Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test

INSTRUCTIONS FOR USE

103750

April 2021

Quanterix Corporation

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Customer Support

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For use under an Emergency Use Authorization (EUA) Only

Prescription Use Only

For In Vitro Diagnostic Use Only

Read this package insert prior to use. Package insert instructions must be carefully followed. Reliability of assay results cannot be assured if there are deviations from the instructions in this package insert.

The clinical applicability of a quantitative or semi-quantitative result is currently unknown and cannot be interpreted as an indication or degree of immunity nor protection from reinfection, nor compared to other SARS-CoV-2 antibody assays.

NAME

Simoa™ Semi-Quantitative SARS-CoV-2 IgG Antibody Test

INTENDED USE

The Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test is an automated paramagnetic microbead-based immunoassay intended for qualitative and semi-quantitative detection of IgG antibodies to SARS-CoV-2 in human serum and dipotassium EDTA plasma using the Quanterix HD-X immunoassay system. The Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. The Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test should not be used to diagnose or exclude acute SARS-CoV-2 infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a, that meet requirements to perform moderate or high complexity tests.

Results are for the detection of SARS CoV-2 IgG antibodies. IgG antibodies to SARS-CoV-2 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.

Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

The sensitivity of the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test 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.

False positive results for the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

Samples should only be tested from individuals that are 15 days or more post symptom onset.

The Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY AND EXPLANATION OF TEST

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a recently identified coronavirus strain responsible for the Coronavirus Disease 2019 (COVID-19) pandemic. SARS-CoV-2 emerged in China in December 2019 and is transmitted mainly through droplets and surface contact routes. The virus infects human cells through interaction between angiotensin converting enzyme 2 (ACE2) on respiratory cells and spike or S-protein on the outer envelope of the virion particle. COVID-19 affects people in different ways. Symptoms can include signs and symptoms of acute respiratory illness, such as fever, cough, shortness of breath, but the infection can also be asymptomatic. Symptomatic, pre-symptomatic and asymptomatic infected individuals can all be sources for viral transmission. The current gold standard for diagnosis of SARS-CoV-2 infection is real-time reverse transcription polymerase chain reaction (rRT-PCR), which detects the presence of SARS-CoV-2 nucleic acid material in upper respiratory specimens, such as nasopharyngeal swab and oropharyngeal swab. In contrast, anti- SARS-CoV-2 antibody detection assay detects antibodies to the virus after the host immune response and seroconversion. Classes of antibodies detected by antibody tests are generally IgM and IgG.

PRINCIPLES OF THE PROCEDURE

The Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test is a 3-step paramagnetic microbead-based sandwich ELISA that uses single molecule array (Simoa) technology.[2] In the first step, sample is drawn from a sample tube or microtiter plate by the instrument pipettor and mixed with COVID-19 spike protein coated paramagnetic capture beads in a reaction cuvette. IgG antibodies in the sample that are specific to COVID-19 spike protein are bound by the capture beads. After an incubation, capture beads are collected with a magnet, and washed. Biotinylated anti-human IgG detector antibodies are then mixed with the capture beads, and the detector antibodies bind to the captured sample IgG. Following a second wash, a conjugate of streptavidin-ß-galactosidase (SBG) is mixed with the capture beads. SBG binds to the biotinylated detector antibodies, resulting in enzyme labeling of captured sample IgG. After a third wash, the capture beads are resuspended in a resorufin ß-D-galactopyranoside (RGP) substrate solution. Digital processing occurs when beads are transferred to the Simoa array disc which is composed of microarrays of femtoliter reaction wells. Individual capture beads are then sealed within microwells in the array through the addition of oil, which forms a liquid seal trapping the labeled immunocomplexes and RGP within the wells. If anti-spike IgG from the sample has been captured and labeled, the ß-galactosidase hydrolyzes the RGP substrate into a fluorescent product that provides the signal for measurement. The concentration of IgG in unknown samples is interpolated from a calibration curve obtained by 4-parameter logistical regression fitting. Total time to first result on a single sample is 80 minutes. Time to perform 96 tests is approximately 2 hours and 45 minutes.

For additional information on system and assay technology, refer to the *Simoa HD-X Analyzer User Guide (EUA Edition) (USER-0105)*.

REAGENTS

Reagent Kit

Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test kit

Reagent Kit	Bead Reagent	1 bottle (4.4 mL)	SARS-CoV-2 spike protein (recombinant) coated capture beads in Tris buffer with a protein stabilizer (bovine) and a surfactant. Preservative: ProClin 300.
	Detector Reagent	1 bottle (12.3 mL)	Biotinylated anti-human IgG antibody (goat polyclonal) in phosphate buffer with a protein stabilizer (bovine) and a surfactant. Preservative: ProClin 300.
	SBG Reagent	1 bottle (12.3 mL)	Conjugate of streptavidin-ß-galactosidase (SBG) in phosphate buffer with a protein stabilizer (bovine). Preservative: ProClin 300.
	Sample Diluent	4 bottles (30 mL ea)	Phosphate buffer with a protein stabilizer (bovine), a heterophilic blocker, and a surfactant. Preservative: Sodium azide.
	RGP Reagent	2 bottles (3.4 mL)	Resorufin ß-D-galactopyranoside (RGP) in phosphate buffer with a surfactant.
Calibrators	Cals A-H (0 plus 7 levels)	8 vials (1 mL each)	Anti-SARS-CoV/CoV-2 IgG in phosphate buffer with a protein stabilizer (bovine), a surfactant, and sodium azide as a preservative.
Controls	Negative control	1 vial (0.125 mL each)	Chimeric monoclonal IgG antibody in pooled normal human serum.
	Positive control	1 vial (0.125 mL each)	Chimeric monoclonal IgG antibody in pooled normal human serum.

Materials Required but Not Provided

- Simoa HD-X Analyzer, Simoa software v3.1 (Item # 103385)
- Simoa HD-X System Wash Buffer 1 (Item # 100486)
- Simoa HD-X System Wash Buffer 2 (Item # 100487)
- Simoa HD-X Sealing Oil (Item # 100206)
- Simoa cuvettes (Item # 103346, 3000 ct box)
- Simoa disposable pipettor tips (Item # 101726)
- Simoa Discs (Item # 100001)
- Simoa microplate shaker (Item # 102899)
- X-Pierce™ XP-100 plate seals (K-1080, Sigma Aldrich item # Z722502)
- Simoa 96 well assay plates (Item # 101457)

WARNINGS AND PRECAUTIONS

For in vitro diagnostic and laboratory professional use only. For emergency authorization use only. For prescription use only.

This test has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a, that meet requirements to perform moderate or high complexity tests.

This test has been authorized only for detecting the presence of IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens.

The emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[3] Biosafety Level 2[4] or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- Simoa reagents contain methylisothiazolones, which are components of ProClin and are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.

R43 May cause sensitization by skin contact.

S24 Avoid contact with skin.

S35 This material and its container must be disposed of in a safe way.

S37 Wear suitable gloves.

S46 If swallowed, seek medical advice immediately and show this container or label.

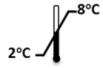
- For a detailed discussion of safety precautions during instrument operation, refer to the S*imoa HD-X Analyzer User Guide (EUA Edition) (USER-0105)*.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be assured if there are deviations from the instructions in this package insert.

Handling Precautions

- The reagents are 1-time use; any remaining material should be discarded after the completion of the assay run.
- Do not use reagent kits beyond the expiration date. When stored and handled as directed, reagents and calibrator are stable until the expiration date.
- Do not pool reagents within a kit or between reagent kits.
- Do not attempt to reuse tips, cuvettes, or Simoa Discs, as this will cause significant data quality deterioration.

Shipping and Storage Instructions

Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test reagents are shipped on cold packs. If the reagents arrive at room temperature, reagent integrity may be suspect. Contact Quanterix® service.

- 

Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test reagents must be stored at 2–8°C in an upright position.
- Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test Calibrators and Controls are shipped on dry ice. If these components arrive in an unfrozen state, their integrity may be suspect. Contact Quanterix service.
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Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test calibrators and controls must be stored at –80°C and should be kept upright.
- When stored and handled as directed, reagents and calibrators are stable until the expiration date.

Indications of Reagent Deterioration

If a control sample returns a concentration value out of the expected range, this may indicate deterioration of reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. Refer to the Calibration and Quality Control Procedures section of this document.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Insufficient sample processing may cause inaccurate results.
- Serum and plasma specimens should be immediately removed from the sample collection tubes (after centrifugation) and put in a separate tube that can then be aliquoted and frozen for future use.
- For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter. Do not use grossly hemolyzed specimens.
- Specimens thawed after frozen storage must always be mixed THOROUGHLY by low speed vortexing or inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- Specimens may be stored for up to 24 hours at 2–8°C prior to being tested. If testing will be delayed for more than 24 hours, specimens should be frozen at –20°C or colder.
- Specimen stability in different storage conditions has not been validated for this assay.
- The Simoa HD-X Analyzer does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen type is used in the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test.
- For freshly drawn serum specimens, ensure that complete clot formation has taken place prior to centrifugation. If specimens are centrifuged before a complete clot forms, the presence of fibrin or particulate matter may cause erroneous results.
- Centrifuge all specimens prior to assay. Centrifugation conditions should be sufficient to efficiently remove particulate matter and to clarify the sample, for example 5 minutes at 10,000 g for serum or plasma.
- Centrifuged specimens with a lipid layer on the top should be transferred to a secondary tube. Care must be taken to transfer only the clarified specimen without the lipemic material.
- For optimal results, inspect all samples for bubbles immediately before placing on the instrument. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross-contamination.
- Use caution when handling patient specimens to prevent cross-contamination. Use of disposable pipettes or pipette tips is recommended.
- Multiple freeze-thaw cycles of specimens should be avoided.
- Specimens with obvious microbial contamination should not be used.

PROCEDURE

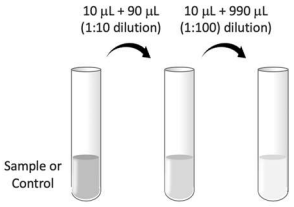
Assay Procedure

- The Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test assay definition must be downloaded from the customer portal and installed on the Simoa HD-X Analyzer prior to performing the assay.
- Calibrators, controls, and samples must be allowed to come to room temperature and mixed thoroughly before loading onto the HD-X Analyzer.
- Simoa reagents can be loaded onto the HD-X straight from refrigerated storage or after equilibration to room temperature.
- Solubilize RGP fully by heating at 30–37°C with constant vigorous shaking on the Simoa microplate shaker set to 800 rpm for a minimum of 30 minutes and a maximum of 4 hours. One RGP bottle is required for up to 48 tests. Two RGP bottles are required for 49-96 tests.
- Immediately before loading on the HD-X Analyzer, the Bead Reagent bottle must be mixed to resuspend the capture beads that may have settled. To resuspend the beads, vortex for a minimum of 30 seconds.

  Note: The bead diluent is formulated with an antifoam agent, but vortexing can still create foaming. If the foam does not dissipate within a few minutes, remove excess foam with a pipette prior to loading bead reagent onto the Simoa HD-X Analyzer.
- Set up the assay run on the instrument (see the *Simoa HD-X Analyzer User Guide (EUA Edition) (USER-0105)*).
  - Remove caps from reagent bottles and load the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test reagents (Bead Reagent, Detector Reagent, SBG Reagent) into the reagent bay.
  - Load samples, calibrators, controls, and RGP into the sample bay. (Note: Two bottles of RGP are needed per 96-test run. One bottle of RGP may be used for multiple smaller runs up to 48 tests, but total time on board the instrument should be limited to no more than 8 hours and one work shift.)
  - In the run set up screen, specify '**neat'** protocol. The samples will be diluted off-line prior to running the assay, and the instrument will not perform additional pre-dilutions. Follow the guidelines in the Specimen Dilution Procedure section of this package insert to set up sample dilutions.
  - Replenish consumables and system resources as needed prior to initiating the run, as described in the *Simoa HD-X Analyzer User Guide (EUA Edition) (USER-0105)*).
  - Initiate the run.

Specimen Dilution Procedure

- Treat specimens and assay Controls the same way.
- Perform a 1:1000 pre-dilution of specimens and controls with Sample Diluent. Given the large dilution, it is recommended to perform the dilution in two steps to maximize pipetting accuracy. A recommended two-step dilution is to dilute 10 µL of sample to 100 µL, followed by a subsequent dilution of a 10 µL aliquot of diluted sample to 1000 µL:



- If an alternative procedure is followed, volume transfers should be performed with appropriately calibrated pipettors and good pipetting technique to ensure proper accuracy.
- The HD-X system accepts two types of sample inputs: sample tubes or Quanterix-supplied 96-well plates. Both types of sample inputs are valid for calibrators, controls, and samples. Each replicate test consumes 100 µL of diluted sample. Multiple replicate tests of the same sample may be run from the same tube or well. The minimum sample volume in the tube or well depends on the number of replicates to be run and the required dead volume for the tube or well.
- **Note:** If samples are left on board the instrument or pipetted samples are left on a lab bench for more than an hour, evaporation effects may influence the results depending on the volume of sample. As a general guideline, at room temperature and normal humidity, a 60 µL sample will lose approximately 5% of its weight per hour. A 120 µL sample may lose 5% of its weight in approximately 3 hours. If pre-pipetted 96-well plates are to be stored prior to assay, it is recommended to seal the plate as described in the section below (see Quanterix-supplied 96-well plates).
  - Sample tubes. The minimum sample volume needed may depend on the type and volume of the tube due to different dead volumes. Samples introduced in 5-mL Nalgene Cryogenic sample tubes have a dead volume of 50 µL.
  - Quanterix-supplied 96-well plates. Quanterix-supplied 96-well plates have a dead volume of 30 µL. The maximum recommended sample volume for sealed Quanterix-supplied 96-well plates is 280 µL. Use of plate seals is necessary to prevent evaporation. X-Pierce Sealing Films (cat # XP-100) are the only plate seals compatible with the Simoa HD-X Analyzer. When placing seals, care must be taken to center the black circular marks over all plate wells. Once seal is placed, do not tip the plate or sample well contents may wick up onto the seal and cause cross-contamination of wells.

Calibration

- To perform a Semi-Quantitative SARS-CoV-2 IgG Antibody Test calibration, test Calibrators A through H in duplicate. All levels of Semi-Quantitative SARS-CoV-2 IgG Controls must be tested to evaluate the assay calibration. Ensure that all assay control values are within expected concentration ranges.
- Refer to the lot-specific Certificate of Analysis (CoA) for the concentrations of the calibrators and update in the assay definition.
- Once a Semi-Quantitative SARS-CoV-2 IgG Antibody Test calibration is accepted and stored, all subsequent samples may be tested without further calibration unless one or more Controls are out of their expected ranges (see Quality Control Procedures) or a reagent kit with a new lot number is used.
- The Semi-Quantitative SARS-CoV-2 IgG Antibody Test utilizes a 4 Parameter Logistic Curve fit data reduction method to generate a calibration curve. Specimen results are interpolated from the calibration curve.

Preparing Calibrators

- Calibrators should be brought to room temperature prior to pipetting. Do not heat the vial to accelerate thawing.
- When the solution is fully thawed, THOROUGHLY mix by multiple gentle inversions or vortexing. Frozen protein solutions can partition during freezing, so complete mixing of thawed material is critical for accurate calibrators.

Preparing Controls

- Controls should be brought to room temperature prior to pipetting. Do not heat the vial to accelerate thawing.
- When the solution is fully thawed, THOROUGHLY mix by multiple gentle inversions or vortexing. Frozen protein solutions can partition during freezing, so complete mixing of thawed material is critical for accurate controls.
- Controls should be included in each run and should follow the same dilution scheme selected for samples.

QUALITY CONTROL PROCEDURES

- Follow the specific quality control procedures in your laboratory.
- The Semi-Quantitative SARS-CoV-2 IgG Antibody Test Negative Control and Positive Control should be included in every batch run to assess calibration curve storage integrity and run validity.
- Control values and ranges listed in the lot-specific Certificate of Analysis should be considered only as guides. Each laboratory should establish statistically based control values and ranges with a sufficiently powered study. For guidance, it is recommended to consult with Clinical and Laboratory Standards, Institute (CLSI) Guideline C24, 4th ed., or other published guidelines for quality control recommendations.
- If the results from one or more of the controls are outside their expected ranges, the stored calibration curve may no longer be valid, and the assay may need to be re-calibrated. The length of time calibration curves may be stored must be validated for each laboratory. Alternatively, calibration can be performed with every batch run. Controls must be run with every calibration to assess calibration accuracy and run validity.

- If quality control results do not meet acceptance criteria defined by your laboratory, sample results may be suspect. Follow the established quality control procedures for your laboratory. For troubleshooting information, refer to the *Simoa HD-X Analyzer User Guide (EUA Edition) (USER-0105)*).

Interpretation of Results

- The assay clinical cutoff is 0.77 µg/mL.

Result Value	Result Reported	Interpretation
< 0.77 µg/mL	Negative for SARS-CoV-2 IgG antibodies.	SARS CoV-2 IgG antibodies were not detected, or IgG antibodies are below the detection limit of the assay.
≥ 0.77 µg/mL	Positive for SARS-CoV-2 IgG antibodies with the numeric value in µg/mL for semi-quantitative measurements.	SARS-CoV-2 IgG antibodies were detected.

- Results of this assay should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings.
- Do not use this assay in patients taking biotin supplements. Testing specimens in patients taking biotin may lead to false results. Therefore, do not report the results with this device unless it is confirmed that the patient is not taking biotin.

  Note: Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the *Simoa HD-X Analyzer User Guide (EUA Edition) (USER-0105)*).

LIMITATIONS OF THE PROCEDURE

- For in vitro diagnostic use on the HD-X under Emergency Use Authorization only. Other Quanterix instrument platforms are not authorized for diagnostic testing.
- The clinical applicability of a quantitative or semi-quantitative result is currently unknown and cannot be interpreted as an indication or degree of immunity nor protection from reinfection, nor compared to other SARS-CoV-2 antibody assays.
- COVID-19 patients may have a delayed antibody response from the time of infection with insufficient levels of antibody to be detectable by the assay.
- This device should not be used to diagnose or exclude acute SARS-CoV-2 infection. Direct testing for SARS-CoV-2 with a molecular assay should be performed to evaluate acute infection in symptomatic individuals.
- Performance has only been established with the specimen types listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.
- Results from antibody testing should not be used as the sole basis for patient management decisions. Test results should be interpreted in conjunction with clinical observations, patient history, epidemiological information, and other laboratory findings.
- A positive result may not indicate previous SARS-CoV-2 infection. Consider other information, including clinical history and local disease prevalence, in assessing the need for a second, but different, serology test to confirm an immune response.
- A negative result means the sample is considered non-reactive for SARS-CoV-2 IgG antibodies by the criteria of the Semi-Quantitative SARS-CoV-2 IgG Antibody Test. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. A negative result may indicate that IgG antibodies are present in concentrations below the clinical cutoff or detection limit of the assay. This can occur during acute infection prior to seroconversion.
- IgG concentration results from the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test may not be used interchangeably with values obtained with different manufacturers' test methods, i.e., numerical results between different methods are not comparable.
- SARS-CoV-2 IgG antibodies may be below detectable levels in samples collected from patients less than 14 days from a positive polymerase chain reaction (PCR) result. In addition, the immune response may be depressed in elderly, immunocompromised, or immunosuppressed patients.
- Specimens with direct evidence of antibodies to non-SARS-CoV-2 coronavirus strains such as HKU1, NL63, OC43, or 229E have not been evaluated with this assay.
- Cross-reactivity may occur due to past or present infection with HSV.
- It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to re-infection.
- This test should not be used for screening donated blood.
- Bacterial contamination of specimens may affect the test results.
- Do not use this assay in patients taking biotin supplements. Testing specimens in patients taking biotin may lead to false results. Therefore, do not report the results with this device unless it is confirmed that the patient is not taking biotin.
- The presence of 20 mg/dL of conjugated bilirubin may result in a decrease of the observed value of approximately 16%. The presence of 30 U/mL of rheumatoid factor may result in a decrease or increase of the observed value of approximately 22%. The presence of 12 g/dL or 15 g/dL of protein may result in an increase of the observed value of approximately 17% and 20%, respectively. Do not test icteric samples.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with immunoassays. People routinely exposed to animals or animal serum products can be prone to this interference, and anomalous values may be observed.
- The performance of this test has not been established in individuals who have received a COVID-19 vaccine. The clinical significance of a positive or negative antibody result following COVID-19 vaccination has not been established, and the result from this test should not be interpreted as an indication or degree of protection from infection after vaccination.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. The samples for the negative agreement study were collected in USA prior to December 2019. The samples for the positive percent agreement were collected in Germany in March and April 2020 and in USA between May and July 2020. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Conditions of Authorization for the Laboratory

The Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

Authorized laboratories using the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test, must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

- Authorized laboratories using the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test must include with result reports of the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test must use the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test are not permitted.
- Authorized laboratories that receive the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test must notify the relevant public health authorities of their intent to run the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test prior to initiating testing.

- Authorized laboratories using the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories must collect information on the performance of the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test and report to DMD/OHT7-OIR/OPEQ/ CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Quanterix Corporation at www.Quanterix.com any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test of which they become aware.
- All laboratory personnel using the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test must be appropriately trained in automated immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit and the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test in accordance with the authorized labeling. All laboratory personnel using the assay must also be trained in and be familiar with the interpretation of results of the product**.**
- Quanterix Corporation, authorized distributors, and authorized laboratories using the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.
- The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate and high complexity tests" as "authorized laboratories."

PERFORMANCE CHARACTERISTICS

Precision

A two-site precision study was conducted in accordance with CLSI Document EP05-A3.[6] Samples and assay controls were assayed in duplicate (samples) or triplicate (controls) in 2 runs per day for 3 days using the HD-X system and one lot of Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test reagents, calibrators, and controls per site. Since different lots of controls were used at two sites, between-site component for controls was not evaluated. Results from the study are exhibited in the tables below:

	n	Mean IgG (µg/mL)	Repeatability %CV	Between-run %CV	Between-day	Total %CV
Lot 1/Site 1						
Control 1	18	0.454	8.6%	2.4%	0.0%	9.0%
Control 2	18	3.236	3.9%	2.4%	4.6%	6.5%
Lot 2/Site 2						
Control 1	18	0.311	4.8%	5.2%	0.0%	7.1%
Control 2	18	2.855	2.2%	2.5%	2.5%	4.2%

	n	Mean IgG (µg/mL)	Repeatability %CV	Between-run %CV	Between-day	Between-lot/site	Total %CV
Lot 1/Site 1							
Serum 1 (Negative)	12	0.250	10.8%	0.0%	5.2%		12.0%
Serum 2 (Low Positive)	12	1.101	9.9%	0.0%	3.6%		10.6%
Serum 3 (moderate Positive)	12	111.1	8.1%	6.5%	9.7%		14.2%
Lot 2/Site 2							
Serum 1 (Negative)	12	0.223	10.3%	0.0%	4.5%		11.2%
Serum 2 (Low Positive)	12	1.032	3.2%	0.0%	1.4%		3.5%
Serum 3 (moderate Positive)	12	117.8	7.8%	10.0%	0.0%		12.7%
Overall							
Serum 1 (Negative)	24	0.236	10.6%	0.0%	4.7%	7.6%	14.0%
Serum 2 (Low Positive)	24	1.066	7.6%	0.0%	2.8%	4.0%	9.0%
Serum 3 (moderate Positive)	24	114.4	7.9%	8.6%	6.0%	0.0%	13.1%

Analytical Sensitivity

The limit of blank (LoB) and limit of detection (LoD) were estimated according to CLSI Document EP17-A2.[7] LoB was determined separately with two lots of reagents, calibrators, and controls as the 95% percentile across 6 replicates each of 5 seronegative samples tested over a 3-day period. The greater LoB calculated from the 2 lots was 0.029 µg/mL. LoD was determined with 2 lots of reagents, calibrators, and controls based on the LoB and standard deviation of a population of repeated measurements of 5 low seropositive samples tested over a 3-day period (30 replicates total per lot). The greater LoD calculated from the 2 lots was 0.051 µg/mL.

Limit of Quantitation

The limit of quantitation (LoQ) was estimated according to CLSI EP17-A2.[7] LoQ was determined with 2 lots of reagents, calibrators, and controls as the lowest concentration of IgG giving ≤±15% inaccuracy and imprecision of %CV ≤20%. A panel of 4 seronegative samples supplemented with known increasing concentrations of anti-SARS-CoV-2 IgG were repeatedly tested over 3 days across 2 lots and 2 HD-X instruments (n = 9 replicates/panel member each lot). Bias from expected IgG values and imprecision were evaluated for each panel member. The greater LoQ determined from the 2 lots was 0.213 µg/mL.

Linearity

Dilution linearity was conducted in accordance with CLSI Document EP06-A.[8] Seropositive serum and/or anti-SARS-CoV-2 IgG were admixed with seronegative serum in different ratios to prepare a dilution series comprised of a minimum of five levels within the linear range of the assay. A linear regression was performed for each dilution series, and the slope, intercept, and R^2 were calculated. Also, polynomial regression fitting of each dilution series was performed comparing linear and non-linear fits. Deviations from linearity were evaluated by comparing the mean concentrations of four replicates vs a linear fit at each dilution. Linearity was demonstrated for the analytical measuring interval of 0.21 µg/mL to 250 µg/mL with deviations from linearity within 15%. Taking into consideration the estimates of LoB, LoD, LoQ, precision, and linearity, the analytical measuring interval is 0.21 µg/mL to 250 µg/mL

Recovery

Accuracy of recovery of known quantities of IgG antibody spiked into negative human samples was examined in two studies. In the first study, 3 µg/mL of anti-SARS-CoV-2 IgG were spiked into 3 serum and 2 K2EDTA plasma samples from pre-pandemic donors. Each sample was measured once, and vendor-provided concentrations of the antibody were used for calculations. Mean recovery was 123% and 124% for plasma and serum, respectively. In the second study, 1.7 µg/mL, 4.2 µg/mL, and 17 µg/mL of anti-SARS-CoV-2 IgG were spiked into 3 serum and 3 K2EDTA plasma samples from pre-pandemic donors. Samples were measured in duplicates, and the concentration of the IgG stock solution was value assigned by assaying four dilutions across the assay range and averaging the results. Mean recovery was 98% and 95% for plasma and serum, respectively.

Class Specificity

The anti-human IgG antibody used in the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test demonstrates class-specific reactivity only to human IgG isotypes. No binding interactions were observed to human IgM.

Cross Reactivity

Analytical specificity of the assay was evaluated for potentially cross reacting antibodies. 50 samples from patients with the following viral infections were tested: HIV (20 samples), Hepatitis B (10 samples), Hepatitis C (10 samples), and herpes simplex virus (10 samples). Among these samples 48 were negative, and 2 out of 10 herpes simplex virus samples were false positive with the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test.

Interfering Substances

Potential interference of endogenous substances commonly found in serum and plasma specimens, including hemoglobin, conjugated bilirubin,

unconjugated bilirubin, triglycerides, and rheumatoid factor (RF) was evaluated. Spiked and unspiked serum and plasma samples were tested, and the testing demonstrated the following changes between quantitative results (acceptance criteria were qualitative agreement >95% and <10% bias):

Substance	Substance Concentration	Qualitative Agreement Between Samples with and without Substance	Average Difference Between Samples with and without Substance, %
Triglycerides	1000 mg/dL	100%	9
Hemolysate	500 mg/dL	100%	10
Bilirubin Conjugated	20 mg/dL	100%	16
Bilirubin Unconjugated	20 mg/dL	100%	8
Rheumatoid Factor	30 U/mL	100%	22
Protein (Human Serum Albumin)	15 g/dL	100%	8

Sixteen percent (16%) negative bias was observed in the presence of 20 mg/dL of conjugated bilirubin while a 22% negative or positive bias was observed with 30 U/mL RF, increasing the risk of false results. The average bias observed in the presence of 15 g/dL human serum albumin across three seropositive and three seronegative serum pools was 8%, however the presence of 12 g/dL or 15 g/dL of protein resulted in positive biases of approximately 17% and 20% respectively in individual sample pools. Do not use this assay in patients taking biotin supplements. Testing specimens in patients taking biotin may lead to false results. Therefore, do not report the results with this device unless it is confirmed that the patient is not taking biotin.

Clinical Performance

Positive Percent Agreement (PPA)

A study was performed to determine the clinical performance of the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test. To estimate the positive percent agreement (PPA) between the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test and the polymerase chain reaction (PCR) comparator, 157 serum and plasma samples from 50 symptomatic patients determined to be SARS-CoV-2 positive by RT-PCR were tested. The PPA and the 95% confidence interval (CI) were calculated using the initial sample collected in each of the 3 designated time frames since positive PCR test (i.e. ≤ 7 days, 8-14 days, and 15-30 days), per subject. The performance summary data is illustrated in the table below.

Positive Agreement Days Post RT-PCR Positivity, considering first bleed in each time period

Days from positive PCR test	Number Tested	Reactive	PPA	95% CI
0 - 7	31	14	45.16%	29.16% – 62.23%
8 - 14	16	14	87.50%	63.98% - 96.50%
≥15	28	28	100.00%	87.94% - 100.00%
Total	75			

Note: A positive PCR result confirms the presence of virus, but seroconversion to IgG reactivity follows a latency period of undetermined length. Therefore, samples collected early in the infection are expected to be non-reactive for anti-SARS-CoV-2 IgG.

Negative Percent Agreement (NPA)

496 serum and plasma samples collected from pre-pandemic donors were tested to determine the Negative Percent Agreement (NPA). Out of 496, 4 serum samples were positive with the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test, which translates into a negative percent agreement of 99.19% (492/496), (95% CI: 97.95% - 99.69%).

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For additional information on Simoa technology, instrument operation maintenance, and data analysis, refer to the *Simoa HD-X Analyzer User Guide (EUA Edition) (USER-0105)*.

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