VirIntel COVID-19 Antibody Test

1 plate - 96 wells 42 Tests

For In Vitro Diagnostics Only

The VirIntel COVID-19 Antibody Test is a qualitative in vitro diagnostic test, in a one-step antigen capture format, for the detection of antibodies to SARS-Cov-2 in human serum samples.

1. Intended Use

The VirIntel COVID-19 Antibody Test is a one-step antigen capture format Enzyme-Linked Immunosorbent Assay (ELISA), intended for the qualitative detection of antibodies (IgG) to two immunogenic SARS-CoV-2 proteins in human serum. It is intended for identifying individuals with an adaptive immune response to SARS-CoV-2, indicating prior infection.

The assay should not be used to diagnose acute SARS-CoV-2 infection. It should not be used to claim improbability of re-infection, since to date no medical data is available on the subject.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a, to perform high complexity tests.

IgG antibodies to SARS-CoV-2 generally begin to be detectable in blood two weeks after initial infection. The duration of time in which they are present differs between patients and is overall 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 the VirIntel COVID-19 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 with the VirIntel COVID-19 Antibody Test may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

2. Description of the Test

Serological antibody test is a diagnostic identification of the presence of certain antibodies in a patient's blood. Antibodies are glycoproteins formed by the B-cells as a specific immune system response to infections and foreign molecules. Serological test allows to tell whether a specific person has been through a certain infection or not, and thus determine their risks of getting sick and/or spreading the infection further. Serological surveys are also used in epidemiological studies to determine the prevalence and spread rate of a disease within a population.

COVID-19 is a disease caused by a virus from the Coronaviridae family known as SARS-CoV-2. Other members of the family, which affect humans, are four "common cold" seasonal coronaviruses, which cause mild upper and lower respiratory syndromes, and widely known SARS (SARS-CoV-1) and MERS (MERS-CoV) coronaviruses, which were responsible for outbreaks in 2003 and 2014 in Asia and Middle East among thousands of people, causing severe acute respiratory syndromes.

SARS-CoV-2 is an enveloped virus; its genome is a single-stranded positive-sense RNA. It was first identified in December 2019, in Wuhan City, China, after several individuals had developed severe pneumonia symptoms resembling SARS-Cov-1 infection. The virus has quickly spread, reaching all the world countries, and in March 2020, WHO officially announced COVID-19 as a pandemic.

The incubation period of the COVID-19 infection usually ranges from 1 to 14 days. The virus is mainly detected in respiratory secretions, and the general transmission of infection is considered airborne. It has been shown that the virus attaches to pulmonary cells using their ACE-2 receptors, followed by endocytosis. Immune response is expected to build starting from a week. Symptoms vary between patients and may include fever, dry cough, anosmia, sputum production, headache, dyspnea, fatigue, nausea, diarrhea, and others. While some cases can be asymptomatic, others lead to acute respiratory distress syndrome (ARDS) and even death.

VirIntel COVID-19 Antibody Test allows detection IgG antibodies to two proteins of SARS-CoV-2, a surface glycoprotein receptor-binding domain (RBD) and a nucleocapsid phosphoprotein (N), providing comprehensive data on the immune response to the infection.

3. Principle of the Procedure

VirIntel COVID-19 Antibody Test is a dual antigen Enzyme-Linked Immunosorbent Assay (ELISA) for qualitative detection of anti-SARS-CoV-2 IgG antibodies in human blood serum. Two SARS-Cov-2 proteins are used for capture and detection of the antibodies: a surface glycoprotein receptor-binding domain (RBD) and a nucleocapsid phosphoprotein (N).

The blood serum samples and the control monoclonal IgG antibodies recognizing the SARS-CoV-2 RBD and N proteins are diluted and incubated with the SARS-Cov-2 proteins (antigens) immobilized in the microplate wells. The anti-SARS-CoV-2 antibodies form complexes with the SARS-Cov-2 proteins on the microplate well surface. All other antibodies present in the blood serum are removed from the microplate well during the washing step.

The secondary (tracer) antibody recognizing human IgG is used to detect the antibodies that remained bound to the microplate well surface after the washing step. The secondary antibody is coupled to a horseradish peroxidase (HRP) enzyme, enabling a colorimetric detection of the immune complex.

The chromogenic substrate, o-phenylenediamine dihydrochloride (OPD), develops a yellow color upon incubation with HRP and hydrogen peroxide. The reaction is stopped by addition of hydrochloric acid, and the optical density is measured at 490 nm. The presence of anti-SARS-CoV-2 antibodies in a blood serum sample is determined by comparing the optical density in the specimen well to the optical density in the wells with the monoclonal antibodies used as a positive control.

4. Reagents

4.1.Kit content

- 1 plate-96 wells 42 tests microplate pre-coated with antigens
- Template for identification of the control and specimen wells
- Reagent 1 (R1): Antibody dilution solution
- Reagent 2 (R2): Monoclonal anti-RBD antibody, cut-off control
- Reagent 3 (R3): Monoclonal anti-RBD antibody, positive control
- Reagent 4 (R4): Monoclonal anti-N antibody, cut-off control
- Reagent 5 (R5): Monoclonal anti-N antibody, positive control
- Reagent 6 (R6): HRP-conjugated anti-human Fc antibody, secondary antibody
- Reagent 7 (R7) Phosphate buffer saline concentrate, for preparation of the Washing Solution
- Reagent 8 (R8) O-phenylenediamine dihydrochloride, chromogenic substrate
- Reagent 9 (R9) Urea hydrogen peroxide, HRP substrate
- Reagent 10 (R10): 3N HCl, stop solution

4.2. Storage and handling requirements

- This kit should be stored at +2-8°C.
- After the package is opened, the microplate and reagents R1- R6, R7, R8 and R9 should be stored at +2-8°C. R8 and R9 are very hygroscopic and light sensitive and should be removed from the foil blisters immediately before use.
- Reagents R7 and R10 can be stored at ambient temperature.

5. Warnings and Precautions

For in vitro diagnostic use only by a professional user in a laboratory environment.

Prescription Use only.

5.1. Health and safety precautions

- This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with the potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with the requisite Good Laboratory Practices.
- No known test method can offer complete assurance that infectious agents are absent. Therefore, all human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious disease, following recommended Universal Precautions for bloodborne pathogens as defined by local, regional and national regulations.
- Biological spills: Human source material spills should be treated as potentially infectious.
- Spills not containing acid should be immediately decontaminated, including the spill area, materials, and any contaminated surfaces or equipment, with an appropriate chemical disinfectant that is effective for the potential biohazards relative to the specimens involved (commonly a 1:10 dilution of household bleach, 70-80% Ethanol or Isopropanol, an iodophor such as 0.5% WescodyneTM Plus, etc.), and wiped dry.
- Spills containing acid should be appropriately absorbed (wiped up) or neutralized, the area flushed with water and wiped dry; materials used to absorb the spill may require biohazardous waste disposal. Then the area should be decontaminated with one of the chemical disinfectants. Caution: Do not place solutions containing bleach into the autoclave!
- Of all specimens and material used to perform the test as though they contain an infectious agent. Laboratory, chemical or biohazardous wastes must be handled and discarded in accordance with all local, regional, and national regulations.
- For hazard and precaution recommendations related to some chemical components in this test kit, please refer to the symbol(s) shown on the labels and described below. The Safety Data Sheet is available on our website.
- This product contains human or animal components. Handle with care.

5.2. Procedural precautions

- DO NOT USE the kit if the packaging of components is damaged.
- DO NOT USE expired reagents.
- DO NOT USE microwell plates if there is no desiccant inside the microplate pouch.
- Bring all reagents to room temperature (18-30°C) before use.
- Carefully prepare working reagents, avoiding any contamination.
- The use of disposable material is recommended for preparation of reagents, except Washing Solution, which can be prepared in a reusable glass or plastic bottle or flask. Wash the glassware thoroughly and rinse with deionized water after each use.
- Do not allow the microplate to dry between the end of a washing step and the addition of reagents.
- Do not mix reagents from different lots within a test run.
- Do not mix reagents from other kits that have different lot numbers.
- The enzyme reaction is extremely sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the conjugate or substrate solutions.
- Do not change the assay procedure.
- Each run of this assay must proceed to completion without interruption after it has been started. A delay of less than 5 minutes between steps is acceptable.
- . Check the pipettes and other equipment for accuracy and correct operation.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzymatic activity of the conjugate.
- Use a new pipette tip for each specimen.
- Microplate washing is a critical step in this procedure: follow the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.

6. Specimens

The test is performed on serum specimens.

Comply with the following guidelines for handling, processing, and storing of blood specimens:

- Collect a blood specimen according to standard laboratory procedures. Allow blood to clot completely before centrifugation.
- Keep tubes sealed all the time to prevent contamination.
- After centrifugation, collect the serum and keep it in a sealed tube.
- The specimens can be stored at +2-8°C if the test is performed within 4 days.
- If the test cannot be completed within 4 days, freeze the specimens at -20°C or lower.
- Serum specimens can be subjected to a maximum of 1 freezing/ thawing cycle.
- Previously frozen specimens should be thoroughly mixed after thawing prior to testing.
- Do not heat the specimens.

7. Procedure

7.1. Materials and equipment required but not provided

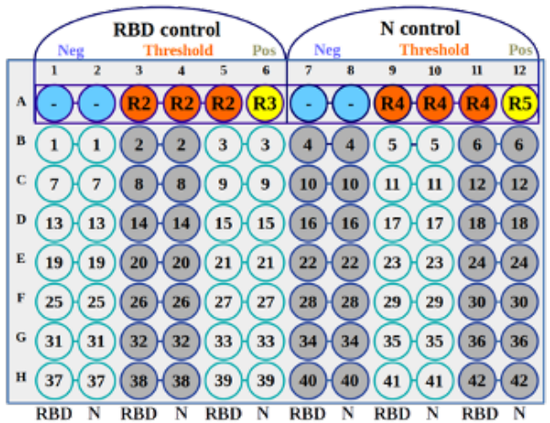
- Sterile deionized water
- Sodium hypochlorite (household bleach)
- Absorbent paper
- Gloves and eye / face protection
- Container for biohazardous waste
- Precision pipettes to measure and dispense 2 µL to 1000 µL
- Multichannel pipettes to measure and dispense 10 µL to 1000 µL
- Graduated cylinders of 25 mL and 1000 mL capacity
- Glass or plastic bottle of 1 L capacity
- Disposable tubes of 1.5 mL and 50 mL capacity
- Disposable reagent reservoirs for multichannel pipettes
- Disposable pipette tips
- Disposable serological pipettes to measure and dispense 1 to 10 mL
- Microcentrifuge
- Microplate reader equipped with a 490 nm filter

7.2. Assay Procedure

7.2.1. Preparation of the plate

- Bring reagents to room temperature (+18-25°C) for at least 30 minutes before use.
- Using a microcentrifuge, briefly spin down the tubes containing reagents R2, R3, R4, R5, and R6to ensure that all the liquid is collected at the bottom of the tube.
- Prepare the antibody threshold dilutions:
  Add 228 µl of R1 (antibody dilution solution) to R2 (monoclonal anti-RBD antibody)
  Add 468 µl of R1 (antibody dilution solution) to R4 (monoclonal anti-N antibody)
- Fill all wells of the reaction plate with 98 µl of antibody dilution solution (R1).
- Identify the individual wells for addition of controls and blood serum specimens, using the provided template as a guide. Two aliquots of each specimen are added to two horizontally neighboring wells (e.g, B1 and B2, B3 and B4, etc.). Note that the first row (A1-A12) is used for negative and positive controls.

7.2.2. Incubation with the blood serum specimens and control antibodies



- Add 2 µl of the first blood serum specimen to wells B1 and B2. Continue with the remaining specimens filling the remaining rows (B-H). Make sure to thoroughly mix the serum samples with the dilution solution in the wells without touching the bottom of the well with a pipette tip.
- Add 10 µl of R2 to each of the wells A3, A4, A5
- Add 10 µl of R3 to well A6.
- Add 10 µl of R4 to each of the wells A9, A10, A11
- . Add 10 µl of R5 to well A12.
- Prepare the Washing Solution by adding Reagent 7 (R7) powder to 950 mL of deionized water. Mix until the powder is completely dissolved. Adjust the volume to 1000 mL and mix again.
- . Incubate the reaction plate at room temperature for 2 hours.

7.2.3. Washing

- Wash the plate 3 times with Washing Solution, using 300 µL of the prepared Washing Solution per well. Invert the microplate and gently tap on absorbent paper to remove the remaining liquid after each washing step.

7.2.4. Incubation with the secondary antibody

- Add 4 µl of the secondary antibody (R6) to 12 ml of antibody dilution solution (R1). Add 100 µl to all wells of the plate using a multi- channel pipette. Avoid touching the tips of the pipette to the walls of the well. Incubate for 1 hour at room temperature.

7.2.5. Washing

- Wash the plate 3 times with Washing Solution, using 300 µL of the prepared Washing Solution (R7 dissolved in 1 L of deionized water, see Section 7.2.1) per well. Invert the microplate and gently tap on absorbent paper to remove the remaining liquid after each washing step.

7.2.6. Plate Development and Reading

- Prepare the HRP development OPD solution by dissolving R8 tablet in 20 mL of deionized water during the incubation with secondary antibody. Add and dissolve R9 tablet after the last wash, immediately before addition of the OPD solution to the plate.
- Add 100 µl to all wells of the plate. After a 10-minute incubation, stop the reaction by adding 50 µl of stop solution (R10) to the wells. Note that the bubbles generated by pipetting interfere with the optical density measurements. Avoid introduction of bubbles or remove them with a pipette tip.
- Determine the optical density at 490 nm using a microplate reader and record the data.

7.3. Quantification, Quality Control, and Interpretation of the Results

7.3.1 Quantify the thresholds and negative controls for RBD and N:

- RBD Threshold (RBDthr) = (A3+A4+A5) / 3
- RBD Negative control (RBDnc) = (A1+A2) / 2
- N Threshold (Nthr) = (A9 + A10 + A11) / 3
- N Negative control (Nnc) = (A7 + A8) / 2

7.3.2. Quality control

The following criteria should be fulfilled for the test results to be considered valid:
The Positive Controls should be at least 3-fold higher than the Negative Controls:

- A6 > RBDnc * 5
- A12 > Nnc * 5

The Thresholds should be at least 1.7-fold higher that the Negative Controls

- RBDthr > RBDnc * 1.7 Nthr > Nnc * 1.7

7.3.3 Interpretation of the results

For two samples representing each blood serum specimen (for first sample it is values B1 and B2), establish the ratio

- SRBD = B1 / RBDthr
- SN = B2 / Nthr

Both SRBD and SN must be 1.0 or over for the test result to be considered positive.
If the ratios are between 0.9 and 1.0, repeating the test in a few days can be recommended.

8. Limitations of the Procedure

- VirIntel COVID-19 Antibody Test is only provided for use by clinical laboratories or to healthcare workers for point-of-care testing, and not for at home testing.
- VirIntel COVID-19 Antibody Test has not been reviewed by the FDA.
- 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.
- 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.
- The detection of anti-SARS-CoV-2 antibodies is dependent on the presence of the analyte in the specimen. A negative or non-reactive result can occur if the quantity of antibodies for the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay. During the acute infection phase and/or for immunosuppressed patients, anti-SARS-CoV-2 anti- bodies might not be detectable. Thus, a negative result does not preclude or rule out COVID-19 infection.
- Performance characteristics of VirIntel COVID-19 Antibody Test have not been evaluated with specimens of serum or plasma originating from newborns or pediatric patients.
- . Positive results of the VirIntel COVID-19 Antibody Test may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
- This test should not be used for screening of donated blood.

9. Analytical Performance Characteristics

9.1 Precision Measurement

The precision of the VirIntel COVID-19 Antibody Test was evaluated by testing 20 specimens 6 times over the course of 7 days. The results are summarized in the table below.

Table 1. Precision Measurement

	SD	SD median	% CV
IgG, anti-N	0.007 — 0.019	0.018	14.6% - 34.6%
IgG, anti-RBD	0.008 — 0.055	0.012	14.5% - 35.7%

9.2. Class Specificity

The monoclonal Mouse Anti-Human IgG Fc Antibody (50B4A9) [HRP] from GenScript is used for IgG detection. The manufacturer does not report cross-reactivity with other antibody types. The available reference is:
https://www.genscript.com/antibody/A01854-Mouse_Anti_Human_IgG_Fc_Antibody_50B4A9_HRP_mAb.html

9.3. Clinical Performance Characteristics

The clinical performance of the VirIntel COVID-19 Antibody Test was assessed with specimens obtained from a general asymptomatic population of pre-epidemic individuals and on specimens from donors recovered from COVID-19.

9.3.1. Clinical Specificity

A total of 83 specimens collected prior to the outbreak of the COVID-19 pandemic were tested. Five of these specimens were HIV positive .The test results were considered positive if both RBD and N analysis results appeared above the respective thresholds. The study yielded no false positives results; thus, the specificity of the test was 100% (83/83).

9.3.2 Clinical Sensitivity

A total of 31 specimens (COVID-19 Panels A-1 and A-2, Access Biologicals, LLC) from donors who were determined COVID-19-positive using a SARS-CoV-2 RT-PCR assay (Taq-Path COVID-19 Combo kit, ThermoFisher Scientific) 4-8 weeks before the blood draw were used for the clinical sensitivity validation. The test results were considered positive if both RBD and N analysis results appeared above respective thresholds. The study yielded 2 false negative results; thus, the sensitivity of the test was 93.6% (29/31).

Table 2. Clinical sensitivity and specificity validation.

Antibody	Performance measure	Estimate of performance	95% Confidence Interval
IgG, anti-RBD	Sensitivity (PPA)	93.6% (29/31)	(80.9%; 98.6%)
IgG, anti-RBD	Specificity (NPA)	98.8% (82/83)	(94.5%; 99.9%)
IgG, anti-N	Sensitivity (PPA)	100% (31/31)	(92.3%; 100%)
IgG, anti-N	Specificity (NPA)	98.8% (82/83)	(94.5%; 99.9%)
IgG, Combined	Sensitivity (PPA)	93.6% (29/31)	(80.9%; 98.6%)
IgG, Combined	Specificity (NPA)	100% (83/83)	(97.0%; 100%)

The confidence interval was calculated by Jeffreys method

9.3.3 Additional specificity and sensitivity analyses conducted during the assay development

One study involving 96 samples confirmed as negative by another ELISA test demonstrated specificity of 98.96% (95/96). An independent study at another site involving 10 of these samples demonstrated specificity of 100% (10/10). The third study involving 12 samples confirmed negative by another ELISA test and 1 ovarian cancer sample collected before 2018 also demonstrated specificity of 100% (13/13).
One study involving 96 samples confirmed as positive by another ELISA test demonstrated sensitivity of 97.91% (94/96). The second study involving 11 samples confirmed positive by unspecified SARS-CoV-2 RT-PCR assays demonstrated sensitivity of 100% (11/11). The third study involving 9 samples confirmed positive by another ELISA demonstrated sensitivity of 100% (9/9).