ACCEL ELISA® COVID-19

For the Detection of Total Antibody to SARS-CoV-2 in Serum

IVD **REF AE301** **Rx Only**

Intended Use

The Plexense ACCEL ELISA® COVID-19 test is a serological microplate-based enzyme linked immunosorbent assay (ELISA) intended for the qualitative detection of total antibodies (including IgG, IgM and/or IgA) to SARS-CoV-2 in human serum. ACCEL ELISA® COVID-19 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. ACCEL ELISA® COVID-19 should not be used to diagnose acute SARS-Cov-2 infection. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 32 U.S.C. §263a, to perform high complexity tests.

Special Conditions for Use

For prescription use only.

For in vitro diagnostic use only.

Principle of Procedure

This assay utilizes the microplate-based enzyme immunoassay technique. Diluted serum and diluted positive and negative controls are added to microtiter wells coated with SARS-CoV-2 recombinant nucleocapsid protein. A horseradish peroxidase (HRP)-labeled polyclonal goat anti-human Immunoglobulin detector conjugate is then added to each well and the wells incubated. During this incubation, anti-SARS-CoV-2 antibodies in the serum bind to the SARS-CoV-2 nucleocapsid antigens bound to the test wells. The HRP conjugate (detector) also attaches to the anti-SARS-CoV-2 antibodies forming the recombinant antigen/human anti-SARS-CoV-2/HRP Conjugate complex. Following incubation, unbound protein is removed from the wells by a washing step. A substrate solution is then added, followed by a brief incubation. Any bound HRP conjugate will catalyze a reaction with the substrate, resulting in a color change that is measured by a spectrophotometric microplate reader. The degree of substrate color change is directly related to the amount of conjugate bound to the microtiter well and is proportional to the amount of the anti-SARS-CoV-2 total antibody level in the tested specimen.

Summary and Explanation of the Test

The 2019 novel coronavirus (COVID-19 or SARS-CoV-2) is a single-stranded RNA coronavirus.[1] Comparisons of the genetic sequences of this virus have shown similarities to SARS-CoV-2 and bat coronaviruses.[2] In humans, coronaviruses cause respiratory infections.[3] Coronaviruses are composed of several proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N).[4] Results suggest that the spike protein retains sufficient affinity to the Angiotensin converting enzyme 2 (ACE2) receptor to use it as a mechanism of cell entry.[5] Human to human transmission of coronaviruses is primarily thought to occur among close contacts via respiratory droplets generated by sneezing and coughing.[6]

SARS-CoV-2 antibodies generally become detectable following infection in immunocompetent individuals.[7] The presence of antibodies indicates seroconversion following SARS-CoV-2 infection. Negative results do not preclude SARS-CoV-2 infection as results may vary from person to person based on immunocompetency. Seroconversion may be variable from person to person therefore the time between infection to positive antibody detection in immunocompetent individuals can vary. It is also presently unknown how long human anti-SARS-CoV-2 circulating antibodies persist following infection and the level of protection these may provide.

Results are for the detection of SARS-CoV-2 antibodies. Antibodies (IgG, IgM and/or IgA) 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 ACCEL ELISA® COVID-19 early after infection is not known. 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 ACCEL ELISA® COVID-19 may occur due to cross-reactivity from preexisting antibodies or other possible causes.

Reagents Provided

1. SARS-CoV-2 antigen-coated Microplate: 96 wells in a 1 x 8 strip format. Each strip is packaged within a frame enclosed in a resealable foil pouch that includes a desiccant. Each well is coated with recombinant SARS-CoV-2 nucleocapsid antigen. Ready for use as supplied. Do not use wells if the foil pouch shows evidence of damage, such as tears or holes in the foil pouch.
2. Conjugate Dilution Buffer (10 mL): Ready to use as supplied.
3. Sample Dilution Buffer (10 mL): Ready to use as supplied.
4. HRP Conjugate (30 µL): HRP-labeled goat anti human globulin. Dilute prior to use.
5. TMB Substrate (13 mL): TMB/H_2O_2. Ready for use as supplied.
6. Positive Control: Prepared from serum from patients with confirmed infection with SARS-CoV-2. Dilute prior to use.
7. Negative Control: Prepared from serum derived from donors shown to be negative for SARS-CoV-2 by PCR. Dilute prior to use.

Materials Required but not Provided

1. Red stoppered blood collection tubes without neutral gel separators
2. General laboratory centrifuge for serum separation
3. Washing Buffer: 1X Phosphate-buffered saline with 0.1% TWEEN® 20 (PBST) -- ([NaCl]: 137 mM, [KCl]: 2.7 mM, [Na2HPO4]: 10 mM, [KH2PO4]: 1.8 mM, [Tween® 20]: 0.1% (w/v))
4. Vortex mixer
5. Interval timer
6. Precision single channel pipettes capable of delivering 10 µL, 25 µL, 100 µL, and 1000 µL, etc.
7. Repeating dispenser suitable for delivering 100 µL
8. Disposable pipette tips suitable for above volume dispensing
9. Disposable microcentrifuge tube or microplate for sample dilution
10. Deionized or distilled water
11. Multichannel pipettor capable of delivering 150-200 µL
12. Spectrophotometric microplate reader capable of reading absorbance at 650 nm

Storage and Stability

Store at 2–8 °C. **Do not freeze**. Return to 2–8 °C immediately after use. Do not use after expiration date indicated on the kit box and/or component and reagent labels.

Warnings and Precautions

1. This test kit is for in-vitro diagnostic use only.
2. The microplate wells contain dried bovine serum albumin.
3. Wear gloves while performing this assay and handle all reagents as if they were potentially infectious. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.
4. Dispose of human specimens and used tests as medical waste.

Specimen Preparation

Samples stored at room temperature (15-30 °C) can be used within 8 hours. Samples stored at 2-8 °C can be tested within 48 hours. Samples stored longer than 48 hours should be placed at ≤ -22 °C. Samples should not be frozen and thawed repeatedly. Severely hemolyzed samples should not be used.

Preparation of Reagents and Sample

Dilution of HRP Conjugate:

1. Vortex the HRP Conjugate thoroughly to homogenize before opening vial and starting the dilution.
2. Before use, dilute HRP Conjugate 1:500 with Conjugate Dilution Buffer (e.g., adding 20 µL of HRP Conjugate stock to 10 mL of the Conjugate Dilution Buffer). Diluted conjugate should be used immediately after dilution. Unused diluted conjugate should be discarded at the end of a test run.

Dilution of Negative Control:

1. Vortex the Negative Control thoroughly to homogenize before opening vial and starting the dilution.
2. Before use, dilute Negative Control 1:10 with Sample Dilution Buffer (e.g., adding 15 µL of Negative Control stock to 135 µL of Sample Dilution Buffer). Diluted Negative Control should be used immediately. Unused diluted Negative Control should be discarded at the end of a test run.

Dilution of Positive Control:

1. Vortex the Positive Control thoroughly to homogenize before opening vial and starting the dilution.
2. Before use, dilute Positive Control 1:10 with Sample Dilution Buffer (e.g. adding 15 µL of Positive Control stock to 135 µL of Sample Dilution Buffer). Diluted Positive Control should be used immediately. Unused diluted Positive Control should be discarded at the end of a test.

Dilution of Samples:

1. Collect blood specimen into a red stoppered collection tube by venipuncture. (Do not use tubes with neutral gel separators.)
2. Allow the blood to clot.
3. Separate the serum from the clot by centrifugation.
4. Carefully transfer the serum into a clean pre-labeled tube.
5. Dilute serum 1:10 with Sample Dilution Buffer (e.g., adding 15 µL of serum to 135 µL of Sample Dilution Buffer). Diluted sample should be used immediately.

Procedural Notes

1. Keep light-sensitive reagents (HRP Conjugate, TMB Substrate) in the original bottles and avoid unnecessary exposure to the light.
2. Store any unused test strips in the resealable foil pouch with desiccant to protect from moisture.
3. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
4. Incubation times or temperatures other than those stated in this insert may adversely affect the results.
5. Avoid introducing air bubbles in the test wells as this could result in lower binding efficiency.
6. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

Assay Procedure:

1. Mix diluted HRP Conjugate and each diluted Negative Control, Positive Control and serum sample for 1:1 ratio (e.g., adding 150 µL of diluted HRP Conjugate to 150 µL of diluted sample or control).
2. Transfer 100 µL of HRP Conjugate-Serum mixture and 100 µL each of the two HRP Conjugate-Control mixtures to individual microplate wells and allow the wells to incubate for 20 minutes at room temperature (15-30 ℃).
3. Wash the wells with Washing Buffer.
   i. Decant the contents of the wells manually with a hard, rapid downward motion. Fluid should be captured in a receptable designed to collect medical waste. Remove all residual reagent from the microplate by tapping it on absorbent paper with the opening facing downwards.
   ii. Fill each well with 120 µL of Washing Buffer with a multichannel pipettor.
   iii. Decant the Washing Buffer from the wells with a hard, rapid downward motion. Remove all residual solution from the microplate by tapping it on absorbent paper with the opening facing downwards.
   iv. Repeat steps ii and iii four more times (total of 5 washings). Do not leave any residual moisture in the wells on each washing step.
4. Add 100 µL of TMB Substrate to each well and mix for 30 minutes by gently tapping or gently swirling the plate.
5. Incubate the wells at room temperature (15-30 °C) for 10 minutes to allow for color (blue) development.
6. After 10 minutes, read the absorbance value of each well using a microplate reader at 650 nm. The OD readings of the test and controls must be read within 1 minute of test completion.

Interpretation of Results

Calculate positive and negative results by dividing each sample's or control's reaction value at OD 650 nm by the cutoff value determined for the kit lot in use. The lot-specific cutoff is identified in the Cutoff Worksheet located in the kit box inner lid.

The cut off value is determined during lot manufacturing and is + 3 SD from the average OD of selected negative samples. Users should not calculate their own cutoff.

Sample and control results should be interpreted as follows:

Calculated Value	Result	Interpretation
< 1.1	Negative	Absence of antibodies to SARS-CoV-2 or that antibody levels are below the detection limits of the assay.
≥1.1	Positive	Presence of antibodies (IgG, IgM or IgA or any combination) indicating exposure to SARS-CoV-2

Test results are qualitative for total antibodies to SARS-CoV-2. Results are reported as either positive or negative. There are no equivocal results associated with this assay. Results of this test can be used only to determine prior exposure to SARS-CoV-2. They cannot be used to make a diagnosis of acute or active infection.

Quality Control

Test the Positive and Negative Controls with each test run. Failure of the controls to produce the expected results indicate either a technical error (i.e., dispensing error, washing error, incubation error, etc.) or that one or more test reagents were inactive or contaminated at the time of testing. When one or both controls fail, repeat both control and sample tests. Do not report results for samples, as they are considered invalid. Contact VEO Diagnostics Product Support for assistance with troubleshooting if controls repeatedly fail on retesting. (See contact numbers at the end of this insert.)

Limitations of the Procedure

1. . This test has not been reviewed by FDA.
2. Negative results do not preclude SARS-CoV-2 infection. If active infection is suspected, direct testing for SARS-CoV-2 is necessary.
3. The sensitivity of the test early after infection is not known.
4. Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection.
5. This test is only for qualitative detection of total antibody to SARS-CoV-2 in human serum. Test results should not be the sole basis for clinical diagnosis and treatment. The confirmation of infection with novel coronavirus (COVID-19 or SARS-Cov-2) must be combined with the patient's clinical signs in conjunction to other tests.
6. It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to reinfection.
7. Positive results may be due to past or present infection with nonSARS-CoV-2 coronavirus strains such as HKU1, NL63, OC43, or 229E.
8. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
9. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.
10. **Residual liquid remaining in test wells after washing can interfere with the substrate and lead to erroneous readings.**
11. **Not for screening of donated blood.**

Conditions of Authorization for the Laboratory

1. The ACCEL ELISA® COVID-19 Fact Sheet for Healthcare Practitioners and the Fact Sheet for Patients and labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19 or at https://www.veo-diagnostics.com.
2. Authorized laboratories* using the ACCEL ELISA® COVID-19 test must adhere to the Conditions of Authorization indicated below:
   A. Authorized laboratories using ACCEL ELISA® COVID-19 will include all authorized Fact Sheets with test results reports of ACCEL ELISA® COVID-19 tests. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
   B. Authorized laboratories using ACCEL ELISA® COVID-19 will use ACCEL ELISA® COVID-19 as outlined in the Instructions for Use. Deviations from authorized procedures, including authorized instruments, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use ACCEL ELISA® COVID-19 are not permitted.
   C. Authorized laboratories that receive ACCEL ELISA® COVID-19 will notify the relevant public health authorities of their intent to run ACCEL ELISA® COVID-19 prior to initiating testing.
   D. Authorized laboratories using ACCEL ELISA® COVID-19 will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
   E. Authorized laboratories will collect information on the performance of ACCEL ELISA® COVID-19 and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and VEO Diagnostics, Inc. at 513-872-1330 (www.veo-diagnostics.com) any suspected occurrence of false reactive and false nonreactive results and significant deviations from the established performance characteristics of ACCEL ELISA® COVID-19 of which they become aware.

F. All laboratory personnel using ACCEL ELISA® COVID-19 must be appropriately trained in enzyme linked immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use ACCEL ELISA® COVID-19 in accordance with the authorized labeling. All laboratory personnel using ACCEL ELISA® COVID-19 must also be trained in and be familiar with the interpretation of results of the product.

G. Plexense, Inc.'s authorized distributor (VEO Diagnostics, Inc.), and authorized laboratories using the ACCEL ELISA® COVID-19, will ensure that any records associated with this product are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection on request.

*Authorized laboratories refers to "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests as authorized laboratories.

Specific Performance Characteristics

Cross-reactivity:
ACCEL ELISA® COVID-19 was tested with 251 natural human serum specimens. Of these, 202 were collected between 2008 and 2013 from Korean hospital patients and were expected to be SARS-CoV-2 negative. The patients were presumed to have been exposed to, and produced antibodies, to other respiratory organisms that could cause symptoms similar to SARS-CoV-2 infection, or to other organisms that might cause infectious diseases (such as influenza A and B, *Haemophilus influenzae*, Respiratory Syncytial Virus, other coronaviruses), or to other organisms that might cause infectious diseases (such as HCV or HBV). Forty-nine (49) samples were collected in March 2020 from patients for whom a SARS-CoV-2 PCR test had been ordered and was found negative. There was greater than 95% agreement between the ACCEL ELISA® COVID-19 test result and the donor's negative status. The data suggest ACCEL ELISA® COVID-19 does not cross-react with antibodies to respiratory or other infecting organisms.

Clinical Agreement Studies:
Sensitivity and specificity studies were performed with samples collected by an affiliate of the Korean Research Institute of Bioscience and Biotechnology. Positive serum samples were obtained from patients identified as positive by RT-PCR and who presented with clinical manifestations of COVID 19 infection. Sera from PCR-positive donors were collected between February and March, 2020. Negative sera were obtained from archived samples collected between 2008 and 2010, when SARs-CoV-2 did not exist.

Positive Percent Agreement (PPA) was calculated for ACCEL ELISA COVID-19 against the comparator method (Seegene Allpex™ nCov-2) results using a 2 x 2 table. (See Table 1.) Negative Percent Agreement (NPA) was calculated by comparing the ACCEL ELISA COVID-19 result to the expected result (See Table 2.)

Table 1. ACCEL ELISA® COVID-19 Sensitivity Compared to RT-PCR

	RT-PCR Result		
ACCEL ELISA® COVID-19 Result	**Positive**	**Negative**	**Total**
Positive	30	0	30
Negative	0	0	0
Total	30	0	30
Percent Positive Agreement (PPA)	30/30 = 100%; 95% CI = 88.4 to 100%		

Table 2. ACCEL ELISA® COVID-19 Specificity Compared to Expected

	Expected Result		
ACCEL ELISA® COVID-19 Result	**Positive**	**Negative**	**Total**
Positive	0	1	1
Negative	0	74	74
Total	0	75	75
Percent Negative Agreement	74/75 = 98.7%; 95% CI = 92.8 to 100%		

Positive serum samples were further stratified by the days between the RT-PCR test and the day the serum sample was collected. This information appears in Table 3.

Table 3. Positive serum samples stratified by the days collected following patient RT-PCR testing.

	ACCEL ELISA® COVID-19 Result		
Days between RT-PCR Positive Test and Serum Sample Collection	**Positive**	**Negative**	**Total**
0-10 days	7	0	7
11-20 days	19	0	19
> 20 days	4	0	4
Total	30	0	30

References

1. CDC (2020). Transmission of Novel Coronavirus (COVID-19) (https//:www.cdc.gov/coronavirus)
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4. Li, F., Li, W., Farzan, M., & Harrison, S. (2005). spike receptor-binding domain complexed with its receptor. doi: 10.2210/pdb2ajf/pdb.
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6. Xu, X., Chen, P., Wang, J., Feng, J., Zhou, H., Li, X., … Hao, P. (2020). Evolution of the novel coronavirus from the ongoing Wuhan outbreak and mode protein for risk of human transmission. Science China Life Sciences 10.1007/s11427-020-1637-5.
7. Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., … Shi, Z. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. doi: 10.1038/s41586-020-2012-7.

Symbols

Description	Symbol
Manufacturer	
In vitro diagnostic	
Storage temperature	
Lot number	
Catalogue Number	
Consult instructions for use	
Prescription Use Only	**Rx Only**
Enough reagents for _____ tests	

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