COVID-19 Trace™ IgG MICRO-ELISA

REF	Antibodies Detected	Ig Class	Platform	Format
S1500	SARS-CoV-2 IgG Antibodies	IgG	Antigen Coated ELISA Plates for 90 Determinations	96 Wells

IVD

For Prescription Use Only
For In Vitro Diagnostic Use Only.

NAME

COVID-19 Trace™ IgG MICRO-ELISA

INTENDED USE

The COVID-19 Trace™ IgG MICRO-ELISA is a test intended for the qualitative detection of human anti SARS-CoV-2 IgG antibodies in human serum or plasma. The COVID-19 Trace™ IgG MICRO-ELISA assay is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The COVID-19 Trace™ IgG MICRO-ELISA assay should not be used to diagnose acute SARS-CoV-2 infection. Use of this test is limited to laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet the requirements to perform high complexity tests. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities. The sensitivity of the COVID-19 Trace™ IgG MICRO-ELISA early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary for confirmation. False positive results from the COVID-19 Trace™ IgG MICRO-ELISA assay may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Results are for the detection of SARS CoV-2 IgG antibodies. SARS-CoV-2 antibodies are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion. **The COVID-19 Trace™ IgG MICRO-ELISA assay has completed the Section IV.D notification process under FDA's "Policy for Coronavirus Disease – 2019 Test During the Public Health Emergency (Revised)" and has not been reviewed by the FDA.**

SUMMARY AND EXPLANATION OF TEST

According to the U.S. Department of Health and Human Services/Centers for Disease Control and Prevention (CDC), Chinese authorities identified an outbreak caused by a novel—or new—coronavirus termed SARS-CoV-2. The virus can cause mild to severe respiratory illness; known as Coronavirus Disease 2019 (COVID-19) formerly called 2019-nCoV.[1] The outbreak began in Wuhan, Hubei Province, China and has spread to a growing number of countries worldwide including the United States. On March 11, 2020, the World Health Organization declared COVID-19 a pandemic. SARS-CoV-2 is different from six other previously identified human coronaviruses, including those that have caused previous outbreaks of Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory

Syndrome (MERS). Additional information on coronaviruses is available on the U.S. Centers for Disease Control and Prevention coronavirus website.

The COVID-19 Trace™ IgG MICRO-ELISA assay is designed to detect immunogloblulin class G (IgG) antibodies to the receptor binding domain (RBD) protein antigen of SARS-CoV-2 in serum and plasma from individuals who are suspected to have had coronavirus disease (COVID-19), or in serum or plasma from subjects infected by the virus SARS-Cov-2 but may be asymptomatic at the time of the test.

The SARS-CoV-2 Spike (S) Protein consists of the S1 and S2 domains.[2] The S1 domain contains the receptor-binding domain (RBD) that can specifically bind to angiotensin-converting enzyme 2 (ACE2) receptors on target cells.[2] The SARS-CoV-2 nucleocapsid (N) protein plays a role in transcription, replication, and packaging of the viral RNA genome, while also affecting host cell responses such as cell cycle and translation.[3] SARS-CoV-2 is closely related to the SARS virus, which was first identified in 2002-2003.[3] In-depth analysis has identified the SARS-CoV-2 RBD as being essential for ACE2 binding.[3] Both SARS-CoV and SARS-CoV-2 utilize the ACE2 cellular receptor to gain entry into cells, with SARS-CoV-2 binding with higher affinity.[4] Vaccine and therapeutic development are targeting portions of the spike protein, including the RBD portion.[5]

BIOLOGICAL PRINCIPLES OF THE TEST

The COVID-19 Trace™ IgG MICRO-ELISA test is designed for the qualitative detection of human anti SARS-CoV-2 IgG antibodies present in human serum and plasma. The test is a solid phase enzyme-linked immunosorbent assay (ELISA), using a chromogenic enzyme substrate. The SARS CoV-2 recombinant receptor binding domain (RBD) antigen is immobilized to polystyrene wells of a microplate (solid phase). The IgG antibodies to SARS-CoV-2 present in the test sample and controls bind to the antigen coated wells. The assay quality controls, cutoff control and diluted test samples are added to the microtiter wells coated with immobilized SARS-CoV-2 antigen. After an incubation period, the wells are washed to remove unbound sample matrix and then the Anti-human IgG Fc specific horseradish peroxidase (HRP) conjugate is diluted in conjugate diluent and added to the wells. After an incubation period, the wells are washed to remove unbound Anti-human IgG-HRP and an enzyme substrate-chromogen (hydrogen peroxide, H_2O_2, and tetramethylbenzidine, TMB) is added to each well and incubated, resulting in the development of a blue color. The intensity of the blue color is directly proportional to the concentration of the SARS CoV-2 IgG antibodies in the test sample. An assay stop solution is added at the 20 minute mark post addition of Substrate Chromogen and the color intensity is read in a microplate reader capable of reading absorbance at 450 nm and validated in compliance with FDA's 21 CFR Part 11. Positive and negative quality controls are provided to ensure the integrity of the test. A cutoff control is provided to calculate the status of the unknown test sample.

REAGENTS AND MATERIALS SUPPLIED

⚠ **CAUTION:** Do not use any reagents where damage to the packaging has occurred.

COMPONENTS IN EACH COVID-19 Trace™ IgG MICRO-ELISA TEST KIT
Volumes listed in the table below indicate the volume per bottle in the kit.

REF	Reagent/Component	Amount	Storage	Symbol
S1500-1	1 Microplate 96-well strips coated with recombinant SARS CoV-2 receptor binding domain (RBD) antigen. Wells are provided in a sealed foil pack with silica gel desiccant.	12 X 8	2° - 8°C until expiration date.	MICROPLATE
S1500-2	1 bottle Positive Control (1X) Buffered protein solution containing recombinant human anti SARS-CoV-2 RBD (human IgG_1) and ProClin-300 as a preservative. The Positive Control is used to monitor the integrity of the test.	0.5 (ml)	2° - 8°C until expiration date.	CONTROL +
S1500-3	1 bottle Negative Control (1X) Negative human plasma containing ProClin-300 as a preservative. The Negative Control is used to monitor the integrity of the test.	0.5 (ml)	2° - 8°C until expiration date.	CONTROL -
S1500-4	1 bottle Cutoff Control (1X) Buffered protein solution containing recombinant human anti SARS-CoV-2 RBD (human IgG_1) and ProClin-300 as a preservative. The Cutoff Control is used to monitor the integrity of the test and determine the cutoff threshold to determine test sample status.	0.5 (ml)	2° - 8°C until expiration date.	CUTOFF CONTROL
S1500-5	1 bottle SAMPLE DILUENT (1X) Buffered protein solution containing ProClin-300 as a preservative.	50 (ml)	2° - 8°C until expiration date.	SAMPLE DILUENT
S1500-6	1 bottle Anti Human IgG Fc Specific ENZYME CONJUGATE (100X) Anti-Human IgG Fc Specific (mouse monoclonal antibody IgG_{2b}) conjugated with horseradish peroxidase in a buffered protein solution containing ProClin-300 as a preservative. Enzyme Conjugate reagent demonstrates class specific reactivity to all human IgG isotypes. Minimal (<1%) cross reactivity to human IgM, IgA or IgE. There is no detectable binding to bovine, goat, horse or sheep IgG.	150 (µl)	2° - 8°C until expiration date.	ENZYME CONJUGATE

REF	Reagent/Component	Amount	Storage	Symbol
S1500-7	1 bottle Conjugate Diluent (1X) A buffered protein solution containing ProClin-300 as a preservative. This diluent is for the purpose of diluting the 100 X Enzyme Antibody Conjugate. The 100X Enzyme Antibody Conjugate should only be diluted into the Conjugate Diluent immediately prior to running the assay. Unused diluted conjugate should be discarded.	12 (ml)	2° - 8°C until expiration date.	CONJUGATE DILUENT
S1500-8	1 bottle WASH BUFFER (20X) Phosphate buffered saline 20X concentrate solution with a surfactant containing ProClin-300 as a preservative.	25 (ml)	2° - 8°C until expiration date.	WASH BUFFER
S1500-9	1 bottle SUBSTRATE CHROMOGEN (1X) Buffered hydrogen peroxide and 3,3',5,5'- tetramethylbenzidine (TMB) solution.	12 (ml)	2° - 8°C until expiration date. Protect from light.	SUBSTRATE CHROMOGEN
S1500-10	1 bottle STOP SOLUTION (1X) 1.0 N sulfuric acid (H_2SO_4).	10 (ml)	2° - 8°C until expiration date.	STOP SOLUTION
S1500-11	2 Adhesive Plate Sealers	2 X 1 Ea.	Non Reagent	PLATE SEALER

ADDITIONAL MATERIALS REQUIRED

- Boxes of Pipette Tips - 0.1 ml, 0.2 ml and 1.0 ml
- Calibrated pipettes capable of accurately delivering volumes from 4 µl – 1.0 ml
- Disposable tubes for making dilutions (micro-centrifuge tubes or borosilicate glass test tubes).
- Precision micro-titer absorbance plate reader wavelength of 450 nm.
- Distilled or deionized water
- Lab timer
- 37°C Incubator without CO_2 supply

Instrumentation

The COVID-19 Trace™ IgG MICRO-ELISA test is to be used with a calibrated ELISA microplate reader with secure software capable of reading absorbance at 450 nm The microplate reader and software should be validated and in compliance with FDA's 21 CFR Part 11.

Antibody Class Specificity

The COVID-19 Trace™ IgG MICRO-ELISA assay uses a mouse anti human IgG Fc specific monoclonal antibody to detect IgG antibodies to the SARS-CoV-2 receptor binding domain (RBD) antigen. This mouse anti-human IgG, Fc Fragment Specific (Leinco clone HP6043) horseradish peroxidase conjugate is validated for use in enzyme immunoassay for the detection of Human IgG, Fc Fragment Specific. Cross-reactivity by ELISA against chimeric antibodies: Human IgG_1: 100%, Human IgG_2: 100%, Human IgG_3: 100%, Human IgG_4: 100%, Human IgM: <0.01%, Human IgA: <0.01%, Human IgE: <0.01%. There is no detectable binding to bovine, goat, horse sheep IgG. CLINICAL AND VACCINE IMMUNOLOGY, May 2009, p. 739–748 and Reimer, C. B. et al. (1984) *Hybridoma* **3**:263 2. Jefferies, R. et al. (1985) *Immunol. Letters* **10**:223 3. Phillips, D. J. et al. (1987) *Immunol. Letters* **17**:159

WARNINGS AND PRECAUTIONS ⚠

- For *In Vitro* diagnostic use only. For prescription use only. A thorough understanding of this package insert is necessary for the successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and following the package insert.
- This test is only for the qualitative detection of IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens.
- The COVID-19 Trace™ IgG MICRO-ELISA assay has completed the Section IV.D notification process under FDA's "Policy for Coronavirus Disease – 2019 Test During the Public Health Emergency (Revised)" and has not been reviewed by the FDA.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Do not use kit components past the expiration date printed on the outside of the kit box.
- All patient specimens should be considered potentially infectious and handled in accordance with good laboratory procedure.
- Wear suitable protective attire, gloves, eye/face protection when handling any of the components of this kit.
- Sample collection and handling procedures require specific training and guidance and should only be performed by properly trained healthcare professionals.
- Dispose of containers and unused contents in accordance with Federal, State, and Local regulatory requirements.
- Follow package insert instructions precisely to obtain accurate test results.
- Some Reagents contain thimerosal; avoid contact with skin.
- Avoid contact with SUBSTRATE-CHROMOGEN (tetramethylbenzidine) solution. It is harmful if inhaled or absorbed through skin (may cause irritation).
- Do not interchange reagents between test kits with different lot numbers.
- Specimen and reagents should be allowed to come to room temperature and mixed thoroughly by gentle inversion or swirling before assay is run.
- Azide inhibits this enzyme reaction. Avoid the use of samples or commercial controls that contain sodium azide.

SAFETY PRECAUSTIONS

⚠ **CAUTION:** This product requires the handling of human specimens. It is recommended that all human-sourced materials and all consumables contaminated with potentially infectious materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate regional, national, and institutional biosafety practices should be used for materials that contain, are suspected of containing, or are contaminated with infectious agents.[10-13]

KIT STORAGE AND STABILITY

UNOPENED: 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.

OPENED: Once opened, the kit reagents are stable when stored at 2 – 8°C. Refer to label on the kit box for expiration date.

INDICATION OF INSTABILITY OR DETERIORATION: Deterioration of kit reagents may be indicated when a quality control value is out of the specified range. Associated test results are invalid, and samples must be retested with a new kit where the control values are within the specified ranges.

SPECIMEN COLLECTION AND PREPARATION

SPECIMEN TYPES The following specimen types listed below may be used with this assay.

Specimen Types	Collection Tubes
Serum	Serum
Plasma (Sodium Citrate)	Sodium Citrate

- Each Laboratory must follow their own internal procedures to establish the use of additional collection tubes.
- This test has not been validated for performance characteristics using cadaveric specimens or the use of bodily fluids other than human serum or plasma.
- Collection tubes containing anticoagulants may be dilutive resulting in a lower Sample Cutoff Ratio.

SPECIMEN CONDITIONS

Do not use:

- Heat inactivated specimens
- Pooled specimens
- Grossly hemolyzed specimens
- Specimens with obvious microbial or contamination
- Specimens with fungal growth

Blood obtained by venipuncture should be allowed to clot at room temperature (18 - 30°C) for 30 to 60 minutes and then centrifuged according to the Clinical and Laboratory Standards Institute (CLSI Approved Guideline – Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; GP44). Testing of serum or sodium citrate plasma should be performed as soon as possible after collection and processing. Do not leave specimens at room temperature for prolonged periods. Separated serum or plasma should remain at 18 -30°C for no longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be refrigerated at 2-8°C. If assays are not completed within seven days, or the separated serum or plasma is to be stored beyond seven (7) days, samples should be frozen at or below -20°C. Avoid repeated freezing and thawing of samples more than four times as this can cause antibody deterioration. Frost-free freezers are not suitable for sample storage. Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use. If specimens are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.

SPECIMEN STORAGE

Specimen Types	Storage Temperature	Storage Time
Serum or Plasma	18 - 30°C	8 hours
Serum or Plasma	2 - 8°C	48 hours
Serum or Plasma	≤ - 20°C	> 7 Days

SPECIMEN SHIPPING If specimens are to be shipped, they should be packed in compliance with Local, State and Federal Regulations covering transportation of clinical specimens and infectious agents.

SPECIMEN CONDITIONS PRIOR TO ANALYSIS

- Follow blood tube manufacturer's processing instructions for test specimens. Do not use gravity separation.
- Recentrifuge specimens if fibrin, red blood cells, or other particulate matter are observed.

PREPARATION OF FROZEN SPECIMENS PRIOR TO ANALYSIS

- Allow frozen samples to come to room temperature 18 - 30°C before processing.
- Thoroughly mix thawed specimens by gentle vortexing or inversion until homogeneous.
- Recentrifuge thawed and mixed specimens.

Test Specimens: Prepared by diluting 1:100 in Sample Diluent. Example: Dilute 4.0 µl serum or plasma into 396 µl of Sample Diluent and vortex at low speed or invert tube 10 times.

TEST PROCEDURE

REAGENT PREPARATION

Upon removal of reagents from refrigerator, allow all reagents to stand for 30 minutes to reach room temperature 18 – 30°C before use. Vortex reagents on low speed or invert 10 times to mix.

- **Microplate** Allow the 96-well polystyrene microplate strips coated with recombinant SARS-CoV-2 antigen and sealed in a foil pack with silica gel desiccant to come to room temperature 18 – 30°C before opening. Once the microplate strips have been opened, if not completely used, reseal with desiccant in the foil pouch and store at 2 - 8°C in a dry place.
- 1 bottle 0.5 ml **Cutoff Control** (1X) Preparation: Mix by vortexing at low speed or invert the tube 10 times. Ready to use. RTU
- 1 bottle 0.5 ml **Positive Control** (1X) Preparation: Mix by vortexing at low speed or invert the tube 10 times. Ready to use. RTU
- 1 bottle 0.5 ml **Negative Control** (1X) Preparation: Mix by vortexing at low speed or invert the tube 10 times. Ready to use. RTU
- 1 bottle 50 ml **SAMPLE DILUENT** (1X) Preparation: Ready to use. RTU
- 1 bottle 150 µl Anti Human IgG Fc Specific (mouse monoclonal) **ENZYME ANTIBODY CONJUGATE** (100X) Preparation: Just prior to running a full 96 well test plate, dilute 120 µl of Enzyme Antibody Conjugate into 12.0 ml of Conjugate Diluent (1X).
- 1 bottle 12.0 ml **Conjugate Diluent** (1X) Preparation: Ready to use. RTU

- 1 bottle 25 ml **WASH BUFFER CONCENTRATE** (20X) Preparation: If crystals appear during storage at 2 - 8°C, warm the concentrate by placing at 37°C until clear. Dilute full bottle of Wash Buffer Concentrate (20X) with 475 ml of deionized water to a final volume of 500 ml and mix well before use.
- 1 bottle 12 ml **SUBSTRATE-CHROMOGEN** Preparation: Ready to use. RTU
- 1 bottle 10 ml **STOP SOLUTION** 1.0 N sulfuric acid (H_2SO_4.) Preparation: Ready to use. RTU

⚠ **CAUTION:** Strong Acid. Wear protective gloves, mask and safety glasses. Dispose of all materials according to all applicable safety rules and regulations.

ASSAY PROCEDURE

⚠ **CAUTION:** The test procedure must be followed as written. Any deviations from this procedure may produce erroneous results.

1. Allow all kit reagents to stand for 30 minutes to reach room temperature 18 – 30°C and gently mix each vial by vortexing on low speed or inverting 10 times.
2. The Positive Control, Negative Control and the Cutoff Control must be assayed in duplicate on the 96 well Microplate each time the test is performed. Up to ninety (90) test specimens may be run in singlicate on each full plate.
3. Place sufficient microplate strip wells in a strip holder to run all assay controls in duplicate and test specimens in singlicate. (Figure 1 below represents a suggested plate layout for the Positive Control, Negative Control, Cutoff Control and each individual Test Specimen.)
4. Pipette **100 µl** of the 1X **Positive Control, Negative Control** and **Cutoff Control** into the individual microplate wells in duplicate according to Figure 1 below.
5. Pipette **100 µl** of the diluted **Test Specimens** into the corresponding individual microplate wells in singlicate as shown in Figure 1 below.
6. Cover the plate with an adhesive plate sealer and incubate for **30 +/- 1 minutes** at **+37 °C ± 1 °C** in an incubator without carbon dioxide. For manual processing of microplate wells, cover the finished test plate with an adhesive protective plate sealer and start incubation. When using automated microplate processors, for incubation, follow the recommendations of the instrument manufacturer.

   ⚠ **Caution:** Do not stack plates on top of each other. They should be spread out as a single layer for even temperature distribution.

7. Plate Washing: Remove the protective adhesive strip and aspirate each well and wash, repeating the process for a total of **four washes**. Wash by filling each well with **300 µl** of 1X **Wash Buffer** using a manual squirt bottle, manifold dispenser, or autowasher leaving the 1X Wash Buffer in each well for **30 – 60 seconds.** Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper toweling.
8. Pipette **100 µl** of diluted 1X **Enzyme Antibody Conjugate** into each micro-well previously incubated with Controls and Test Specimens.
9. Place an adhesive plate sealer over the wells and incubate without carbon dioxide at (37°C ± 1°C) for **30 Minutes ± 1 minute**.

⚠ **Caution:** Do not stack plates on top of each other. They should be spread out as a single layer for even temperature distribution.

10. Plate Washing: Remove the protective adhesive strip and aspirate each well and wash, repeating the process for a total of **four washes**. Wash by filling each well with **300 µl** of 1X **Wash Buffer** using a manual squirt bottle, manifold dispenser, or autowasher leaving the 1X Wash Buffer in each well for **30 – 60 seconds.** Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper toweling.
11. Add **100 µl** of **Substrate Chromogen** to each well. Incubate for 20 minutes at room temperature (18 – 30°C) protected from direct light.
12. Immediately upon concluding the 20 minute incubation, add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
13. Immediately after adding stop solution, at the 20 minute mark +/- 1 minute, read the absorbance from the color intensity of each well at 450 nm. Prior to measuring, carefully shake the microplate to ensure a homogeneous distribution of the solution in the wells.

⚠ **Caution:** The plate should be read at 20 minute +/- 1 minute. If not read within this time period, results may not be accurate. The test must be repeated.

Figure 1: COVID-19 Trace™ IgG Micro-ELISA Suggested Pipetting Scheme

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83
B	PC	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84
C	NC	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
D	NC	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
E	CC	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
F	CC	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
G	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
H	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90

PC = Positive Control
NC = Negative Control
CC = Cutoff Control
S = Sample

QUALITY CONTROL

Each kit contains one positive control, one negative control and a cutoff control. The controls are intended to monitor for substantial reagent failure. The test is invalid and must be repeated if the control samples do not meet the

specifications listed in this procedure. If the test is invalid, the results cannot be used. Quality Control (QC) requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to CLSI C24 and 42 CFR 493.1256 for guidance on appropriate QC practices. The results below are given strictly for guidance purposes only and applicable for spectrophotometric readings only. (See Examples Below.)

Example 1: COVID-19 Trace™ IgG Negative Control

Negative Control	OD 450
Replicate 1	0.20
Replicate 2	0.19
Negative Control Sum	0.39
Average Negative Control (Sum/2)	0.195

Example 2: COVID-19 Trace™ IgG Positive Control

Positive Control	OD 450
Replicate 1	0.95
Replicate 2	1.05
Positive Control Sum	2.0
Average Positive Control (Sum/2)	1.0

Example 3: COVID-19 Trace™ IgG Cutoff Control

Cutoff Control	OD 450
Replicate 1	0.70
Replicate 2	0.60
Cutoff Control Sum	1.3
Average Cutoff Control (Sum/2)	0.65

COVID-19 Trace™ IgG Quality Control Requirement

Assay Control	Requirement OD 450nm
Positive Control	OD ≥ 1.0
Negative Control	OD < 0.25
Cutoff Control	0.6 < OD <0.8

Quality Control: The results on the table above must be obtained for the assay to be considered valid. Non-fulfillment of these Quality Control criteria is an indication of deterioration of reagents or an error in the test procedure and the assay must be repeated.

INTERPRETATION OF RESULTS

The assay Cutoff Control value was determined by screening a large number (>500) human plasma samples that were collected prior to the COVID-19 outbreak (Prior to December 1, 2019). The cutoff selection was performed by estimating the mean of the negative specimens plus four times the standard deviation.

The COVID-19 Trace™ IgG assay determines the status of an unknown test specimen by determining the average assay Cutoff Control value as shown in Example 3 above. This is followed by calculating the Sample Cutoff Ratio of the OD450 nm obtained from the test sample divided by the OD_{450} nm of the average Cutoff Control value.

Sample Cutoff Ratio (SCR)	Test Result	Interpretation
≥ 1.2	Positive	Indicates the presence of detectable IgG antibodies targeting the SARS-CoV-2 antigen.
< 0.8	Negative	Indicates no detectable IgG antibodies targeting SARS-CoV-2 antigen were found. The result does not rule out the possibility of SARS-CoV-2 infection.
0.8 ≤ <1.2	Borderline	In case of a borderline test cutoff ratio, a definitive test result is not possible. It is recommended that the patient be re-drawn one to two weeks later and tested in duplicate along with controls using the COVID-19 Trace™ IgG serology test.

LIMITATIONS OF TEST PROCEDURE

- IVD For In vitro Diagnostic Use.
- Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43 or 229E.
- Not for the screening of donated blood.
- Results from this antibody test should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
- This test is for clinical laboratory use only. It is not for home use.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as SARS-CoV-2 IgG that employ mouse monoclonal antibodies.[7, 8]
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed.[9]
- Rheumatoid factor (RF) in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[9]

PERFORMANCE CHARACTERISTICS

SPECIMENS

Both natural and contrived specimens were used to validate the COVID-19 Trace™ IgG MICRO-ELISA Test. When contrived samples were used, they were made by spiking negative human plasma collected prior to December 1, 2019 with a cocktail of human recombinant monoclonal antibodies (human IgG_1), all of which were sequenced from plasma B-cells isolated from COVID-19 survivors and screened for specificity to the recombinant SARS-CoV-2 receptor binding domain (RBD) protein.

PRECISION

Laboratory Precision

Studies on laboratory precision and assay repeatability were carried out according to CLSI guideline EP05-A3.[6] The precision is given as standard deviation (SD) and coefficient of variation (CV). Testing was performed with one lot of COVID-19 Trace™ IgG MICRO-ELISA kit containing Positive Control, Negative Control and Cutoff Control. The two controls and one human plasma panel were assayed in triplicates at two separate times per day and on 5 different days.

Days Post PCR Confirmation	n	COVID-19 Trace™ IgG MICRO-ELISA Kit				
		Mean Cutoff Ratio	Repeatability Within-Assay		Within-Laboratory*	
			SD	%CV	SD	%CV
Negative Control	30	0.16	0.01	N/A[a]	0.04	N/A[a]
Positive Control	30	4.63	0.13	2.9%	0.48	10.4%
Positive Panel	30	8.11	0.39	4.9%	0.89	11.0%

*Includes repeatability within-assay, between assays and between day variability.
[a] Not Applicable

INTERFERENCE

Potentially Interfering Endogenous Substances

A study was performed based on guidance from CLSI EP07, 3rd edition.[15] Each potentially interfering substance was tested at or above physiological levels to determine whether they could cause false positives or false negatives using the COVID-19 Trace™ IgG MICRO-ELISA assay. Each Interfering substance was tested at 2 levels using a seronegative sample spiked with recombinant anti human SARS-CoV-2 receptor binding domain (RBD) IgG (high negative and low positive levels, approximately 0.8 SCR and 1.7 SCR respectively) in replicates of ten. The study showed no interference from these endogenous substances.

Potential Interfering Endogenous Substances	Concentration of Potentially Interfering Endogenous Substance
Unconjugated Bilirubin	40 mg/dL
Conjugated Bilirubin	40 mg/dL
Hemoglobin	1000 mg/dL
Triglycerides	2000 mg/dL
Cholesterol	40 mg/dL
Albumen	15 g/dL

POSITIVE AGREEMENT TO PCR

The positive percent agreement (PPA) of the COVID-19 Trace™ IgG MICRO-ELISA assay was estimated by testing a panel of fifty (50) specimens collected from individuals by day of symptom-onset and who tested positive for SARS-CoV-2 using an EUA polymerase chain reaction (PCR) assay at an earlier time point. The positive percent agreement (PPA) and the 95% confidence interval (CI) were calculated. The test results are presented in the following table.

Days Post Symptom Onset	n	COVID-19 Trace™ IgG MICRO-ELISA Kit				
		POS.	BORD.	NEG.	Borderline Counted as Negative	
					PPA	95% CI
0 - 7	10	5	0	5	50.0%	23.7% - 76.3%
8 - 14	10	7	1	2	70.0%	39.7% - 89.2%
> 15	30	30	0	0	100.0%	88.7% - 100%

*95% Confidence Interval is calculated using the Wilson Method[1]

NEGATIVE AGREEMENT

To evaluate the negative percent agreement of the COVID-19 Trace™ IgG MICRO-ELISA Test for presumed negative samples, samples from unselected apparently healthy US plasma donors were used. The negative percent agreement (NPA) and 95% confidence interval (CI) were calculated. The results are presented in the following table.

Panels Before December 1, 2019	n	COVID-19 Trace™ IgG MICRO-ELISA Kit				
		POS.	BORD.	NEG.		
					NPA	95% CI*
Plasma Donors (USA)	531	3	9	519	99.4%	98.36% - 99.81%

*95% Confidence Interval is calculated using the Wilson Method[14]

SAMPLE MATRIX COMPARISON

The COVID-19 Trace™ IgG MICRO-ELISA assay has been validated for use with human serum and sodium citrate plasma. Human serum and plasma was tested using five (5) seronegative sample pairs spiked with a natural plasma sample from a confirmed positive donor by an EUA positive PCR. All samples, spiked and seronegative, were run in triplicate. The Passing-Bablok regression was performed for the comparison of plasma to serum. The results from the assays are presented in the following table.

COVID-19 Trace™ IgG MICRO-ELISA Kit	**Serum**
n =	15
Sample Cutoff Ratio Range - Plasma	0.3 – 1.8
Sample Cutoff Ratio Range - Serum	0.3 – 1.7
Regression Equation (*x* = plasma, *y* = serum)	0.9953*x* + 0.0028
95% C.I. of Intercept	-0.068 – 0.074
95% C.I. of Slope	0.899 – 1.012
Coefficient of Determination (R^2)	0.9904

The COVID-19 Trace™ IgG MICRO-ELISA has been validated for use with human serum and sodium citrate plasma. Human serum and plasma was tested using fifteen (15) seronegative sample pairs spiked with a cocktail of recombinant SARS-CoV-2 human monoclonal antibodies (IgG) sequenced from plasma B-cells of COVID-19 survivors at moderate and low concentrations. All samples, spiked and seronegative, were run in duplicate. The results of the assays are presented in the following table.

Sample Type	**COVID-19 Trace™ IgG MICRO-ELISA Kit**								
	Low Positive			**Mid Positive**			**Seronegative**		
	NEG	**POS**	**Percent Agreement**	**NEG**	**POS**	**Percent Agreement**	**NEG**	**POS**	**Percent Agreement**
Plasma (Sodium Citrate)	0	15	100%	0	15	100%	15	0	100%
Serum	0	15	100%	0	15	100%	15	0	100%

ANALYTICAL SPECIFICITY

Potentially Cross Reacting Antibodies

The COVID-19 Trace™ IgG assay has been evaluated for potentially cross-reacting antibodies. A total of 58 serum or plasma specimens from 12 different categories were tested. Fifty eight (58) samples tested negative and zero samples tested positive. The data are summarized in the following table.

Cross Reactivity Panel	n	COVID-19 Trace™ IgG MICRO-ELISA Kit		
		SCR	NEG.	Negative (%)
HCoV-NL63 Infection (Serum)	5	SCR < 0.8	5	100%
HCoV-229E Infection (Serum)	5	SCR < 0.8	5	100%
HCoV-OC43 Infection (Serum)	5	SCR < 0.8	5	100%
HCoV-HKU1 Infection (Serum)	5	SCR < 0.8	5	100%
Anti influenza A (Plasma)	5	SCR < 0.8	5	100%
Anti Influenza B (Plasma)	5	SCR < 0.8	5	100%
Anti HCV (Serum)	5	SCR < 0.8	5	100%
Anti HBV (Serum)	5	SCR < 0.8	5	100%
Anti Haemophilus Influenzae (Plasma)	5	SCR < 0.8	5	100%
Anti Respiratory Syncytial Virus (Plasma)	5	SCR < 0.8	5	100%
ANA (1XPlasma 2XSerum)	3	SCR < 0.8	3	100%
Anti HIV (Serum)	5	SCR < 0.8	5	100%

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SYMBOLS KEY

ISO 15223 SYMBOLS		OTHER SYMBOLS	
	Caution	MICROPLATE	Microplate Strips
	Consult instructions for use	CONTROL +	Positive Control
	Manufacturer	CONTROL -	Negative Control
	Manufacturer date	CUTOFF CONTROL	Cuttoff Control
	Temperature limitations	SAMPLE DILUENT	Sample Diluent
	Use by/Expiration date	ENZYME CONJUGATE	Enzyme Conjugate
	Total number of IVD tests that can be performed with the IVD medical device	CONJUGATE DILUENT	Conjugate Diluent
LOT	Lot Number	WASH BUFFER	Wash Buffer
REF	Product Number	SUBSTRATE CHROMOGEN	Substrate Chromogen
IVD	*In Vitro* Diagnostic Medical Devices	STOP SOLUTION	Stop Solution
	Sensitive to direct sunlight	PLATE SEALER	Plate Sealer
	Biological hazards	RTU	Ready to Use

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