**1.0 INTRODUCTION**

**Intended Use:** The Qualitative Determination of Anti-SARS-CoV-2 Specific Antibodies in Human Serum or Plasma by Microplate Enzyme Immunoassay

**2.0 SUMMARY AND EXPLANATION OF THE TEST**

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), discovered at the end of 2019, is the cause of the disease COVID-19. Both SARS-CoV-2 and SARS-CoV, the cause of the 2002 SARS epidemic, are of the genus betacoronavirus and are closely related. Transmission of SARS-CoV-2 is primarily through close contact with infected patients via expelled respiratory droplets, usually from coughing or sneezing.

Due to its high transmission rate and severity, COVID-19 has emerged as a global pandemic that has forced lockdowns and quarantine protocols from countries all over the world. Though diagnoses are primarily conducted using viral nucleic acid detection via real-time reverse transcription polymerase chain reaction (RT-PCR), antibodies can be a good marker for efficacy of treatment and can also be used to determine the presence of the virus.

Tests for immunoglobulin G (IgG) antibodies are of particular interest since they are produced in high amounts and indicate immunity to a pathogen. Additionally, IgG antibodies can be a good marker for efficacy of treatment and can also be used to determine the presence of the virus.

**3.0 MATERIALS**

**Materials provided:**
- A. Anti-SARS-CoV-2 IgG Controls – 1 ml/vial - Ions PC, NC, CC
- Three (3) vials of ready-to-use references for anti-SARS-CoV-2 at positive, negative, and cut-off levels of IgG.
- Store at 2-8°C.
- Anti-IgG Enzyme Reagent – 12 ml/vial - Ions PC
- One (1) vial of anti-human IgG-horseshad peroxidase (HRP) conjugate in a buffering matrix.
- A preservative has been added. Store at 2-8°C.
- SARS-CoV-2 Antigen Coated Plate – 96 wells - Ions PC, NC, CC
- One (1) vial contains a strong acid (0.5 M H2SO4). Store at 2-8°C.
- SARS-CoV-2 Antigen Coated Plate – 96 wells - Ions PC, NC, CC
- One well-96 microplate coated with recombinant nucleocapsid protein from SARS-CoV-2 and packaged in an aluminum bag with a drying agent.
- Store at 2-8°C.
- Serum Diluent Concentrate – 20ml
- One (1) vial contains 100 ml of serum diluent containing buffer salts and a dye. Store at 2-8°C.
- Wash Solution Concentrate – 20ml - Ions PC, NC, CC
- One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.
- Substrate – 12ml/vial - Ions PC
- One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide (H2O2) in buffer. Store at 2-8°C.
- Stop Solution – 8ml/vial - Ions PC, NC, CC
- One (1) vial contains a strong acid (0.5 M H2SO4). Store at 2-8°C.
- **Product Instructions**
- **Note:** Do not reagents beyond the kit expiration date.
- **Note:** Avoid extended exposure to heat and light. Do not use reagents beyond the kit expiration date.
- **Note:** The results should be read within fifteen (15) minutes of adding the stop solution.

**4.0 REAGENTS**

**Materials provided:**
- A. Anti-SARS-CoV-2 IgG Controls – 1 ml/vial - Ions PC, NC, CC
- Three (3) vials of reference for anti-SARS-CoV-2 at positive, negative, and cut-off levels of IgG.
- Store at 2-8°C.
- Anti-IgG Enzyme Reagent – 12 ml/vial - Ions PC
- One (1) vial of anti-human IgG-horseshad peroxidase (HRP) conjugate in a buffering matrix.
- A preservative has been added. Store at 2-8°C.
- SARS-CoV-2 Antigen Coated Plate – 96 wells - Ions PC, NC, CC
- One well-96 microplate coated with recombinant nucleocapsid protein from SARS-CoV-2 and packaged in an aluminum bag with a drying agent.
- Store at 2-8°C.
- Serum Diluent Concentrate – 20ml
- One (1) vial contains 100 ml of serum diluent containing buffer salts and a dye. Store at 2-8°C.
- Wash Solution Concentrate – 20ml - Ions PC, NC, CC
- One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.
- Substrate – 12ml/vial - Ions PC
- One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide (H2O2) in buffer. Store at 2-8°C.
- Stop Solution – 8ml/vial - Ions PC, NC, CC
- One (1) vial contains a strong acid (0.5 M H2SO4). Store at 2-8°C.
11.0.4 PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:
1. Maximum Absorbance (Positive control) > 1.8
2. Positive control RV > 15
3. Highly lipemic, hemolyzed or grossly contaminated specimen with over 3.0 units of absorbance.
4. For valid test results, adequate controls and other parameters should not be used incorrectly. Monobind shall have no liability which could produce false test results, or if results are particularly if the results conflict with other determinants.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. The clinical significance of the result should be used in evaluating the possible presence of SARS-CoV-2 infection or COVID-19. However, clinical and laboratory conclusions should not be used solely based on this test but rather as an adjunct to the clinical manifestations of the patient and other relevant tests such as a history, nasopharyngeal swab, etc. A positive result does not indicate active COVID-19 infection and does not distinguish between infected patients of contagiousness of COVID-19. Similarly, a negative result does not eliminate the absence COVID-19 infection but rather a low titer of antibody that may be related to the early stages of disease.
7. The Anti-SARS-CoV-2 IgG AccuBind® ELISA test system does not necessarily predict immunity to the SARS-CoV-2. The data is shown in Table 3 below.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance
1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes. Do not remove the cover from the plates.
3. High lipemic, hemolyzed or grossly contaminated specimen(s) should not be used. If more than one (1) plate is used, it is recommended to repeat the Cut-off control.
4. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
5. Plate readers measure vertically. Do not touch the bottom of the wells.
6. If the anti-SARS-CoV-2 IgG AccuBind® ELISA test system does not unnecessarily predict immunity to the SARS-CoV-2. There has not been a conclusive study to indicate that the presence of IgG antibodies confirms immunity to the SARS-CoV-2 virus.
7. There have not been sufficient studies to determine the longevity of Anti-SARS-CoV-2 IgG in human patients.
8. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution.
9. Plate readers measure vertically. Do not touch the bottom of the wells.
10. The Anti-SARS-CoV-2 (COVID-19) IgG AccuBind® ELISA Test System is a qualitative assay and does not necessarily give an indication of quantities of IgG antibody.
11. Samples, which are contaminated microbiologically, should not be used.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision
The within and between assay precision of the Anti-SARS-CoV-2 (COVID-19) AccuBind® ELISA Test System were determined by analysis on three different levels of pool control sera. The number, mean value, standard deviation (σ), and coefficient of variation for each of these control sera are presented below.

15.0 EXPECTED RANGES OF VALUES
A study of apparently healthy population (>150) from prior to December 2019 was undertaken to determine expected values for the Anti-SARS-CoV-2 AccuBind® ELISA test system. Based on the data, the cut-off point was established.

16.0 REFERENCES