in culture, wherein the non-ionic surfactant is tergitol and polyethylene glycol sorbitan monolaurate and the medium comprises about 0.1% (v/v) tergitol and about 0.1% (v/v) polyethylene glycol sorbitan monolaurate.

[0218] Clause 112. The improvement of clause 111, wherein the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

[0219] Clause 113. The improvement of any one of clause 111 or clause 112, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

[0220] Clause 114. The improvement of any one of clauses 111-113, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

[0221] Clause 115. The improvement of any one of clauses 111-114, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

[0222] Clause 116. The improvement of any one of the clauses of 111-115, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

What is claimed is:

1. A method of inactivating any SARS-CoV-2 in a biological sample prior to testing the sample for the presence of a SARS-CoV-2 antigen or antibody, the method comprising maintaining the sample in a medium comprising at least about 0.1% (v/v) of at least one non-ionic surfactant under conditions appropriate for and for an amount of time sufficient for the at least one non-ionic surfactant to inactivate the SARS-CoV-2 in the sample as determined by an inability of the SARS-CoV-2 to replicate in culture, without substantially interfering with testing the sample for the presence of a SARS-CoV-2 antigen or antibody.
2. The method of claim 1, wherein the at least one non-ionic surfactant is a secondary alcohol ethoxylate.
3. The method of claim 1 or claim 2, wherein the at least one non-ionic surfactant is tergitol.
4. The method of any one of claims 1-3, wherein the at least one non-ionic surfactant is tergitol and polyethylene glycol sorbitan monolaurate.
5. The method of any one of claims 1-4, wherein the medium comprises from about 0.1% to about 1.0% (v/v) of the at least one non-ionic surfactant.
6. The method of claim 5, wherein the medium comprises from about 0.1% to about 0.75% (v/v) of the at least one non-ionic surfactant.
7. The method of claim 6, wherein the medium comprises from about 0.1% to about 0.5% (v/v) of the at least one non-ionic surfactant.
8. The method of claim 7, wherein the medium comprises about 0.2% (v/v) of the at least one non-ionic surfactant.
9. The method of claim 8, wherein the medium comprises about 0.1% (v/v) tergitol and about 0.1% (v/v) polyethylene glycol sorbitan monolaurate.

10. The method of any one of claim 1-9, wherein the at least one non-ionic surfactant is: (a) present in or added to a viral transport medium (VTM) or universal transport medium (UTM); (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

11. The method of any one of claims 1-10, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, an anal swab specimen, or a nasal mucus specimen.

12. The method of any one of claims 1-11, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

13. The method of any one of claims 1-12, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of care assay.

14. The method of any one of claims 1-13, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

15. In an improvement of a method of testing a biological sample for the presence of a SARS-CoV-2 antigen or antibody,

the improvement comprising maintaining the sample in a medium comprising at least about 0.1% v/v of at least one non-ionic surfactant prior to testing the sample for the presence of a SARS-CoV-2 antigen or antibody,

wherein the sample is maintained under conditions appropriate for and for an amount of time sufficient for the at least one non-ionic surfactant to inactivate any SARS-CoV-2 present in the sample as determined by an inability of the SARS-CoV-2 to replicate in culture, without substantially interfering with testing the sample for the presence of a SARS-CoV-2 antigen or antibody.

16. In the improvement of claim 15, wherein the at least one non-ionic surfactant is a secondary alcohol ethoxylate.

17. In the improvement of claim 15 or claim 16, wherein the at least one non-ionic surfactant is tergitol.

18. In the improvement of any of claims 15-17, wherein the at least one non-ionic surfactant is tergitol and polyethylene glycol sorbitan monolaurate.

19. In the improvement of any one of claims 15-18, wherein the medium comprises from about 0.1% to about 1.0% (v/v) of the at least one non-ionic surfactant.

20. In the improvement of claim 19, wherein the medium comprises from about 0.1% to about 0.75% (v/v) of the at least one non-ionic surfactant.

21. In the improvement of claim 20, wherein the medium comprises from about 0.1% to about 0.5% (v/v) of the at least one non-ionic surfactant.

22. In the improvement of claim 21, wherein the medium comprises about 0.2% (v/v) of the at least one non-ionic surfactant.

23. In the improvement of claim 22, wherein the medium comprises about 0.1% (v/v) tergitol and about 0.1% (v/v) polyethylene glycol sorbitan monolaurate.

24. In the improvement of any one of claims 15-23, wherein the at least one non-ionic surfactant is: (a) present in or added to a VTM or UTM; (b) present in or added to a pretreatment solution; (c) present in or added to an assay-specific reagent; or (d) any combination of (a) to (c).

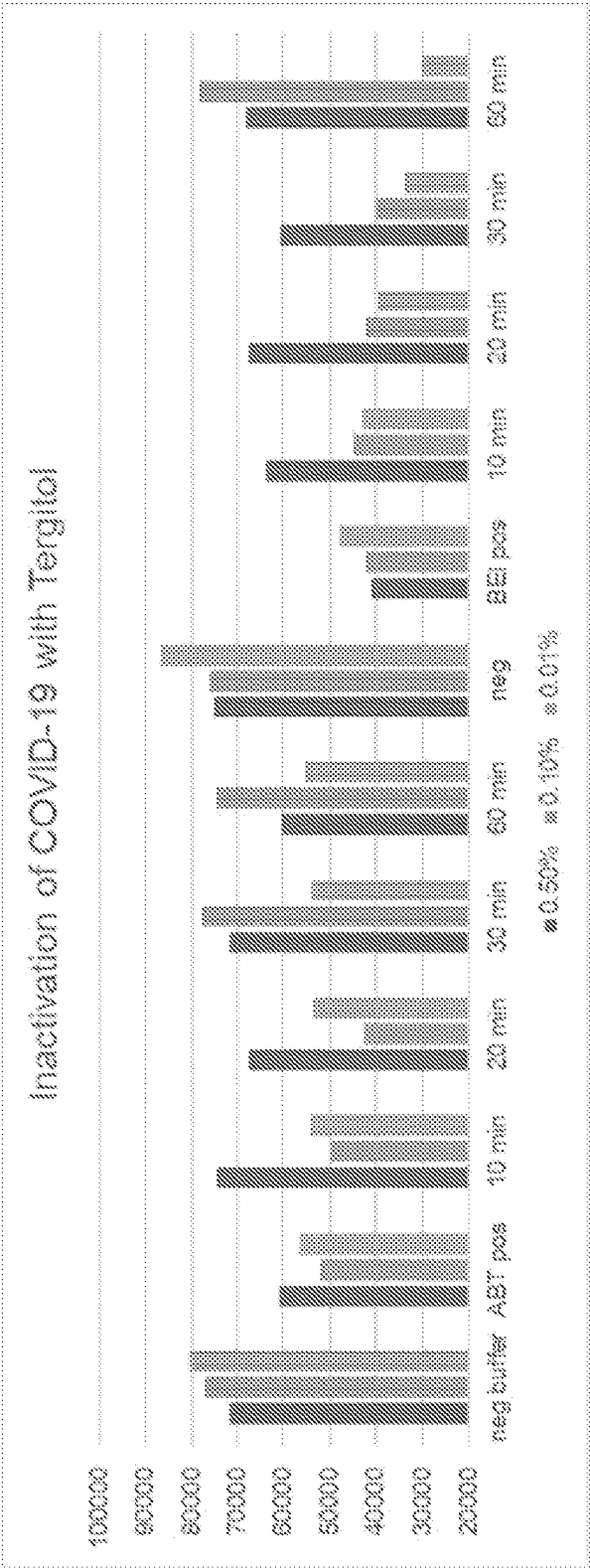
25. In the improvement of any one of claims 15-24, wherein the sample comprises saliva, an oropharyngeal specimen, a nasopharyngeal specimen, or an anal swab specimen, or a nasal mucus specimen.

26. In the improvement of any one of claims 15-25, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using an immunoassay or a clinical chemistry assay.

27. In the improvement of any one of claims 15-26, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is performed using single molecule detection, a lateral flow assay, or a point-of-care assay.

28. In the improvement of any one of claims 15-27, wherein testing the sample for the presence of a SARS-CoV-2 antigen or antibody is adapted for use in an automated system or a semi-automated system.

FIG. 1



INTERNATIONAL SEARCH REPORT

International application No

PCT/US2021/065581

A. CLASSIFICATION OF SUBJECT MATTER**INV. G01N33/569****ADD.**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LIIS HALJASMÄGI ET AL: "LIPS method for the detection of SARS-CoV-2 antibodies to spike and nucleocapsid proteins", EUROPEAN JOURNAL OF IMMUNOLOGY, WILEY-VCH, HOBOKEN, USA, vol. 50, no. 8, 6 July 2020 (2020-07-06), pages 1234-1236, XP071228668, ISSN: 0014-2980, DOI: 10.1002/EJI.202048715	1, 2, 5, 10-16, 19, 24-28
Y	abstract ; Fig 1 ; pg 1236, col 1, para 1	8, 9, 22, 23
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Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

21 March 2022

Date of mailing of the international search report

30/03/2022

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2021/065581

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WELCH STEPHEN R. ET AL: "Analysis of Inactivation of SARS-CoV-2 by Specimen Transport Media, Nucleic Acid Extraction Reagents, Detergents, and Fixatives", JOURNAL OF CLINICAL MICROBIOLOGY, vol. 58, no. 11, 21 October 2020 (2020-10-21), XP055902926, US ISSN: 0095-1137, DOI: 10.1128/JCM.01713-20	1-5, 10-12, 14-19, 24-26,28
Y	abstract ; Table 1, 4 ; Fig 1 ; pg 8, para 1 -----	8, 9,22, 23
X	PATTERSON EDWARD I ET AL: "Methods of Inactivation of SARS-CoV-2 for Downstream Biological Assays", JOURNAL OF INFECTIOUS DISEASES, vol. 222, no. 9, 1 October 2020 (2020-10-01), pages 1462-1467, XP055903105, US ISSN: 0022-1899, DOI: 10.1093/infdis/jiaa507 Retrieved from the Internet: URL:https://academic.oup.com/jid/article-pdf/222/9/1462/33828459/jiaa507.pdf>	1-7, 10-12, 14-21, 24-26,28
Y	abstract ; pg 1463, col 1, para bridging col 2 ; pg 1464, col 2, para bridging pg 1465 ; pg 1466, col 1, para 1 ; Fig 1 -----	8, 9,22, 23
Y	HIROYUKI KOGAKI ET AL: "Novel rapid immunochromatographic test based on an enzyme immunoassay for detecting nucleocapsid antigen in SARS-associated coronavirus", JOURNAL OF CLINICAL LABORATORY ANALYSIS., vol. 19, no. 4, 1 January 2005 (2005-01-01), pages 150-159, XP055716274, US ISSN: 0887-8013, DOI: 10.1002/jcla.20070 abstract ; pg 154, col 1, para 2 -----	8, 9,22, 23
X,P	CN 113 755 556 A (UNKNOWN) 7 December 2021 (2021-12-07) examples 1-5 ----- -/--	1-8, 10-12, 14-22, 24-26,28

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2021/065581

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	LIU DAN ET AL: "Comparative research on nucleocapsid and spike glycoprotein as the rapid immunodetection targets of COVID-19 and establishment of immunoassay strips", MOLECULAR IMMUNOLOGY, PERGAMON, GB, vol. 131, 9 January 2021 (2021-01-09), pages 6-12, XP086484723, ISSN: 0161-5890, DOI: 10.1016/J.MOLIMM.2021.01.005 [retrieved on 2021-01-09] abstract ; pg 8, para 2.3.6 -----	1-28

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2021/065581

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 113755556	A	07-12-2021	NONE
