FORM 2

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PROVISIONAL SPECIFICATION
(See Section 10 and Rule 13)

COVID-19 NEUTRALIZING ANTIBODY DETECTION TEST KIT AND METHODS OF USE

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The following specification particularly describes the invention and the manner in which it is to be performed

COVID-19 NEUTRALIZING ANTIBODY DETECTION TEST KIT AND METHODS OF USE

RELATED APPLICATION

This application is being filed as a Provisional Application with the Indian Patent Office.

FIELD OF THE INVENTION

The present invention relates to a test kit for detecting the presence of neutralizing antibodies to COVID-19 virus in a subject and a method for using the same.

BACKGROUND OF THE INVENTION

Covid-19 first emerged in December 2019 in Wuhan, China. The cause of the disease was confirmed as a new kind of coronavirus, this virus is transmitted through air from people to people. The infection has quickly spread in China and to a number of countries around the world.

Covid-19 virus causes over 2% mortality, there is 7 to 14 days incubation period. The current primary diagnostic method for the presence of virus is majorly by RT-PCR of the viral RNA. There also are tests to detect the virus through what are called “antigen tests”. Antigen tests detect one or more of the proteins in the viral particle. Both RT-PCR and viral antigen tests are currently used widely in multiple formats and several variations of the two test themes. The antigen tests primarily use a test that detects the viral NP protein (Nucleoprotein), due to its abundance (1,000 copies per virion). The surface glycoprotein (S protein) of the virus is also used to detect the virus, albeit, less desirable due to decreased sensitivity, since ~100 copies are present in each virion particle. Alternatively, researchers have developed methods to detect antibodies in persons who have been infected, as a measure of past exposure.

Currently, with the introduction of vaccines, large numbers of asymptomatic infected individuals and those who have recovered from infection, it is important to learn if these persons have mounted an immune response against the virus and have antibodies that neutralize the virus. Essentially, the questions that warrants an answer are; “Is the person responsive to vaccine”, “Is the individual protected from vaccine or prior infection”? If so, “for how long?”

With the recent discovery that presence of neutralizing antibodies is key to protection, there is an urgent need to develop a simple and fast test to screen large population and asymptomatic and symptomatic and previously infected people.

The viral coat primarily consists of S protein, the M (membrane) protein and E (envelope protein). The S protein embedded in the viral membrane which is represented by about 100 copies per virion is responsible for initial interactions with the ACE-2 (Angiotensin Converting Enzyme) on the human cell surface (viral receptor). The interaction between the S protein and ACE-2 occurs through the RBD (Receptor Binding Domain) of the S protein, and disruption of this interaction is crucial to preventing viral entry, infection and disease severity. Presence of antibodies to the RBD is therefore virus neutralizing and protective. This was borne out in a recent publication demonstrating the correlate of protection being the virus neutralizing antibodies against the RBD of the virus (McMahan *et al.* Nature, December 4, 2020. https://doi.org/101038/s41586-020-03041-6).

Infection or vaccination typically produces specific IgM antibody initially, detectable at 2 to 4 days after the infection or vaccination, while specific IgG antibody appears after 5 to 7 days. Compared to other test, this test is more convenient with low cost, it can be performed in field, mobile hospital and laboratory. IgM is the first type of antibody made in the body as the first response to an infection by the immune system. It is the largest antibody found in the body and less abundant (5 to 10%) than the other antibodies. IgM exists as a pentamer consisting of identical heavy and light chains. There are ten antigen binding sites for the IgM. However, due to the conformational constraints of the IgM, only five sites are available for antigen binding. While IgM is somewhat selective, its selectivity is not sufficient enough to be protective, however, its presence indicates beginnings of an immune response in an individual. IgG is another type of antibody produced by white blood cells and are found in all body fluids. It is the most abundant antibody found in blood (80%). IgGs are produced at later stages of the infection and remain in the body for longer periods to fight against repeated infections. IgGs exist as monomers with two antigen binding sites for each antibody. IgG is more specific or selective to a particular antigen compared to IgM, and affords protection from infection in vaccinees and those previously infected.

Therefore, there is a need for a more selective and sensitive test for detecting the presence of neutralizing antibodies against COVID-19 virus in a subject.

BRIEF DESCRIPTION OF FIGURES

Figure 1. Concept 1: Detection of RBD binding Human IgG in blood (Immobilized RBD).

Figure 2. Concept 2: Detection of RBD binding Human IgG in blood (anti-human IgG, IgY or IgY against IgG-Fc).

Figure 3. Concept 2: Detection of RBD binding Human IgG in blood (nanobody, nanobody against IgG-Fc).

DESCRIPTION OF THE INVENTION

The present invention provides a test kit for determining the presence of COVID-19 RBD antibodies in a subject.
The method includes analyzing the sample that is obtained from the subject using a test kit comprising a COVID-19 RBD and anti-human IgG and/or anti-human IgM to determine a possible presence of COVID-19 RBD antibody.

The invention as described herein provides a method for detecting the presence of antibodies against COVID-19 virus in a subject is conducted as follows. A sample is obtained from a subject to be tested for the presence of antibodies against COVID-19 RBD. The sample from the subject as described herein includes but not limited to serum, blood, plasma or a mixture thereof.

The method includes analyzing the sample that is obtained from the subject using a test kit comprising a COVID-19 RBD and anti-human IgG and/or anti-human IgM to determine a possible presence of COVID-19 RBD antibody.

The concepts of the invention are provided in Figure 1, Figure 2 and Figure 3 and are described in the foregoing sections.

In a key aspect, invention provides a method for detecting the presence of neutralizing antibodies against COVID-19 virus in a subject. The method includes:

1.1. analyzing a sample obtained from said subject using COVID-19 RBD (“RBD”) and anti-human IgG and/or anti-human IgM to determine a possible presence of COVID-19 RBD

antibody; and the said analysis indicates a possible presence of COVID-19 RBD antibody, wherein the presence of neutralizing antibodies against COVID-19 RBD is indication that the said subject has neutralizing antibodies and is protected from infection.

1.2. analyzing for the presence of neutralizing antibodies against COVID-19 RBD as provided in step 1.1.

1.3. the steps of 1.1 and/or 1.2 include certain subdomains and peptides of RBD that are critical for ACE-2 binding.

In particular, the test includes determining the presence of antibodies to peptides derived from RBD or to RBD of COVID-19 comprising amino sequence including but not limited to SEQ ID NO. 1 represented by:

HMASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIR
GDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNL
KPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAP
ATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDP
QTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTG
SNVFQTLE

The diagnostic test is carried out in a direct or indirect ELISA or using a dot blot. In another aspect, the invention provides steps for analyzing for the presence of neutralizing antibodies against COVID-19 RBD by LFA (lateral Flow assay) carried out as follows:

- applying said sample to a lateral flow device to flow laterally towards a distal end of the lateral flow device, wherein the lateral flow device sequentially comprises:
    - a filter pad;
    - a conjugate pad, comprising a mixture of gold colloid having anti-human IgG antibody attached thereto to gold colloid having biotin or streptavidin attached thereto;
    - a sample pad; and
    - a membrane comprising:
    - a test region, wherein one test region comprises RBD, and a control region, comprising streptavidin/biotin-bovine serum albumin;

- detecting any binding at the test region and the control region,
- wherein binding at the test region and the control region is indication that antibodies against COVID-19 RBD is present in said sample.

The detection of signal at the test site with anti-human IgG is indicative of neutralizing antibodies in the subject.

In another aspect there is one or two test regions may be available wherein one test region comprises anti-human IgG and the other test region comprises anti-human IgM, and a control region, comprises streptavidin or biotin linked to bovine serum albumin; and the detection and binding occurs at both the test regions and the control region. The detection of signal at the test site with anti-human IgG is indicative of neutralizing antibodies in the subject.
In another aspect, the RBD or its peptide thereof is isolated from a virus and recombinantly expressed in mammalian, insect or bacterial cell.
Furthermore, it is provided that, biotin is attached to the RBD and streptavidin to the bovine serum albumin.
The invention also provided that for anti-human IgM is substituted with anti-human IgG-Fc fragment or a portion thereof.

In another aspect, anti-human IgM is substituted with antibody to human IgM-Fc fragment or a portion thereof.

Furthermore, the anti-IgG is any IgG from a mammal (goat, sheep, rat, mouse, donkey, horse etc.) or is an IgY from avian (chicken, goose, ostrich etc.) or is a nanobody (NB) from camel or llama or a monoclonal or polyclonal antibody derived from a mammal, avian, camel or llama.

In another embodiment the anti IgM is an IgG from a mammal (goat, sheep, rat, mouse, donkey, horse etc.) or is an IgY from avian (chicken, goose, ostrich etc.) or is a nanobody (NB) from camel or llama or a monoclonal or polyclonal antibody derived from a mammal or avian or camel or llama.

In another aspect, the assays described in the present disclosure detect anti-RBD antibodies in serum, blood, plasma or a mixture thereof obtained from the said subject requiring the detection.

In another aspect, a kit of the invention includes the assay elements described above with a needle to prick the finger, a buffer solution to facilitate migration of the sample to the test and

control windows and a capillary to facilitate application of the sample from the subject to the sample window.

Additional objects, advantages, and novel features of this invention will become apparent to those skilled in the art.

The invention in brief provides the following:

- A method for detecting the presence of antibodies against COVID-19 RBD in a subject, said method comprising:

  analyzing a sample obtained from said subject using a COVID-19 RBD and anti-human IgG and anti-human IgM to determine a possible presence of COVID-19 RBD antibody; and wherein the presence of COVID-19 RBD antibody is indication that neutralizing antibody to COVID-19 is present in said subject.

The above method of claim 1, wherein RBD of COVID-19 S-protein comprise amino acid sequence provided belowas SEQ ID NO: 1:

HMASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQ
IAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIY
QAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNK
CVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPG
TNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTLE

- The protein sequence of RBD of COVID-19 S-protein is a subset of protein sequences that are neutralizing epitope of COVID-19 S-protein

- The method detecting the presence of antibodies against COVID-19 RBD in a subject wherein said steps comprise analyzing for the presence of COVID-19 RBD antibody by following the steps as below:

  (i) applying said sample to a lateral flow device to flow laterally towards a distal end of the lateral flow device, wherein the lateral flow device sequentially comprising:

  (a) a filter pad;

  (b) a conjugate pad, comprising a mixture of gold colloid having COVID-19 RBD attached thereto and gold colloid having biotin or streptavidin attached thereto;

(c) a sample pad; and

(d) a membrane comprising:

One or two test regions, wherein one test region comprises anti-human IgG and the other test region comprises anti-human IgM, and

a control region, comprising streptavidin or biotin-bovine serum antigen;

(ii) detecting any binding at both of the test regions and the control region, wherein binding at any test region and the control region is indication that antibody against COVID-19 RBD is present in said sample.

- The method detecting the presence of antibodies against COVID-19 RBD in a subject wherein said steps comprise analyzing for the presence of COVID-19 RBD antibody by following the steps as below:

(i) applying said sample to a lateral flow device to flow laterally towards a distal end of the lateral flow device, wherein the lateral flow device sequentially comprising:

(a) a filter pad;

(b) a conjugate pad, comprising a mixture of gold colloid having anti-human IgG attached thereto and gold colloid having streptavidin or biotin attached thereto;

(c) a sample pad; and

(d) a membrane comprising:

One test region, wherein one test region comprises RBD, and

a control region, comprising streptavidin or biotin-bovine serum antigen;

(ii) detecting any binding at both of the test regions and the control region, wherein binding at any test region and the control region is indication that antibody against COVID-19 RBD is present in said sample.

- The methods described herein, wherein anti-human IgG is an IgY polyclonal antibody.
- The methods described herein, wherein anti-human IgG is an IgY monoclonal antibody.
- The methods described herein, wherein anti-human IgG is nanobody (NB) polyclonal antibody.

- The methods described herein, wherein anti-human IgG is a nanobody (NB) monoclonal antibody.
- The methods described herein, where anti-human IgG is a Fab fragment of IgG, IgY or nanobody (NB) antibody.
- The methods described herein, where anti-human IgM is an IgY polyclonal antibody.
- The methods described herein, where anti-human IgM is an IgY monoclonal antibody.
- The methods described herein, where anti-human IgM is nanobody (NB) polyclonal antibody.
- The methods described herein, where anti-human IgM is a nanobody (NB) monoclonal antibody.
- The methods described herein, where anti-human IgM is a Fab fragment of IgG, IgY or nanobody (NB) antibody.
- The methods described herein, where anti-human IgG is an IgY polyclonal antibody.
- The methods described herein, where anti-human IgG is an IgY monoclonal antibody.
- The methods described herein, where anti-human IgG is nanobody (NB) polyclonal antibody.
- The methods described herein, where anti-human IgG is a nanobody (NB) monoclonal antibody.
- The methods described herein, where anti-human IgG is a Fab fragment of IgG, IgY or nanobody antibody.
- The methods described herein, where anti-human IgM is an IgY polyclonal antibody
- The methods described herein, where anti-human IgM is an IgY monoclonal antibody
- The methods described herein, where anti-human IgM is nanobody polyclonal antibody
- The methods described herein, where anti-human IgM is a nanobody (NB) monoclonal antibody

- The methods described herein, where anti-human IgM is a Fab fragment of IgG, IgY or nanobody (NB) antibody.

The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, e.g., as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter. All references cited herein are incorporated by reference in their entirety.

Dated 10th day of December 2020

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CLAIMS

We Claim:

1. A method for detecting the presence of antibodies against COVID-19 RBD in a subject, said method comprising:

analyzing a sample obtained from said subject using a COVID-19 RBD and anti-human IgG and anti-human IgM to determine a possible presence of COVID-19 RBD antibody; and wherein the presence of COVID-19 RBD antibody is indication that neutralizing antibody to COVID-19 is present in said subject.

2. The method of claim 1, wherein RBD of COVID-19 S-protein comprise amino sequence of SEQ ID NO. 1:

HMASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQ
IAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIY
QAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNK
CVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPG
TNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTLE (SEQ ID NO.1)

3. The method of claim 2 wherein the protein sequence is a subset of protein sequences that are neutralizing epitope of COVID-19 S-protein.

4. The method of claim 1, wherein said step of analyzing for the presence of COVID-19 RBD antibody comprises the steps of:

(i) applying said sample to a lateral flow device to flow laterally towards a distal end of the lateral flow device, wherein the lateral flow device sequentially comprising:

(a) a filter pad;

(b) a conjugate pad, comprising a mixture of gold colloid having COVID-19 RBD attached thereto and gold colloid having biotin or streptavidin attached thereto;

(c) a sample pad; and

(d) a membrane comprising:

One or two test regions, wherein one test region comprises anti-human IgG and the other test region comprises anti-human IgM, and

a control region, comprising streptavidin or biotin-bovine serum antigen;

(ii) detecting any binding at both of the test regions and the control region,

wherein binding at any test region and the control region is indication that antibody against COVID-19 RBD is present in said sample.

5. The method of claim 1, wherein said step of analyzing for the presence of COVID-19 RBD antibody comprises the steps of:

(i) applying said sample to a lateral flow device to flow laterally towards a distal end of the lateral flow device, wherein the lateral flow device sequentially comprising:

(a) a filter pad;

(b) a conjugate pad, comprising a mixture of gold colloid having anti-human IgG attached thereto and gold colloid having streptavidin or biotin attached thereto;

(c) a sample pad; and

(d) a membrane comprising:

One test region, wherein one test region comprises RBD, and

a control region, comprising streptavidin or biotin-bovine serum antigen;

(ii) detecting any binding at both of the test regions and the control region,

wherein binding at any test region and the control region is indication that antibody against COVID-19 RBD is present in said sample.

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COVID-19 NEUTRALIZING ANTIBODY DETECTION TEST KIT AND METHODS OF USE

ABSTRACT

The invention relates to several methods of detection of neutralizing and RBD binding antibodies in patient samples as a method to understand the patient's ability to fight the disease, success of a vaccine and in selection of patients for vaccination.

Dated 10th day of December 2020

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