Manual: COVID19 IgG/IgM/IgA ELISA Kit

Product CV5003

INTENDED USE

LifeSensors' COVID-19 IgG/IgM/IgA ELISA Assay is a plate-based assay intended for the qualitative detection of IgG, IgM and IgA antibodies to SARS-CoV-2 in serum and plasma from patients suspected of COVID-19 infection by a healthcare provider`. The COVID-19 IgG/IgM/IgA ELISA Assay is a companion piece in the diagnosis of patients with suspected SARS-CoV-2 infection along with clinical symptoms and the results of other laboratory tests. Results from the COVID-19 IgG/IgM/IgA ELISA Assay should not be used as the only source of COVID-19 diagnosis.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. 263a, to perform moderate and high complexity tests. Results are for the detection of SARS-CoV-2 antibodies. IgM antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although levels over the course of infection are not well characterized. IgG antibodies to SARS-CoV-2 become detectable later following infection. Positive results for IgG, IgM and for IgA could occur after infection and can be indicative of acute or recent infection. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities. A CLIA categorization of this assay would be consistent with other high complexity serology immunoassays.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Antibodies may not be detected in the first few days of infection; the sensitivity of the COVID-19 IgG/IgM/IgA ELISA Test in early infection phase is unknown.

False positive results for IgG, IgM and IgA antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes. At this time, it is unknown for how long IgG, IgM, or IgA antibodies may persist following infection.

For research use only.

BACKGROUND

Coronaviruses represent a family of viruses grouped together based on their crown-like appearance by electron microscopy. Coronaviruses are the pathogenic agents for different illnesses such as the common cold, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). In 2019, a new coronavirus, coronavirus 2 (SARS-CoV-2) was identified as the cause of a disease outbreak that originated in China causing severe acute respiratory syndrome. In March 2020, the World Health Organization (WHO) declared the COVID-19 outbreak a pandemic. Coronavirus disease (CoVID-19) is highly infectious disease primarily transmitted through direct contact with an infected person via droplets from sneezing or coughing.

Structurally, coronaviruses are spherically enveloped particles containing single-stranded (positive-sense) RNA associated with a nucleoprotein (N protein). The resulting helical nucleocapsid is anchored to the membrane protein. The viral envelope bears club-shaped glycoprotein projections in the form of S glycoproteins, which play a crucial role in the infection process of the host cell. Following the attachment of the viral S protein to host receptors, the virus enters the host cell via endocytosis. Once the Fusion of virus membrane with the endosomal membrane occurs, viral ssRNA(+) is released into the cytoplasm. The viral RNA hijacks the protein expression machinery of the infected cell for the purposes of replicating as well as producing viral proteins that are needed for new virion assembly, which exit the infected cell through exocytosis. Structural proteins such as the N protein are encoded by sub-genomic mRNAs. The N protein is required for coronavirus RNA synthesis and has RNA chaperone activity that may be involved in template switch. Nucleocapsid protein is not only the most abundant of the coronavirus proteins but is also a highly immunogenic phosphoprotein. More importantly, because its amino acid sequence is highly conserved, the coronavirus N protein represents an ideal diagnostic tool for trapping and detecting antibodies for SARS-CoV-2 in serum of patients suspected of being Infected.

ABOUT THE ASSAY

In order to control any pandemic, the scientific/medical community not only needs to develop vaccines and viral inhibitors, but it also needs to be equipped with an accurate Infection detection assays in the form antibody detection tests. Currently, viral detection during the SARS-CoV-2 pandemic utilizes real time reverse transcriptase polymerase chain reaction (rRT-PCR) assay that amplifies and detects the viral RNA. The rRT-PCR assay requires several days to generate test results. In addition to that, an isothermal amplification method has been approved by the FDA for the purpose of faster testing. Although these tests are helpful in differentiating between infected and non-infected individuals, they do present some challenges. Both types of assay have low specificity and low sensitivity. They are also not compatible with screening of a large population that leads to the neglect of the asymptomatic infected population. For all these reasons, LifeSensors focused on developing an antibody- and viral protein-based serological screening tool for a fast and accurate detection of immunity-generated antibodies for SARS-CoV-2 in the form of COVID-19 IgG/IgM/IgA ELISA kit. It is a high-throughput assay that employs a standard ELISA format utilizing the highly conserved N antigen for immobilizing SARS-CoV-2 antibodies that are present in the serum of infected patient. The detection of SARS-CoV-2 IgGs, IgMs and IgAs Is mediated by using a mix of anti-Human IgG, IgM and IgA antibodies.

REAGENTS AND MATERIALS NOT PROVIDED

1. Precision pipettors and disposable tips to deliver 10-1000 µL. **A multi-channel pipette is desirable for large assays.**
2. PBS and PBS-T
3. Tubes to prepare sample dilutions.
4. Absorbent paper.
5. Microplate reader capable of measuring absorbance at 450 nm.
6. Centrifuge capable of 3000 × g.
7. Washing bottle or microplate washer for large assays.
8. Data analysis and graphing software.

REAGENTS AND MATERIALS PROVIDED

	MATERIALS	SIZE	QUANTITY
1	Microtiter Strip well Plate	96 wells	1 Plate
2	Detection Antibody (200X)	100 µL	1 Vial
3	Negative Control	0.5 mL	1 Vial
4	Positive Control	0.5 mL	1 Vial
5	Sample Dilution Buffer	10 mL	1 Vial
6	Antibody Dilution Buffer Concentrate (5X)	12 mL	1 Vial
7	Detection reagent	12 mL	1 Vial
8	Stop Solution	12 mL	1 Vial
10	Plate Sealer		4 Sealers
11	Plate Holder		1 Plate Holder

STORAGE AND STABILITY

Store all materials at 2 to 8°C, avoid repeated freeze/thaw cycles. The pre-coated plate must be used within 30 days of receiving the kit when stored at 2 to 8°C. The pre-coated plate is stable for 1 year when stored at - 20°C.

SPECIMEN COLLECTION AND PREPARATION

NOTE: Consider any materials of human origin as infectious and handle using standard biosafety procedures.

PLASMA

1. Collect blood specimen into a lavender or blue top collection tube (containing EDTA or citrate, respectively, in a Vacutainer®) by venipuncture.
2. Separate the plasma by centrifugation.

3. Carefully withdraw the plasma into a new pre-labeled tube.

SERUM

1. Standard collection and serum separation procedures are needed to collect specimen, briefly blood is collected in a mottled red/gray gel-barrier tubes with clot activator.
2. Draw whole blood at volumes 2 ½ times the required volumes of serum into the collection tube.
3. Place collection tube upright position in the rack and allow the blood to clot at room temperature for 30-60 minutes.
4. Insert the tube in the centrifuge and spin for 10 min at recommended speed to avoid hemolysis.
5. Aspirate serum into storage tubes.

SERUM AND PLASMA STABILITY

Test specimens as soon as possible after collection. If specimens are not tested immediately, store at 2-8°C for up to 3 days. The specimens should be frozen at -20°C for long term storage.

For frozen samples, avoid more than 4 freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently.
Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference on result interpretation.

TEST PROCEDURE

REAGENTS PREPARATION

1. Bring all kit components to room temperature (20-25 °C) before use.
2. Antibody Dilution Buffer - Dilute 1 mL of Antibody Detection Buffer Concentrate (5X) with 4 mL of PBS. Vortex.
3. Perform a 200-fold dilution of the Detection Antibody in the Antibody Dilution Buffer (e.g. add 50 µL of Detection Antibody in 10 mL of Antibody Dilution Buffer). Mix Gently.

SUGGESTED SAMPLE PREPARATION

Bring samples to room temperature (20-25 °C). Dilution of samples will be determined by the user. At least 2 different dilutions are suggested starting from 1:100. Dilute sample 100-fold with the sample dilution buffer (e.g.,3 µL of serum + 297 µL of sample dilution buffer). Vortex.

ASSAY PROTOCOL

1. Prepare a plate map and prepare the required volume for each reagent and solution.
2. Secure the needed number of nucleocapsid protein pre-coated wells in the plate holder.
3. Add 100 µL of diluted sample, positive control, and negative control to the appropriate well according to the plate map.
4. Cover and incubate the plate for 30 min at room temperature.
5. Wash the microtiter plate with PBS-T using one of the specified methods indicated below:
   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with PBS-T, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure 3 times for a total of 4 washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears.

   NOTE: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.

   Automated Washing: Wash plate 4 times with PBS-T (350-400µL/well/wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.
6. Add 100 µL of Detection Antibody Solution to each well. Mix well. Cover and incubate the plate for 25 min at room temperature
7. Wash the microtiter plate 4 times as described in step 5.
8. Add 100 µL of Substrate Solution/ detection reagent to each well including the negative and

positive control wells. Cover with foil and incubate for 5 minutes at room temperature. (Avoid light).

9. Add 50 µL of Stop Solution to each well including the negative and positive control wells. Mix well.
10. Determine the Optical Density (O.D.) at 450 nm using a microplate reader **immediately**.

CONTROLS TO MINIMIZE ASSAY VARIANCE

1. It is recommended that all controls and samples be run in a triplicate format (or at least duplicate). Controls and samples must be assayed at the same time. Shake the plate after adding samples, standards, or the detection antibody to the wells manually or with a vortex. But do not shake during the incubation step as this might result in higher backgrounds and worse precision.
2. Cover or cap all kit components and store at 2-8° C when not in use.
3. Pre-coated plates should be allowed to come to room temperature before opening the foil bags. Once the needed number of strips has been removed, immediately reseal the bag with desiccants and store at 2-8°C or at -20°C to maintain plate integrity.
4. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
5. Do not mix or interchange different reagent lots from various kit lots.
6. Incomplete washing will adversely affect the test outcome. All residual PBS-T must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
7. Since the components of the Substrate Solutions are light sensitive, avoid prolonged exposure to light. Also avoid contact between the Substrate Solutions and metal, otherwise color may develop.

CALCULATION AND INTERPRETATION OF ASSAY RESULTS

1. Calculate the average value of the absorbance of the negative control (xNC).
2. Calculate the cutoffs using the following formulas:

   a. Positive cutoff = 1.1 X (xNC + 3*SD)
   b. Negative cutoff = 0.9 x (xNC + 3*SD)

3. Determine the results of the sample by comparing the OD of the sample to the negative and positive cutoffs:

   a. Negative Result: Sample OD ≤ Negative cutoff. Sample does not contain COVID-19 IgG-, IgM-, nor IgA-related antibody.
   b. Positive Result: Sample OD ≥ Positive cutoff. Sample contains COVID-19 IgG-, IgM-, and/or IgA-related antibody.
   c. Inconclusive Result: Negative cutoff < Sample OD < Positive cutoff. Sample needs to be retested in association with other clinical tests.

CERTIFICATE OF ANALYSIS

To assure the validity of the results each assay must include both negative and positive controls. The average value of the absorbance of the negative control must be less than 0.2, and the absorbance of the positive control must be higher than 0.3.

The percent coefficient of variation (CV%) for assays from the same lot was shown to be less than 5% whereas the CV% for assays from different lots was shown to be less than 8%.

We also recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

LIMITATIONS OF THE PROCEDURE

1. The Assay Procedure and the Interpretation of Assay Result must be followed closely when testing for the presence of SARS-CoV-2 virus specific antibodies in the serum or plasma from individual subjects. For optimal test performance, proper sample collection is critical.

Failure to follow the procedure may give inaccurate results.

2. The COVID-19 IgG/IgM/IgA ELISA Kit is limited to the qualitative detection of antibodies specific for the SARS-CoV-2 virus.

3. A negative or non-reactive result can occur if the quantity of antibodies for the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay, or the virus has undergone minor amino acid mutation(s) in the epitope recognized by the antibody detected by the test.

4. If symptoms persist and the result from the COVID-19 IgG/IgM/IgA ELISA Kit is negative or non-reactive, it is recommended to re-sample the patient a few days later or test with an alternative test device.

5. The results obtained with this test should only be interpreted in conjunction with clinical findings, and the results from other laboratory tests and evaluations.

6. **This test should not be used for screening of donated blood.**

GENERAL INFORMATION OR TECHNICAL INQUIRIES

Please visit website www.lifesensors.com or email: customerservice@lifesensors.com

ORDERING

1. Contact LifeSensors via email: orders@lifesensors.com
2. Contact LifeSensors' distributors.

ASSAY PROCEDURE SUMMARY

Read the manual thoroughly before using the product

Prepare all reagents, controls, and samples as described.

Add 100 µL of controls and diluted samples to the pre-coated plates.

Incubate for 30 minutes at room temperature

Wash 4 times with PBS-T

Add 100 µL of Detection Antibody Serum
Incubate for 25 minutes at room temperature.

Wash 4 times with PBS-T

Add 100 µL of Substrate Solution.
Incubate for 5 minutes at room temperature.

Add 50 µL of Stop Solution.

Read Plate at 450 nm
Immediately.

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