SARS-CoV-2 Total Antibody Test System

REF SM9Z7901 Σ 96

IVD

CE

Rx Only

INTENDED USE

The ZEUS ELISA SARS-CoV-2 Total Antibody Test System is an Enzyme-Linked Immunosorbent Assay (ELISA) intended for the qualitative detection of human antibody (IgG, IgM or IgA) to the SARS-CoV-2 virus in human serum and plasma (Dipotassium EDTA, Lithium Heparin and Sodium Citrate). The ZEUS ELISA SARS-CoV-2 Total Antibody Test System is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. The ZEUS ELISA SARS-CoV-2 Total Antibody Test System is for *In Vitro* diagnostic use only.

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, rapid detection of cases and contacts, 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 ZEUS ELISA SARS-CoV-2 Total Antibody Test System is an indirect, antibody capture enzyme-linked immunosorbent assay designed to detect IgG, IgM or IgA class 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 Ig (IgG, IgM, or 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 depends upon the antibody concentration in the original test sample.

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®.**

Component	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 Ig (IgG, IgM or IgA) in 15mL, white-capped bottle(s). Ready to use.
CONTROL +	1	Positive Control (SARS-CoV-2 IgG in Human Serum): 0.5mL, red-capped vial(s).
CAL	1	Calibrator (SARS-CoV-2 IgG in Human Serum): 0.5mL, blue-capped vial(s).
CONTROL -	1	Negative Control (Human Serum): 0.5mL, green-capped vial(s).
DIL SPE	1	SAVe Diluent®: 30mL, green-capped, bottle(s) 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(s) containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN STOP	1	Stop Solution: 15mL, red-capped, bottle(s) containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASHBUF 10X	1	Wash Buffer Concentrate (10X): Dilute 1-part concentrate + 9 parts deionized or distilled water. 100mL, clear-capped, bottle(s) 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.**

NOTES:

1. **The following components are not Test System Lot Number dependent and may be used interchangeably within 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.**
2. **Test System also contains a Component Label containing lot specific information inside the Test System box.**

PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. This test has been authorized only for the presence of antibody against SARS-CoV-2, not for any other viruses or pathogens.
3. 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.
4. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
5. 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).
6. 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.
7. 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.
8. 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.

9. 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.
10. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
11. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
12. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
13. Dilution or adulteration of these reagents may generate erroneous results.
14. Do not use reagents from other sources or manufacturers.
15. 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.
16. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
17. Avoid microbial contamination of reagents. Incorrect results may occur.
18. Cross contamination of reagents and/or samples could cause erroneous results.
19. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
20. Avoid splashing or generation of aerosols.
21. Do not expose reagents to strong light during storage or incubation.
22. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
23. 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.
24. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
25. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
26. 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.
27. 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.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm.
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 remains blue.
	Conjugate – DO NOT FREEZE.
	Unopened Test System, Calibrator, Positive Control, Negative Control, TMB, SAVe 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

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 (MANUAL TEST METHOD)

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 1:21 diluted 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.

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 found in each kit 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.

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.

	Index Value/OD Ratio
Negative Specimens	≤0.90
Equivocal Specimens	0.91 - 1.09
Positive Specimens	≥1.10

   a. An OD ratio ≤0.90 indicates antibodies to SARS-CoV-2 were not detected.

b. An OD ratio ≥1.10 indicates antibodies specific to SARS-CoV-2 were detected.
c. Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimen using an alternate serological method and/or re-evaluate by drawing another sample one to three weeks later.

LIMITATIONS OF THE ASSAY

1. Samples collected too early in the course of an infection may not have detectable levels of antibody.
2. 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.
3. 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.
4. Reactive results must be confirmed with another available method and interpreted in conjunction with the patient's clinical information.
5. 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.
6. It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to reinfection.
7. 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.
8. 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.
9. Samples with excessive hemolysis, lipids, or bacterial contamination should be avoided. False results may occur.
10. This test is only used for the detection of antibodies to SARS-CoV-2 in human serum and plasma. This test should not be used for screening of donated blood.
11. This assay cannot be utilized to test pooled (mixed) serum or plasma. The kit has been evaluated only with individual serum or plasma specimens.

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:**

1. **Specificity; Pre-Pandemic Healthy Blood Donors**: Ninety normal healthy blood donors collected in the North Eastern US prior to November of 2019 were tested on the ZEUS ELISA SARS-CoV-2 Total Antibody Test System. All 90 specimens were negative on the ELISA. The specificity was determined to be 90/90 = 100%.
2. **Specificity; Pre-Pandemic Patients with Various Respiratory Illnesses**: Ninety specimens were collected from patients with a variety of respiratory illnesses and tested on the ZEUS ELISA SARS-CoV-2 Total Antibody Test System. 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 on the ELISA. The specificity in this cohort was determined to be 90/90 = 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 matricies were tested on the ELISA unspiked and spiked with two different levels of anti-SARS-CoV-2 antibody (low spike and moderate spike). For all five donors, the unspiked specimens were clearly negative. The low positive spike produced low positive results in all four matricies for all five donors and the moderate positive spike produced a moderate positive result in all four matricies of all five donors. The study showed that samples collected as serum, K2-EDTA plasma, Lithium Heparin plasma and/or Sodium Citrate plasma are compatible with the ZEUS ELISA SARS-CoV-2 Total Antibody Test System.

c. **Interfering Substances Study:**

Three specimens were obtained for this study; one clearly negative, one clearly positive and one moderately postive for antibody to the SARS-CoV-2 virus. These three specimens were spiked with two different concentrations (low spike and high spike) of various potential interferents; albumin, hemoglobin, intralipid, bilirubin, cholesterol and triglycerides. None of these substances appeared to interfer with the results of the ELISA test. A summary of the data appears in the table below.

	Moderate Positive Serum		Low Positive Serum		Negative Serum	
Sample	**Index Value**	**Result**	**Index Value**	**Result**	**Index Value**	**Result**
Serum Matrix Contorl	2.11	Positive	1.34	Positive	0.54	Negative
Albumin (3.5 g/dL)	2.13	Positive	1.30	Positive	0.49	Negative
Albumin (5.0 g/dL)	2.15	Positive	1.32	Positive	0.46	Negative
Hemoglobin (10 g/dL)	2.08	Positive	1.22	Positive	0.47	Negative
Hemoglobin (20 g/dL)	2.10	Positive	1.19	Positive	0.45	Negative
Intralipid (300 mg/dL)	2.20	Positive	1.29	Positive	0.52	Negative
Intralipid (750 mg/dL)	2.02	Positive	1.25	Positive	0.52	Negative
Bilirubin (1 mg/dL)	2.15	Positive	1.28	Positive	0.54	Negative
Bilirubin (15 mg/dL)	2.14	Positive	1.26	Positive	0.50	Negative
Cholesterol (150 mg/dL)	2.12	Positive	1.20	Positive	0.49	Negative
Cholesterol (250 mg/dL)	2.10	Positive	1.22	Positive	0.53	Negative
Triglycerides (150 mg/dL)	2.12	Positive	1.26	Positive	0.56	Negative
Triglycerides (500 mg/dL)	2.09	Positive	1.26	Positive	0.50	Negative

d. **Precision Study**:

Five specimens were identified that produced a variety of results on the ELISA test; clear negative, high negative, borderline positive, low positive and moderate positive. These five specimens were tested in triplicate, two times per day on five different days, resulting in 30 different results for each of the five samples. The five day precision study of the ZEUS ELISA SARS-CoV-2 Total Antibody Test System is summarized below:

Sample	Mean Index Value	Standard Deviation	Percent CV
Moderate Positive	2.29	0.12	5.38
Low Positive	1.69	0.09	5.53
Borderline Positive	1.16	0.08	6.56
High Negative	0.81	0.07	8.23
Clear Negative	0.26	0.02	8.35

2. **Clinical Performance Studies:**

a. Two separate cohorts of clinically characterized specimens were assembled as follows:

i. COVID-19 RT-PCR Positive Patient Specimens (n=50): The time frame between the onset of clinical symptoms and the date the sample was collected ranged from a minimum of eight days to a maximum of 97 days with a mean of 31 days and a median of 29.5 days.

ii. COVID-19 RT-PCR Negative Patient Specimens (n=84).

b. Using PCR as the reference, the ZEUS ELISA SARS-CoV-2 Total AntibodyTest System performed as follows:

		PCR Testing		
		Positive	Negative	Total
ZEUS ELISA SARS-CoV-2 Total Antibody Test System	Positive	50	0	50
	Negative	2	81	83
	Equivocal	0	3	3
	Total	48	84	

Clinical Sensitivity = 48/50 = 96.0% (95% CI = 86.3% - 99.5%) Clinical Specificity = 81/84 = 96.4% (95% CI = 89.9% - 99.3%)

c. For the PCR positive specimens; stratifying patients into "days between onset of symptoms and sample draw".

		ZEUS ELISA SARS-CoV-2 Total Antibody Test System		
Days Between Onset of Symptoms and Specimen Draw	**Number PCR Positive**	**Number Positive on ZEUS ELISA**	**Positive Percent Agreement**	**95% CI**
≤ 7	0	0	N/A	N/A
8 to 14	7	6	85.7%	42.3% to 99.6%
≥ 15	43	42	97.7%	87.7% to 99.9%

d. **Comparison to Serology:**

Two separate cohorts of serologically characterized specimens were assembled as follows:

i. Fifty-one specimens that were tested and found to be positive using a FDA, EUA-approved serological immunoassay.

ii. Seventy-seven specimens that were tested and found to be negative using a FDA, EUA-approved serological immunoassay.

In the positive cohort, ZEUS ELISA SARS-CoV-2 Total Antibody Test System was also positive for 50/51 for a Positive Percent Agreement of 98.0% (95% CI of 89.6% to 99.9%). In the negative cohort, ZEUS ELISA SARS-CoV-2 Total Antibody Test System was also negative for 77/77 for a Negative Percent Agreement of 100% (95% CI of 95.3% to 100%).

e. **Donor-Matched and Longitudinal Sample Testing:**

Four of the PCR confirmed postive patients provided both serum and matched K2 EDTA plasma. All four patients were positive for both the serum and plasma. Three other PCR confirmed positive patients provided specimens over multiple days. One 79 year old male provided five different specimens 15 to 27 days post onset of symptoms. One 74 year old female provided four different specimens eight to 19 days post onset of symptoms. Finally, a 43 year old female provided two specimens; one at 28 and one at 29 days post onset of symptoms. All 11 specimens from the three patients providing the longitudinal donations were positive on the ZEUS ELISA SARS-CoV-2 Total Antibody Test System.

REFERENCES

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3. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens, Final Rule. Fed. Register 56:64175-64182, 1991.

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