CoVisa™IgG Test

Instructions for Use

For *In Vitro* Diagnostic Use Only

Prescription Use Only

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Intended Use

- The CoVisa™ IgG Test is an Enzyme-Linked Immunosorbent Assay (ELISA) intended for qualitative detection of IgG antibodies to SARS-CoV-2 in human serum and plasma (EDTA-treated). The CoVisa™ IgG 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. 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, to perform moderate or high complexity tests.
- Results are for the detection of SARS CoV-2 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 positive results to the appropriate public health authorities.
- The sensitivity of CoVisa™ IgG 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 CoVisa™ IgG Test may occur due to cross-reactivity from pre-existing antibodies or other possible causes.
- This test is not cleared, CLIA waived, approved, or subject to an approved investigational device exemption.

Introduction

- The Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2) is a single-stranded RNA virus which belongs to the family of coronaviruses. Coronaviruses are composed of several proteins including the Spike (S), Envelope (E), Membrane (M), and Nucleocapsid (N).
- This novel coronavirus is believed to have originated in China, in the city of Wuhan, Hubei Province. In humans, SARS-CoV-2 causes respiratory infections and is mainly transmitted by respiratory droplets and aerosols from infected patients.

Principles of the Procedure

The CoVisa™ IgG Test allows the qualitative detection of the human anti SARS-CoV-2 IgG antibodies present in human serum or plasma (EDTA-treated), utilizing the Enzyme Linked Immunosorbent Assay (ELISA) principle.

The CoVisa™ IgG Test Plate is coated with SARS-CoV-2 recombinant protein. The operator applies assay controls and diluted human serum or plasma (EDTA-preserved) specimens to the SARS-CoV-2 coated-plate. Human anti SARS-CoV-2 antibodies may specifically bind to the coated SARS-CoV-2 protein. Horseradish Peroxidase (HRP)-conjugated anti-human IgG antibody solution is applied to each well for detection of immobilized human anti SARS-CoV-2

antibodies. Upon application of HRP-substrate a colorimetric reaction proportional in strength to the concentration of anti SARS-CoV-2 antibodies present in the respective specimen will occur. Optical Density quantitation allows to determine anti SARS-CoV-2 antibody containing specimens (Samples with Optical Density at 450nm (OD450) at 0.5 or greater are defined as anti-SARS-CoV-2 IgG positive).

Warnings and Precautions

- Biohazard: biological samples such as tissues, body fluids, and blood have the potential to transmit infectious diseases; when handling of these substances and assay waste, follow all applicable local, state/provincial, and/or national regulations.
- Use routine Good Laboratory Practices (GLP). Do not eat, drink, or smoke in designated work areas.
- Care should be taken to avoid contact of skin or eyes with specimens and kit reagents in general. In case of contact, wash immediately with plenty water. Seek medical attention if necessary.
- Do not use kit component that appears damaged or irregular.
- Do not use expired reagents.
- The CoVisa™ IgG Test Developing Solution contains small amount of 3,3',5,5'-Tetramethylbenzidine (TMB), and the CoVisa™ IgG Test Stop Solution is an acid solution. In case of skin contact, eye contact, ingestion, or inhalation, immediately use a shower, eyewash fountain, hand/face spray unit, and other emergency equipment to flush affected area(s) with plenty of water. Seek medical attention if necessary.
- Read the test instructions carefully before using the product.

Specimen

- The specimens compatible with this test are human serum and plasma (EDTA-treated).
- Use of specimen precipitated, contaminated by bacteria or protein suspension should be avoided.
- Do not heat the specimens.

Storage

Appropriate storage conditions and associated stability data of the CoVisa™ IgG Test are summarized below.

Note: during shipping, exposure to temperatures up to 28°C is permitted for up to 72 hours.

Table 1. Kit Storage Conditions

CoVisa™ IgG Test	Temperature	Duration
Part #2100000	2°C to 8°C	See product label for actual expiration date

Storage of Specimen

- The Clinical and Laboratory Standards Institute (CLSI GP44-A4) recommends that specimens should be stored at room temperature no longer than 8 hours. Specimens can be stored refrigerated at +2°C to +8°C for 48 hours. Beyond 48 hours, specimens should be frozen at -20°C or lower.
- Samples should not be repeatedly frozen and thawed.
- Frozen samples must be mixed well after thawing and prior to testing.
- Diluted samples should be incubated within 8 hours.
- Do not use bacterially contaminated samples.
- The individual laboratory is responsible for managing its own studies to determine its own specific stability criteria.

Materials Provided

Table 2. CoVisa™ IgG Test Kit Contents (each kit contains reagents to process 94 specimens x 5 = 470 specimens total)

CoVisa™ IgG Test Item	Quantity
Plate	5 x 96-well plate
Specimen Dilution Buffer	4 bottles of 60 mL
Detector Concentrate	1 bottle of 8 mL
Detector Dilution Buffer	1 bottle of 60 mL
20X Wash Solution	1 bottle of 60 mL
Developing Solution	1 amber bottle of 60 mL
Stop Solution	1 bottle of 60 mL
Negative Control	5 vials
Instructions For Use (IFU)	1 booklet
Quick Guide	1 sheet

Equipment Required but Not Provided

- Microplate reader: wavelength of 450 nm, reference wavelength range from 620 nm to 650 nm
- Calibrated multi-channel pipette to deliver between 100 µL and 200 µL
- Calibrated single-channel micropipettes to deliver between 10 µL and 1000 µL
- Pipette tips
- Serological pipettes
- Timer
- Vortexer
- Thermometer
- Distilled or deionized water to dilute the concentrated 20X Wash Solution
- Microtiter plate cover or equivalent
- Test tubes or microtiter plate to handle dilutions. Non-protein binding (polypropylene)
- 500 mL glass or plastic container, or equivalent to prepare the 1X Wash Solution
- 15 mL Falcon polypropylene tube or equivalent to prepare the Detector Solution
- Reagent Reservoirs
- Absorbent paper / paper towel

Equipment Recommended

- Automatic microplate washer.

  Note: The 20X Wash Solution provided in the CoVisa™ IgG Test allows manual washing of the microplates. Bulk 20XWash Solution is available to accommodate the automatic wash process.

Procedure

- **Make sure all the reagents are equilibrated to room temperature (15°C-25°C) before use.**
- Do not run test below 15°C or above 25°C.
- Pipet all solutions slowly, and avoid the formation of bubbles for accuracy.
- Change pipette tips between all pipetting steps.
- Cover or cap all kit components and store at 2-8° C when not in use.
- Do not allow the microplate to dry between changes of solutions in the wells.
- The Developing Solution is light-sensitive, avoid prolonged exposure to light.
- Any unused well of the CoVisa™ IgG Test Plate cannot be used during a subsequent experiment.

  Note: the instructions provided below are to run one (1)CoVisa™ IgG Test Plate. Volumes need to be adapted if more than one CoVisa™ IgG Test Plate is run.

1. Assay Controls

Note: It is mandatory to perform one positive and one negative control per CoVisa™ IgG Test Plate.

- The Positive Control is located on the CoVisa™ IgG Test Plate, well position (row / column) H/12.
- The Negative Control is applied onto the CoVisa™ IgG Test Plate in well position (row / column) H/11.
- Wells H/11 and H/12 cannot be used for specimen testing.

a) Add 500 µL of CoVisa™ IgG Test Specimen Dilution Buffer to one (1) CoVisa™ IgG Test Negative Control vial.

b) Vortex for 5 seconds.

c) Incubate the CoVisa™ IgG Test Negative Control vial at 15°C to 25°C, for 10min.

d) Vortex for 5 seconds. Keep for use under step 3.

2. SPECIMEN PREPARATION

a) Specimens can be serum or plasma (EDTA-treated). Use a Dilution Tube for each patient specimen and label appropriately.

b) Prior to application to the test, patient specimens are to be diluted 1:100 in Specimen Dilution Buffer.

Example: 5 µL of serum specimen is added to 495 µL of CoVisa™ IgG Test Specimen Dilution Buffer.

c) Vortex for 5 seconds after each step and avoid forming bubbles.

3. SPECIMEN AND ASSAY CONTROLS APPLICATION

a) Remove the CoVisa™ IgG Test Plate(s) from the pouch.

b) Label CoVisa™ IgG Test Plate(s) appropriately.

c) Using an appropriate single-channel pipette, transfer 90 µL from the CoVisa™ IgG Test Negative Control vial (prepared under step 1) into well H/11.

d) Using an appropriate single-channel pipette, add 90 µL of CoVisa™ IgG Test Specimen Dilution Buffer into H/12 well (positive control).

e) Using an appropriate single-channel pipette, transfer for each prepared Specimen (from step 2) 90 µL from the "Dilution Tube" into the appropriate wells.

f) Visually check that all wells applied for testing contain solution. Gently tap the CoVisa™ IgG Test Plate to ensure that the solutions are evenly distributed over the bottom of all wells used, and cover the CoVisa™ IgG Test Plate with a microtiter plate cover.

g) Incubate the CoVisa™ IgG Test Plate at 15°C to 25°C, for 1 hour.

Note: towards the end of the incubation, prepare 1X Wash Solution and Detector Solution (step 4).

4. 1X WASH AND DETECTOR SOLUTIONS PREPARATION

1X Wash Solution

a) If CoVisa™ IgG Test Plate washes are performed manually, prepare 200 mL of 1X Wash Solution by adding 10mL of the CoVisa™ IgG Test 20X Wash Solution to 190mL of distilled or deionized water. Mix thoroughly before use. Keep for used under step 5.

b) If CoVisa™ IgG Test Plate washes are performed with an automated plate washer, prepare 400 mL of 1X Wash Solution by adding 20mL of the CoVisa™ IgG Test 20X Wash Solution to 380mL of distilled or deionized water. Mix thoroughly before use. Keep for use under step 5.

Detector Solution

c) Obtain a 15 mL polypropylene tube or equivalent and label it as “1X Detector Solution”.

d) Obtain the CoVisa™ IgG Test Detector Concentrate and the CoVisa™ IgG Test Detector Dilution Buffer. Gently but thoroughly shake both solutions before use.

e) Using an appropriate single-channel pipette, add 1 mL of CoVisa™ IgG Test Detector Concentrate to 11 mL of CoVisa™ IgG Test Detector Dilution Buffer into the tube labelled as "1X Detector Solution”.

f) Mix the “1X Detector Solution” tube by repeated gentle inversion. Keep for use under step 6.

5. First Wash Step

a) After completion of the 1-hour incubation at 15 to 25°C under step 3.g., obtain the CoVisa™ IgG Test Plate and empty its contents into a sink or proper waste container.

If the CoVisa™ IgG Test Plate washes are performed manually, follow step b); if CoVisa™ IgG Test Plate washes are performed with an automated plate washer, follow step c).

b) Manual wash procedure:

1. Obtain a reagent reservoir, label it as "Wash Solution", and pour into it 1X Wash Solution (prepared under step 4.b). Approximately 22 mL of “Wash Solution” are needed per wash step and per CoVisa™ IgG Test Plate.
2. Using an appropriate multi-channel pipette, transfer 200µLof 1X Wash Solution into each well.
3. Tap the CoVisa™ IgG Test Plate gently for 5 seconds.
4. Allow the 1X Wash Solution to soak for 1 minute.
5. Empty the CoVisa™ IgG Test Plate content into a sink or proper waste container, using a “wrist snap” action.
6. Remove residual liquid by tapping the CoVisa™ IgG Test Plate upside down on a stack of absorbent paper.
7. Repeat steps b.2 to b.6 for 3 more times.

c) Wash procedure using an automated plate washer:

1. Ensure that the plate washer reservoir contains at least 400 mL of 1X Wash Solution (prepared at step 4.c).
2. Set wash volume to 0.40 mL to 0.45 mL of Wash Solution per CoVisa™ IgG Test Plate well.
3. Allow the 1X Wash Solution to soak for 1 minute.
4. Set aspirate function to empty ≥ 95% volume from the wells.
5. Repeat steps c.2 to c.4 for 3 more times.

Remove the CoVisa™ IgG Test Plate from the automated plate washer and tap the CoVisa™ IgG Test Plate upside down on a stack of absorbent paper to remove residual liquid.

6. Detector Application

a) Obtain a reagent reservoir, label it as “Detector”, and pour the content of the "1X Detector Solution" tube (prepared at step 4) into it.

b) After completion of the First Wash (step 5), obtain the CoVisa™ IgG Test Plate, and using an appropriate multi-channel pipette, transfer 100 µL of 1X Detector Solution into each well.

c) Gently tap the plate to ensure that the Detector Solution is evenly distributed over the bottom of each well, and cover the plate with a microtiter plate cover.

d) Incubate the plate at 15°C to 25°C for 1 hour.

7. Second Wash Step

a) When the 1-hour incubation at 15 to 25°C of step 6.d. is over, obtain the CoVisa™ IgG Test Plate and empty its contents into a sink or proper waste container.

b) Follow the same washing procedure performed during the First Wash (step 5):

-if wash step is performed manually, follow steps 5.b.

-if wash step is performed with an automated plate washer, follow step 5.c.2 to 5.c.6.

8. Development and Stop Steps

a) Obtain a reagent reservoir, label it as “Developing Solution” and fill it with 12 mL of CoVisa™ IgG Test Developing Solution.

b) After completion of the Second Wash (step 7), use a multi-channel pipette, and transfer 100 µL of CoVisa™ IgG Test Developing Solution to each well of the CoVisa™ IgG Test Plate.

c) Gently tap the plate to ensure that the CoVisa™ IgG Test Developing Solution is evenly distributed over the bottom of each well.

d) Cover the CoVisa™ IgG Test Plate with a microtiter plate cover and incubate, in the dark, for 20 min at 15°C to 25°C.

e) During the incubation time of step 8.d., obtain a reagent reservoir, label it as "Stop Solution", and transfer 12 mL of CoVisa™ IgG Test Stop Solution into it.

When the 20 min incubation of step 8.d is completed, transfer 100 µL of CoVisa™ IgG Test Stop Solution from the "Stop Solution" reservoir into each well on the plate applied in testing (including H/11 and H12).

Note: wells already contain 100 µL of CoVisa™ IgG Test Developing Solution, and the Stop Solution is added to the Developing Solution in the wells.

Results must be read immediately after addition of the CoVisa™ IgG Test Stop Solution (step 9).

9. TEST RESULTS INTERPRETATION

a) Ensure homogeneity of the colour solution by carefully tapping the CoVisa™ IgG Test Plate for 10 seconds, and immediately read results with a microplate spectrophotometer at 450 nm.

b) Interpret results following Tables 3 and 4.

- CoVisa™ IgG Test outcome interpretation requires that the "Negative Control" (in well position H/11) reads OD_{450} 0.25 or less. If the "Negative Control" reads OD_{450} greater than 0.25, the entire CoVisa™ IgG Test Plate is invalid.
- The CoVisa™ IgG Test Plate "Positive Control" needs to read OD_{450} 0.50 or greater; otherwise the entire CoVisa™ IgG Test Plate is invalid.
- A sample is called "**Positive**", if the OD_{450} is determined at 0.5 or greater.
- A sample is called "**Negative**", if the OD_{450} is determined at lesser than 0.5.

Table 3. System Suitability Evaluation

System Suitability	Description	Instructions
CoVisa™ IgG Test is **Valid**	-If the Positive Control H/12 well reads $OD_{450} \geq 0.50$ **and** -If the Negative Control H/11 well reads $OD_{450} \leq 0.25$	Interpret results following Table 4
CoVisa™ IgG Test is **Invalid**	-If the Positive Control H/12 well reads $OD_{450} < 0.5$ **or** -If the Negative Control H/11 well reads $OD_{450} > 0.25$	Repeat entire plate

Table 4. Test Results Interpretation

System Suitability	Sample Optical Density	Test Result
CoVisa™ IgG Test is **Valid**	Sample reads $OD_{450} < 0.5$	**Negative** for anti SARS-CoV-2 IgG antibody
CoVisa™ IgG Test is **Valid**	Sample reads $OD_{450} \geq 0.5$	**Positive** for anti SARS-CoV-2 IgG antibody

Limitations of the Procedure

- This test has not been reviewed by the FDA.
- Use in conjunction with other clinical patient evaluations.
- Failure to follow the test procedure and instructions on test results interpretation may adversely affect test performance and/or invalidate the test result.

- If the level of antigen in a specimen is below the limit of detection of the test, a negative test result may occur.
- Negative results do not exclude the presence of other coronavirus or non-coronavirus viral infections and should not be used as the sole basis for treatment or other patient management decisions.
- 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.
- A Negative can be because the test has been performed earlier than 14 days after COVID-19 diagnosis.
- Positive results do not exclude co-infections with other viral or bacterial pathogens.
- 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.
- Monoclonal antibodies may fail to detect, or detect with less sensitivity, SARS-CoV-2 viruses that have undergone amino acid changes in the target epitope region.
- 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.
- Not for screening of donated blood.

Assay Performance Summary

a) Cross-Reactivity

To determine cross-reactivity of the CoVisa™ IgG Test to non-SARS-CoV-2 pathogens, serum specimens derived from subjects believed to be negative for SARS-CoV-2 (specimen collected before the assumed emergence of COVID-19) and with confirmed infections or conditions, as shown in the following table, were tested.

Table 5. Cross-reactivity results

Human serum: pathogen	# samples applied / # positive
Hepatitis B	5/0
Hepatitis C	4/0
HIV	2/0
Influenza A	2/0
ANA	5/0

b) Class specificity

The anti-human IgG antibody used in the CoVisa™ IgG Test demonstrates class-specific reactivity only to human IgG isotypes. No binding interactions were observed to human IgM, human IgA. (CoA available upon request).

c) Clinical Agreement Study

Table 6. Clinical data results and scatter blot

		COMPARATOR METHOD (SARS-COV-2 RT-PCR OR PRIOR 09-2019)	
		Positive	Negative
CoVisa™ IgG Test	Positive	38	1
	Negative	0	130

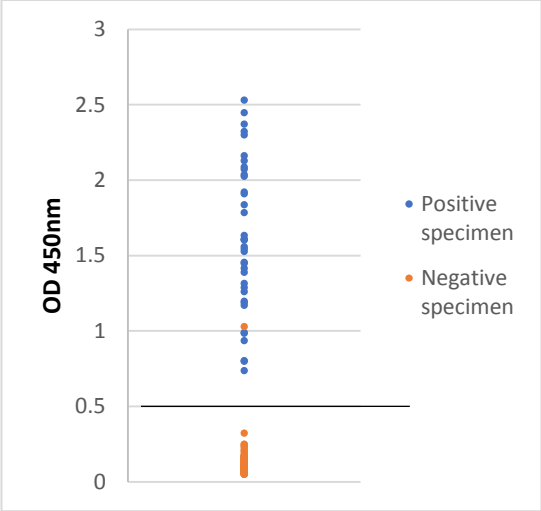

Figure 1: Scatter blot of study outcome for specimens called “negative” and “positive”, with suggested cut-off

Clinical performance of the CoVisa™ IgG Test was evaluated by testing 38 positive serum specimens (11 of these were contrived) from patients more than 14 days after a positive diagnosis for SARS-CoV-2 infection by RT-PCR. 131 negative specimens were confirmed negative for infection with SARS-CoV-2. 100 of the negative specimens were collected on or before September 2019. The remaining SARS-CoV-2 “negative” had a negative RT-PCR for SARS-CoV-2 RNA and no clinical history consistent with possible COVID-19. All RT-PCR tests were conducted with a FDA EUA COVID-19 real-time reverse transcriptase PCR test.

Results from the CoVisa™ IgG Test showed 38 of 38 SARS-CoV-2 positive serums tested positive and 130 of 131 SARS-CoV-2 negatives serum tested negative.

The PPA is 100% and NPA is 99.24%.

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