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COVID-19 IgG NOVEL CORONAVIRUS ELISA TEST SYSTEM

REF EL36-1360R **Σ 96 TESTS**

INTENDED USE

The Monocent, Inc.'s COVID-19 IgG ELISA Test System is intended for the qualitative detection of human anti-COVID19 IgG antibody in human serum.
This ELISA Test System is used as an aid for the detection of novel COVID-19. Patients with suspected clustering cases require diagnosis or differential diagnosis of novel coronavirus infection.

SUMMARY AND EXPLANATION

2019 novel coronavirus (COVID-19) is a single-stranded RNA coronavirus2. Comparisons of the genetic sequences of this virus have shown similarities to SARS-CoV and bat coronaviruses7. In humans, coronaviruses cause respiratory infections3. Coronaviruses are composed of several proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N)4. Results suggest that the spike protein retains sufficient affinity to the Angiotensin converting enzyme 2 (ACE2) receptor to use it as a mechanism of cell entry6. Human to human transmission of coronaviruses is primarily thought to occur among close contacts via respiratory droplets generated by sneezing and coughing1. IgG is the most abundantly found immunoglobulin to be produced in response to an antigen and will be maintained in the body after initial exposure for long term response5.

PRINCIPLE OF THE TEST

Monocent, Inc.'s ELISA Test System is designed, developed, and produced for the qualitative measurement of the human anti-COVID-19 IgG antibody in serum. This assay utilizes the microplate based enzyme immunoassay technique.
Assay controls and 1:100 diluted human serum samples are added to the microtiter wells of a microplate that was coated with COVID-19 recombinant full length nucleocapsid protein. After the first incubation period, the unbound protein matrix is removed with a subsequent washing step. A horseradish peroxidase (HRP) labeled polyclonal goat anti-human IgG tracer antibody is added to each well. After an incubation period, an immunocomplex of "COVID-19 recombinant antigen – human anti-COVID-19 IgG antibody - HRP labeled anti human IgG tracer antibody" is formed if there is specific coronavirus IgG antibody present in the tested specimen. The unbound tracer antibody is removed by the subsequent washing step. HRP-labeled tracer antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to the anti-COVID-19 IgG on the wall of the microtiter well is proportional to the amount of the anti-COVID-19 IgG antibody level in the tested specimen.

MATERIALS AND COMPONENTS

1. COVID-19 antigen coated Microplate
Microplate coated with COVID-19 recombinant protein.
Qty: 1 x 96 well microplate, Ready to use.
2. COVID-19 IgG Sample Diluent
A ready-to-use sample dilution buffer. 1 x 120 mL.
3. HRP labeled Anti-hIgG Tracer Antibody
HRP labeled polyclonal goat anti-human IgG antibody in a stabilized protein matrix. 1 x 11 mL, Ready to use.
4. ELISA Wash Concentrate
Surfactant in a phosphate buffered saline with non-azide preservative.
1 x 30 mL, 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.
5. ELISA HRP Substrate
Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
Qty: 1 x 15 mL, Ready to use.
6. ELISA Stop Solution
0.5 M sulfuric acid. Qty: 1 x 15 mL, Ready to use.
7. COVID-19 IgG Negative Control
Negative control with a bovine serum albumin based matrix with non-azide preservative. Control products do not contain any serum from patients with new type of coronavirus infection.
Qty: 1 x 1 mL, Ready to use.
8. COVID-19 IgG Positive Control
Positive control with a bovine serum albumin based matrix with non-azide preservative. Control products do not contain any serum from patients with new type of coronavirus infection.
Qty: 1 x 0.5 mL, Ready to use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 µL, 25 µL, 100 µL, and 1000 µL, etc.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm or 13 x 100 glass tubes.
5. Disposable plastic 1000 mL bottle with caps.
6. Aluminum foil.
7. Deionized or distilled water.
8. Plastic microtiter well cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

STORAGE CONDITIONS

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

PRECAUTIONS

Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

SPECIMEN COLLECTION

Only 10 µL of human serum is required for measurement in duplicate. Samples should only be used on the same day. Severe hemolytic samples should not be used.

REAGENT PREPARATION

Reagent Preparation
1. Prior to use, allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate must be diluted to working solution prior to use. Please see MATERIALS AND COMPONENTS section for details.

Sample Preparation
1. Dilute sample by a 1:100 dilution ratio with the COVID-19 IgG Sample Diluent. For each 10 µL of sample, 1000 µL of COVID-19 IgG Sample Diluent is needed.
2. Mix well prior to performing the assay.

TEST PROCEDURE

1. Place a sufficient number of microwell strips in a holder to run controls and samples in duplicate.
2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Negative Control	SAMPLE 3	SAMPLE 7
B	Negative Control	SAMPLE 3	SAMPLE 7

C	Negative Control	SAMPLE 4	SAMPLE 8
D	Positive Control	SAMPLE 4	SAMPLE 8
E	SAMPLE 1	SAMPLE 5	SAMPLE 9
F	SAMPLE 1	SAMPLE 5	SAMPLE 9
G	SAMPLE 2	SAMPLE 6	SAMPLE 10
H	SAMPLE 2	SAMPLE 6	SAMPLE 10

3. Add 100 µL of controls and 1:100 diluted samples into the designated microwells.
4. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at room temperature (20-25 ºC) for 30 minutes.
5. Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of diluted wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
6. Add 100 µL of the HRP labeled Anti-hIgG Tracer Antibody into the microwells.
7. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at room temperature (20-25 ºC) for 30 minutes.
8. Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of diluted wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
9. Add 100 µL of the substrate into the microwells.
10. Mix gently and cover the plate with aluminum foil. Incubate at room temperature (20-25 ºC) for 20 minutes.
11. Remove the aluminum foil and add 100 µL of stop solution into each of the microwells. Mix by gently by tapping the plate.
12. Read the absorbance at 450 nm within 10 minutes with a microplate reader.

PROCEDURAL NOTES

1. It is recommended that all samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original bottles and avoid unnecessary exposure to the light.
3. Store any unused antibody-coated strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

QUALITY CONTROL

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 is less than 0.25, and the absorbance of the positive control is not less than 0.50. We also recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

INTERPRETION OF RESULTS

1. Calculate the average value of the absorbance of the negative control (xNC).
2. Calculate the Background Adjustment Factor (BAF) using the following formulas:
- Positive cutoff = 1.1 X (xNC + 0.18)
- Negative cutoff = 0.9 X (xNC + 0.18)
3. Determine the interpretation of the sample by comparing the OD to the following table:

Interpretation	Interval	Results
Negative	Measured value ≤ negative cutoff	The sample does not contain the new coronavirus (COVID-19) IgG related antibody.
Positive	Measured value ≥ positive cutoff	The sample contains novel coronavirus (COVID-19) IgG associated antibodies.
Borderline	Negative cutoff < Measured value < Positive cutoff	Retest the sample in conjunction with other clinical tests.

PERFORMANCE CHARACTERISTICS

Limit of Detection
The limit of detection is not higher than 5 U/mL.
Repeatability
The assay control is tested in 10 replicates with a CV of OD values less than 15%.
Reproducibility
Three lots were tested with the same samples 10 times with a CV less than 20%.

CLINICAL TESTING

Serum samples from two cohorts of patients were tested using the IgG ELISA Test System at the Jiaxing City Center for Disease Control and Prevention and Zhejiang University Hospital. The combined cohort consisted of normal healthy patients with samples collected prior to the COVID-19 outbreak [December 3, 2019] (n = 54) and RT-PCR confirmed positive patients in after the second week of the onset of the disease (n = 30). The results are as follows:

	Test Positive	Test Negative
Confirmed Positive	30	0
Confirmed Negative	0	54

The diagnostic sensitivity is 100%.
The diagnostic specificity is 100%.

LIMITATIONS OF THE TEST

1. This test is only for qualitative detection. Test results should not be the sole basis for clinical diagnosis and treatment. The confirmation of infection with novel coronavirus (COVID-19) must be combined with the patient's clinical signs in conjunction to other tests.
2. In the first week of the onset of the infection with the novel coronavirus (COVID-19) patients results may be negative for IgG. In addition, patients with low immunity or other diseases that affect immune function, failure of important systemic organs, and use of drugs that suppress immune function can also lead to negative results of new coronavirus IgG. Previous infection of SARS or other coronavirus strain may cause a light IgG positive in view of similarity of different strains.
3. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
4. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.
5. This test has not been reviewed by the FDA.
6. Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.
7. Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
8. Positive results may be due to past or present infection with nonSARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
9. Not for the screening of donated blood.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Monocent Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Monocent Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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