INSTRUCTIONS FOR USE

EN

Anti-SARS-CoV-2 Total

REF SM9Z7901 | IVD | Rx Only | Σ 96

INTENDED USE

Anti-SARS-CoV-2 Total is an ELISA-based test system intended for the qualitative detection of total antibodies to the SARS-CoV-2 virus in human serum and plasma (dipotassium EDTA, lithium heparin and sodium citrate) run manually or using the Dynex AGILITY, Dynex DSX or Dynex DS2 automated ELISA Systems. The Anti-SARS-CoV-2 Total is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The Anti-SARS-CoV-2 Total should not be used to diagnose or exclude acute SARS-CoV-2 infection. 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 (automated method) or high (manual and automated method) complexity tests.

Results are for the detection of SARS CoV-2 total antibodies. 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 Anti-SARS-CoV-2 Total 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 Anti-SARS-CoV-2 Total 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 Anti-SARS-CoV-2 Total is only for use under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY AND EXPLANATION

There is currently an outbreak of respiratory disease caused by a novel coronavirus that was first detected in Wuhan City, Hubei Province, China, which has now been designated a pandemic by the World Health Organization (WHO) and which has been detected internationally, including cases in the United States. The virus has been named "SARS-CoV-2" and the disease it causes has been named "Coronavirus Disease 2019" (COVID-19). SARS-CoV-2 has demonstrated the capability to spread rapidly, leading to significant impacts on healthcare systems and causing societal disruption. The potential public health threat posed by COVID-19 is high, both globally and to the United States. To respond effectively to the COVID-19 outbreak, appropriate clinical management and infection control, and implementation of community mitigation efforts are critical. The results from this test may help address these urgent public health concerns by helping to identify those individuals who possess antibodies to the SARS-CoV-2 virus.

PRINCIPLE OF THE ASSAY

The Anti-SARS-CoV-2 Total is an indirect, antibody capture enzyme-linked immunosorbent assay designed to detect total antibodies) to SARS-CoV-2 (novel 2019 Coronavirus) in human serum or plasma collected in CLIA certified laboratories. The wells of the plastic microwell strips are coated with a mixture of recombinant S1 receptor binding domain (RBD) viral protein and recombinant nucleoprotein as the antibody capture antigens. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in the antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen on the surface of the wells. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgG/IgM/IgA is added to the wells and the plate is incubated. The Conjugate will react with antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped, and the color intensity of the solution is measured photometrically. The color intensity of the solution indicates that antibody is present in the original test sample.

The assay steps outlined above can be performed manually using existing laboratory equipment or it can be automated on the Dynex Agility®, Dynex DSX or Dynex DS2.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE:** The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrator, and SAVe Diluent®.

Kit Component	Quantity (Σ 96)	Description
PLATE	1	Plate: 96 wells configured in twelve, 1×8-well, strips coated with a mixture of recombinant S1 RBD viral proteins and recombinant COVID-19 Coronavirus nucleoprotein as antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ	1	Conjugate: Conjugated (horseradish peroxidase) anti-human IgG/M/A (FHeavy chain specific) in 15mL, white-capped bottle(s). Ready to use.
CTRL +	1	Positive Control (anti SARS-CoV-2 Human Serum): 0.5mL, red-capped vial. 21X concentrate.
CAL	1	Calibrator (anti SARS-CoV-2 Human Serum): 0.5mL, blue-capped vial. 21X concentrate.
CTRL -	1	Negative Control (Human Serum): 0.5mL, green-capped vial. 21X concentrate.
DIL SPE	1	SAVe Diluent®: 30mL, green-capped, bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Ready to use. **NOTE: The SAVe Diluent® will change color when combined with serum.**
SOLN TMB	1	TMB: 15mL, amber-capped, amber bottle containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN STOP	1	Stop Solution: 15mL, red-capped bottle containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASH 10X	1	Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. 100mL, clear-capped bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). **NOTE: 1X solution will have a pH of 7.2 ± 0.2.**

NOTE: The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS ELISA Test Systems: TMB, Stop Solution, and Wash Buffer. SAVe Diluent® may be used interchangeably with any ZEUS ELISA Test System utilizing Product No. 005CC.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm. **NOTE: Use of a single (450nm), or dual (450/620 - 650nm), wavelength reader is acceptable. Dual wavelength is preferred, as the additional reference filter has been determined to reduce potential interference from anomalies that may absorb light.**
2. Pipettes capable of accurately delivering 10 - 200µL.
3. Multichannel pipette capable of accurately delivering 50 - 200µL.
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One-liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.

11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant (i.e., 10% household bleach - 0.5% sodium hypochlorite).

STORAGE CONDITIONS

2°C – 8°C	Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening, strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remain blue.
	Conjugate – DO NOT FREEZE.
	Unopened Kit, Calibrator, Positive Control, Negative Control, TMB, Sample Diluent.
2°C – 25°C	Stop Solution: 2 – 25 °C Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days Wash Buffer (10X): 2 - 25°C

PRECAUTIONS

1. For Emergency Use Authorization (EUA) only.
2. For *in vitro* diagnostic use only.
3. This test has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
4. This test has been authorized only for detecting the presence of total antibodies to SARS-CoV-2, not for any other viruses or pathogens.
5. 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* diagnostics 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. § 360bbb3(b)(1), unless the declaration is terminated, or authorization is revoked sooner.
6. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
7. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
8. The Controls are **potentially biohazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, handle these products at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": Current Edition; and OSHA's Standard for Bloodborne Pathogens (3).
9. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay**. Return unused reagents to refrigerated temperature immediately after use.
10. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
11. The SAVe Diluent®, Controls, and Calibrator contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing
Sodium Azide.
12. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
13. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
14. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
15. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
16. Dilution or adulteration of these reagents may generate erroneous results.

17. Do not use reagents from other sources or manufacturers.
18. Do not use any reagents beyond their expiration date.
19. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
20. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
21. Avoid microbial contamination of reagents. Incorrect results may occur.
22. Cross contamination of reagents and/or samples could cause erroneous results.
23. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
24. Avoid splashing or generation of aerosols.
25. Do not expose reagents to strong light during storage or incubation.
26. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
27. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
28. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
29. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
30. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate's enzymatic activity.
31. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.
3. Use only freshly drawn and properly refrigerated serum/plasma obtained by approved aseptic venipuncture procedures in this assay (1,2). Avoid using hemolyzed, lipemic, or bacterially contaminated sera/plasma.
4. Store sample (serum/plasma) at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, samples may be stored between 2 - 8°C, for no longer than 48 hours. If a delay in testing is anticipated, store samples at –20°C or lower. They are stable at –20°C or lower for a maximum of 12 months. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (2).

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).
2. Determine the number of microwells needed. Allow for six Control/Calibrator determinations (one Reagent Blank, one Negative Control, three Calibrators and one Positive Control) per run. Run a Reagent Blank on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2 - 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Negative Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Positive Control	
G	Patient 1	
H	Patient 2	

3. For human serum or plasma samples, prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAVe Diluent®) of the Negative Control, Calibrator, Positive Control, and each patient sample. **NOTE: The SAVe Diluent® will undergo a color change confirming that the specimen has been combined with the diluent**.

4. To individual wells, add 100µL of each diluted Control, Calibrator, and patient specimen. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of SAVe Diluent® to well A1 as a Reagent Blank. Check software and reader requirements for the correct Reagent Blank well configuration.
6. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5 times.
   a. **Manual Wash Procedure**:
      1. Vigorously shake out the liquid from the wells.
      2. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
      3. Repeat steps 1. and 2. for a total of 5 washes.
      4. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day's run.
   b. **Automated Wash Procedure**:
      If using an automated microwell wash system, set the dispensing volume to 300 - 350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.
8. Add 100µL of the Conjugate to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
9. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including the Reagent Blank well, at the same rate and in the same order as the TMB. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the Reagent Blank. Read the plate within 30 minutes of the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21.
2. Add diluted sample to microwell - 100µL/well.
3. → *Incubate 25 ± 5 minutes.*
4. Wash.
5. Add Conjugate - 100µL/well.
6. → *Incubate 25 ± 5 minutes.*
7. Wash.
8. Add TMB - 100µL/well.
9. → *Incubate 10 - 15 minutes.*
10. Add Stop Solution - 50µL/well - Mix.
11. READ within 30 minutes.

15. When using the Dynex Agility, Dynex DSX or Dynex DS2 automated systems, program the above assay steps according to the instrument manufacturer's recommendations. Only instrument programs validated by the laboratory should be utilized. If performing the assay using the Dynex DSX, Dynex DS2 or Dynex Agility instruments, consult the respective Operator's Manual for guidance on how to set up and operate the instrument.

QUALITY CONTROL

1. Each time the assay is performed, the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.

3. The mean OD value for the Calibrator, and Negative Control OD should fall within the following ranges. Index Values of the Negative Control and Positive Control
should fall within a lot-specific range that is found on the Component Label located in the Test System box:

	OD Range
Negative Control	≤0.250
Calibrator	≥0.300
Positive Control	The Index Value should meet the lot specific requirements found on the Component Label.
Negative Control	The Index Value should meet the lot specific requirements found on the Component Label.

4. If the above conditions are not met the test should be considered invalid and should be repeated.
5. The Positive Control and Negative Control are intended to monitor for substantial reagent failure but will not ensure precision at the assay Cutoff.
6. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
7. Refer to CLSI document C24: Statistical Quality Control for Quantitative Measurement Procedures for guidance on appropriate QC practices.
8. NOTE: When using the Dynex Agility, Dynex DSX or Dynex DS2 assay files provided by ZEUS Scientific, these QC criteria are automatically assessed by the Dynex software. If any of the above QC criteria are not met, the software will identify the outlier on the report and automatically suppress patient results.

INTERPRETATION OF RESULTS

1. **Calculations:**
   a. *Correction Factor*: The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. It will also correct for slight day-to-day variations in test results. The Correction Factor is determined for each lot of components and is printed on the Component Label located in the Test System box.
   b. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
   c. *Cutoff OD Value*: To obtain the Cutoff OD Value, multiply the CF by the mean OD of the Calibrator determined above.
   (CF x Mean OD of Calibrator = Cutoff OD Value)
   d. *Index Values/OD Ratios*: Calculate the Index Value/OD Ratio for each specimen by dividing its OD Value by the Cutoff OD from step c.

Example:	Mean OD of Calibrator	=	0.793
	Correction Factor (CF)	=	0.25
	Cutoff OD	=	0.793 x 0.25 = 0.198
	Unknown Specimen OD	=	0.432
	Specimen Index Value/OD Ratio	=	0.432/0.198 = 2.18

2. **Interpretations:** Index Values/OD Ratios are interpreted as follows.

Initial Results				**Retested Results**		
	Index Value/ OD Ratio	**Result**	**Interpretation**	**Index Value**	**Result**	**Interpretation**
Negative Specimens	≤0.90	Negative	Antibodies to SARS-CoV-2 are NOT detected. No additional testing needed.	N/A	N/A	N/A
Equivocal Specimens	0.91 – 1.09	Equivocal/ Indeterminate	Detection of antibodies to SARS-CoV-2 is indeterminate/equivocal. Sample should be retested in duplicate	2 out of 3 results: ≤0.90	Negative	Antibodies to SARS-CoV-2 are NOT detected. No additional testing needed.
				2 out of 3 results: 0.91 to 1.09	Equivocal/ Indeterminate	Detection of antibodies to SARS-CoV-2 is indeterminate or equivocal. Collect and test a new sample in 1 to 3 weeks.
				2 out of 3 results: ≥1.10	Positive	Antibodies to SARS-CoV-2 ARE detected. No additional testing needed.

Positive Specimens	≥1.10	Positive	Antibodies to SARS-CoV-2 ARE detected. No additional testing needed.	N/A	N/A	N/A

NOTE: When using the Dynex Agility, Dynex DSX or Dynex DS2 assay files provided by ZEUS Scientific, all of the above calculations and interpretations are automatically performed with no user intervention. The report will show the calculation of the cut off OD, the resulting patient Index Values and the qualitative interpretation, but only the qualitative result (i.e., positive or negative) should be reported to end users.

LIMITATIONS OF THE ASSAY

1. Use of Anti-SARS-CoV-2 Total is limited to laboratory personnel who have been trained. Not for home use.
2. False positive results may occur due to cross-reactivity from pre-existing antibodies or other possible causes.
3. This assay has not been evaluated with fingerstick specimens. This test is not authorized for use with fingerstick whole blood.
4. The performance of this test has not been established in individuals that 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.
5. The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with 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.
6. SARS-CoV-2 antibodies may be below detectable levels in serum samples collected from patients who have been exhibiting symptoms for less than 15 days. Samples should be collected from individuals that are 15 days or more post symptom onset. Samples should not be tested if collected from individuals less than 15 days post symptom onset.
7. The results of this test are qualitative and are reported as either positive or negative for the presence of anti-SARS-CoV-2 antibody. The intensity of the index value has no bearing on the concentration of antibody present.
8. Performance has only been established with the specimens listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.
9. The Anti-SARS-CoV-2 Total is authorized for use with a manual assay procedure and with the Dynex Agility, Dynex DSX or Dynex DS2 automated ELISA system. Assay performance has not been established for use on other automated instrument platforms.
10. Positive results must be confirmed with another available method and interpreted in conjunction with the patient's clinical information.
11. Results from antibody testing should not be used diagnose or exclude acute SARS-CoV-2 infection or to inform infection status. A molecular assay should be used to evaluate symptomatic patients for acute COVID-19.
12. It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to reinfection.
13. A positive result may not indicate previous SARS-CoV-2 infection. Consider other information, including clinical history, local disease prevalence, and results of a second but different serology test to confirm an adaptive immune response. 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.
14. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. False positive may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnostic determination is made. A negative result can occur if the quantity of the anti-SARS-CoV-2 antibodies present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
15. Samples with excessive hemolysis, lipids, or bacterial contamination should be avoided. False results may occur.
16. This test is only used for the detection of antibodies to SARS-CoV-2 in human serum and plasma.
17. This test should not be used for screening of donated blood.
18. This assay cannot be utilized to test pooled (mixed) serum or plasma. The kit has been evaluated only with individual serum or plasma specimens.

CONDITIONS OF AUTHORIZATION FOR LABORATORIES

The Anti-SARS-CoV-2 Total Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Recipients, 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/in-vitro-diagnostics-euas. Authorized laboratories using the Anti-SARS-CoV-2 Total ("your product" in the conditions below), must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

- Authorized laboratories* using your product must include with test result reports, 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 your product must use your product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, the authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
- Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using your product 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 your product and report to Division of Microbiology Devices (DMD)/Office of Health Technology 7 (OHT7) - Office of In Vitro Diagnostics and Radiological Health (OIR)/Office of Product Evaluation and Quality (OPEQ)/Center for Devices and Radiological Health (CDRH) (via email: CDRH-EUA-Reporting@fda.hhs.gov) and to ZEUS Scientific (support@zeusscientific.com), any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
- All laboratory personnel using your product must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit and use this product 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.
- ZEUS Scientific, authorized distributors, and authorized laboratories using your product 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, that meet requirements to perform moderate (automated method) or high (manual and automated method) complexity tests" as "authorized laboratories."

EXPECTED RESULTS

The normal expected result is negative unless the individual has been previously exposed to the SARS-CoV-2 virus. The frequency of antibody prevalence in the population is highly dependent upon geographical location, social distancing practices and population density.

PERFORMANCE CHARACTERISTICS

1. **Non-Clinical Performance Studies:**
   a. **Cross-Reactivity:**
      i. Cross-Reactivity Study 1: Ninety normal healthy blood donors collected in the Northeastern US prior to November of 2019 were tested on the Anti-SARS-CoV-2 Total. All 90 specimens were negative. The specificity was determined to be 90/90 = 100% (95% CI = 95.9% to 100%)
      ii. Cross-Reactivity Study 2: Ninety specimens were collected from patients with a variety of respiratory illnesses and tested on the Anti-SARS-CoV-2 Total. These specimens had been evaluated for the following infectious agents; MERS, RSV, FluA, FluB, Parainfluenza, Adenovirus, Enterovirus, *Mycoplasma pneumoniae*, Legionella, *B. pertussis*, and *C. pneumoniae*. Many specimens were positive for antibody to multiple agents. All 90 specimens were negative. The specificity in this cohort was determined to be 90/90 = 100% (95% CI = 95.9% to 100%).
   b. **Specimen Matrix Study:**
      Five donors were identified who had no antibody to SARS-CoV-2. These five individuals donated a tube of serum, K2-EDTA plasma, Lithium Heparin plasma and Sodium Citrate plasma. The four different sample matrices were tested with the Anti-SARS-CoV-2 Total unspiked and spiked with two different levels of anti-SARS-CoV-2 antibody (low positive and moderate positive). For all five donors, the unspiked specimens were clearly negative. The low positive spike produced low positive results in all four matrices for all five donors and the moderate positive spike produced a moderate positive result in all four matrices of all five donors. The study showed that samples collected as serum, K2-EDTA plasma, Lithium Heparin plasma and Sodium Citrate plasma are compatible with the Anti-SARS-CoV-2 Total.
2. **Clinical Performance Studies:**
   a. Two separate cohorts of clinically characterized specimens were assembled as follows:
      COVID-19 RT-PCR Positive Patient Specimens (n=50): Fifty serum or plasma samples were obtained from donors that had previously tested positive via an EUA approved RT-PCR test system. COVID-19 RT-PCR Negative Patient Specimens (n=264): Eighty-four serum samples were obtained from donors that had previously tested negative via an EUA approved RT-PCR test

system. In addition, 90 negative serum samples obtained from healthy donors and 90 negative serum samples obtained from febrile donors suspected of respiratory or other illnesses were collected prior to November of 2019.

b. Percent Positive Agreement:
For the PCR positive specimens, the following table shows those results after stratifying patients into "days between onset of symptoms and serology sample draw".

Days Between Onset of Symptoms and Specimen Draw	Number PCR Positive	Number Positive on Anti-SARS-CoV-2 Total	Positive Percent Agreement	95% CI
≤ 7	0	N/A	NA	N/A
8 to 14	7	6	85.7%	48.7% to 97.4%
≥ 15	43	42	97.7%	87.9% to 99.6%
Total:	50	48	96.0%	86.5% to 98.9%

c. Negative Percent Agreement: The following table shows the results for negative specimens either PCR-confirmed negative or collected prior to the COVID-19 pandemic (collected prior to November of 2019).

Number of Negative Samples Tested	Number Negative on Anti-SARS-CoV-2 Total	Negative Percent Agreement	95% CI
264	261	98.9%	96.7%-99.6%

d. Independent Clinical Agreement Validation Study: The Anti-SARS-CoV-2 Total was tested on February 26, 2021, at the Frederick National Laboratory for Cancer Research (FNLCR) sponsored by the National Cancer Institute (NCI). The test was validated against a panel of previously frozen samples consisting of 30 SARS-CoV-2 antibody-positive serum samples and 80 antibody-negative serum and plasma samples. Each of the 30 antibody-positive samples were confirmed with a nucleic acid amplification test (NAAT) and both IgM and IgG antibodies were confirmed to be present in all 30 samples. The presence of antibodies in the samples was confirmed by several orthogonal methods prior to testing with the Anti-SARS-CoV-2 Total. The presence of IgM and IgG antibodies specifically was confirmed by one or more comparator methods. Antibody-positive samples were selected at different antibody titers. All antibody-negative samples were collected prior to 2020 and include: i) Seventy (70) samples selected without regard to clinical status, "Negatives" and ii) Ten (10) samples selected from banked serum from HIV+ patients, "HIV+". Testing was performed by one operator using one lot of the Anti-SARS-CoV-2 Total. Confidence intervals for sensitivity and specificity were calculated per a score method described in CLSI EP12-A2 (2008). Study results and summary statistics are presented in the following table:

Measure	Estimate	95% CI
Sensitivity	93.3% (28/30)	(78.7% - 98.2%)
Specificity	100% (70/70)	(94.8% - 100%)
Combined PPV for prevalence = 5.0%	100%	(44.3% - 100%)
Combined NPV for prevalence = 5.0%	99.7%	(98.8% - 99.9%)
Cross-reactivity with HIV+	10.0% (1/10), Detected	

Important Limitations of the Independent Validation Study:

1. **Samples were not randomly selected, and sensitivity and specificity estimates may not be indicative of the real-world performance of the device.**
2. **These results are based on serum and ACD plasma samples only and may not be indicative of performance with other sample types, such as whole blood, including finger stick blood.**
3. **The number of samples in the panel is a minimally viable sample size that still provides reasonable estimates and confidence intervals for test performance, and the samples used may not be representative of the antibody profile observed in patient populations.**

3. **Comparison of Manual Verus Automated Procedures:**
To verify the correlation between the manual and the automated systems a panel of 51 samples with index values corresponding to negative, equivocal, and positive results were run using the manual method and then were run on the Dynex Agility, Dynex DSX and Dynex DS2 automated systems. In addition, 90 well replicates each of a known SARS-CoV-2 negative control sample and a known SARS-CoV-2 positive control sample were tested in full 96 well plates on each automated system. All wells on each plate generated the expected qualitative result without processing errors. The results of the regression analysis of the assay Index Values for the automated and manual runs are summarized below:

System	Slope	Intercept
Dynex Agility	1.03	0.08
Dynex DSX	0.92	0.03
Dynex DS2	1.04	0.04

REFERENCES

1. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard – Sixth Edition. CLSI document GP41-A6 (ISBN 1-56238-650-6). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2007.
2. Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline—Fourth Edition. CLSI document H18-A4 (ISBN 1-56238-724-3). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2010.
3. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens, Final Rule. Fed. Register 56:64175-64182, 1991.

GLOSSARY OF SYMBOLS

The following symbols **may** have been used in the labelling of this product or products associated with this product.

Symbol	Description	Symbol	Description
	Manufacturer		Keep away from sunlight
IVD	*In vitro* diagnostic medical device	PLATE	Plate
REF	Catalogue number	CONJ	Conjugate
Σ n	Sufficient for *n* tests	CTRL +	Positive Control
LOT	Batch code	CTRL -	Negative Control
	Use by	CAL	Calibrator
	Temperature limitation	DIL SPE	Sample Diluent
CONT	Contents	SOLN TMB	TMB
UDI	Unique Device Identifier	SOLN STOP	Stop Solution
	Consult the warnings and precautions	WASH 10X	Wash Buffer Concentrate (10X)
	Consult electronic instructions for use	EN	English
	Store in the upright position	Made in the USA	Made in the USA
RX Only	Applicable for U.S.A: Prescription *in vitro* diagnostic product		Corrosive
	Hazardous Communication		

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