Cardiolipin IgG/IgM/IgA Test System

REF 2Z51051/SM2Z51051

IVD

CE

Rx Only

INTENDED USE

The ZEUS ELISA Cardiolipin IgG/IgM/IgA Test System is intended for the in vitro, qualitative measurement of IgG, IgM and/or IgA antibodies directed to Cardiolipin in human serum to aid in the diagnosis of primary antiphospholipid syndrome (PAPS) and secondary antiphospholipid syndrome (SAPS) in conjunction with other laboratory and clinical findings.

SIGNIFICANCE AND BACKGROUND

Autoantibodies directed against phospholipids, and anti-Cardiolipin (aCL) in particular, have been associated with recurrent thrombosis, thrombocytopenia, and spontaneous abortions (1, 2, and 3). aCL is observed in patients with systemic lupus erythematosus, in patients with other connective tissue disease (4), in individuals undergoing chlorpromazine treatment (5), as well as in persons who do not have chronic illness.

PRINCIPLE OF THE ASSAY

The ZEUS ELISA Cardiolipin IgG/IgM/IgA Test System is designed to detect IgG/IgM/IgA class antibodies to Cardiolipin in human sera. Wells of plastic microwell strips are sensitized by passive adsorption with Cardiolipin antigens. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. HRP 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 Conjugate are incubated with 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 Sample Diluent.**

PLATE		1. Plate: 96 wells configured in twelve, 1x8-well, strips coated with Cardiolipin antigen form bovine heart. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ		2. Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG/IgM/IgA. One, 15mL, white-capped bottle. Ready to use.
CONTROL	+	3. Positive Control (Human Serum): One, 0.35mL, red-capped vial.
CAL		4. Calibrator (Human Serum): One, 0.5mL, blue-capped vial.
CONTROL	-	5. Negative Control (Human Serum): One, 0.35mL, green-capped vial.
DIL	SPE	6. Sample Diluent: One, 30mL, green-capped, bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Green solution, ready to use.
SOLN	TMB	7. TMB: One, 15mL, amber-capped, amber bottle containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN	STOP	8. Stop Solution: One, 15mL, red-capped, bottle containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASHBUF	10X	9. Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. One, 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.**

NOTES:

1. **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.**
2. **Test System also contains a Component Label containing lot specific information inside the Test System box.**

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. 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.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
4. 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, these products should be handled 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 (5).
5. 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.
6. 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.
7. The Sample 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 upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. 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.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.

13. Do not use reagents from other sources or manufacturers.
14. 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.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. 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.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. 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.
26. 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. **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 remains blue.
	Conjugate – DO NOT FREEZE.
	Unopened Test System, 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

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 sera obtained by approved aseptic venipuncture procedures in this assay (8, 9). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
   Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. If a delay in testing is anticipated, store test sera at –20°C or lower. 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 (13).

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. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent) of the Negative Control, Calibrator, Positive Control, and each patient serum.
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 Sample 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 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, Positive Control, and Negative Control should fall within the following ranges:

	OD Range
Negative Control	≤0.250
Calibrator	≥0.300
Positive Control	≥0.500

   a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤0.9.
   b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥1.25.
   c. If the above conditions are not met the test should be considered invalid and should be repeated.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure, but will not ensure precision at the assay Cutoff.
5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. 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. *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)
   c. *Index Values/OD Ratios:* Calculate the Index Value/OD Ratio for each specimen by dividing its OD Value by the Cutoff OD from step b.

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 to 1.09
Positive Specimens	≥1.10

   a. An OD ratio ≤0.90 indicates no detectable antibodies to Cardiolipin IgG/IgM/IgA and should be reported as negative for IgG/IgM/IgA Cardiolipin antibodies.
   b. An OD ratio ≥ 1.10 is positive for IgG/IgM/IgA antibody to Cardiolipin.
   c. Retest specimens with OD Ratio Values in the equivocal range (0.91 – 1.09) in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimens using an alternate serological method and/or re-evaluate by drawing another sample one to three weeks later.

LIMITATIONS OF THE ASSAY

1. A diagnosis should not be made on the basis of the ZEUS ELISA Cardiolipin IgG/IgM/IgA Test System results alone. Test results for anti-Cardiolipin should be interpreted in conjunction with clinical evaluation and results of other diagnostic procedures.
2. The performance characteristics of this device have not been established for lipemic, hemolyzed and icteric specimens. Therefore, these specimens should not be tested with this assay.
3. Although aCL has been associated with certain SLE subsets (1 - 3), the clinical significance of aCL in SLE and other diseases remains under investigation.
4. The clinical significance of any test result depends upon its relationship to other medical patient data. Disease diagnosis and management should be based on an evaluation of all relevant patient information.
5. A high percentage of confirmed active or seropositive syphilis patients may have elevated aCL levels (10). Confirmatory procedures should be performed to rule out syphilis.
6. aCL may also be found in infections such as hepatitis C (11), malaria, lyme disease and HIV; leukemias and solid-organ malignancies; and frequently in alcoholic cirrhosis (12).
7. Performance characteristics of this device have not been established for matrices other than serum.

EXPECTED RESULTS

1. Demographics And Age Distribution Of The Intended Use Populations:

Three-hundred and three (303) samples submitted for Cardiolipin antibody testing, 294 samples from blood donors, and 503 clinically defined samples were tested at three sites. Site One was the manufacturer, located in New Jersey. Site Two was a hospital laboratory also located in New Jersey. Site Three was a hospital laboratory located in Pennsylvania. Each site tested a third of the samples from each population group. The total volume of samples tested was 1223. The patient demographics are summarized in Table 1. Ninety-eight (98) samples from patients diagnosed with PAPS and 25 samples from patients diagnosed with SAPS were also tested at Site One. Table 2 summarizes these results.

Table 1: Demographics for Populations Tested

Populations	Number Tested	Male	Female	Unknown	Mean Age
Samples Submitted for Cardiolipin Testing	303	102	201	0	48.3
Blood Donors	294	145	149	0	41.9
Clinically Defined Samples	503	65	430	8	41.3
PAPS Samples	98	*	*	*	*
SAPS Samples	25	6	19	0	42.1

*Not available

2. Characterized Samples from Patients with Primary Anti-Phospholipid Syndrome and Secondary Anti-Phospholipid Syndrome:

Ninety-eight (98) samples from patients with PAPS and 25 samples from patients with SAPS were acquired from a medical laboratory and evaluated. Agreement with the clinical diagnosis is presented in Table 2.

Table 2: Testing of Characterized Sera from Patients Diagnosed with PAPS and SAPS

Test System	PAPS Samples			% Agreement with Clinical Diagnosis Presented with 95% CI	
	Positive	Normal	Total		
ZEUS ELISA Cardolipin IgG/IgM/IgA	83	15	98	84.7 (83/98)	74.0 - 91.2
	SAPS Samples				
ZEUS ELISA Cardolipin IgG/IgM/IgA	23	2	25	92.0 (23/25)	74.0 - 99.0

3. Prevalence in Selected Populations:

Prevalence in selected populations for which Cardiolipin antibody testing was requested and for normal blood donors was calculated. Additionally, the prevalence for selected populations of patients with various autoimmune or clotting disorders and pregnant women with a history of pre-eclampsia were also calculated.

Table 3: Reference Range Study for Cardiolipin Antibodies

Population	ZEUS ELISA Cardiolipin IgG/IgM/IgA Results			Observed % Prevalence Cardiolipin Antibodies
	Positive	Negative	Total	
Sera Submtted for Cardiolipin Antibody Testing	47	256	303	15.50
Sera from Healthy Population of Blood Donors	10	284	294	3.40

Table 4: Clinical Study for Cardiolipin Antibodies

Population	ZEUS ELISA Cardiolipin IgG/IgM/IgA Results			Observed % Prevalence Cardiolipin Antibodies
	Positive	Negative	Total	
Thrombocytopenia	2	8	10	20.0
Pre-Eclampsia	0	25	25	0.0
MCTD	4	39	43	9.3
PSS	2	74	76	2.6
Rheumatoid Arthritis	22	249	271	8.1
Sjogren's	0	11	11	0.0
SLE	13	30	43	30.2
Vasculitis	0	17	17	0.0
Disease States Total	43	453	496	8.7

PERFORMANCE CHARACTERISTICS

1. Comparative Study:

The ZEUS ELISA Cardiolipin IgG/IgM/IgA Test System was compared to commercially marketed individual antibody ELISA test systems for detection of IgG, IgM and IgA antibody to Cardiolipin. The clinical study consisted of 1100 serum samples. Three-hundred and three (303) samples from patients for which Cardiolipin antibody testing was requested were purchased from a serum vendor. Two-hundred and ninety-four (294) samples were from healthy blood donors purchased from a serum vendor. Five-hundred and three (503) samples were from patients with various autoimmune diseases, thrombocytopenia, pre-eclampsia and sera requested to be Cardiolipin antibody positive and were acquired from commercial sources.

Table 5: Summary of Comparative Testing

		Predicate Test Systems				
		Positive	**Negative**	**Site Total**	**PPA/NPA**	**95% CI**
ZEUS ELISA Cardiolipin IgG/IgM/IgA	**Positive**	78	20	98	86.7%	77.9 - 92.9
	Negative	12	696	708	97.2%	95.7 - 98.3
	Site Total	90	716	806		

Table 6:

	Predicate Test Systems				
ZEUS ELISA Cardiolipin IgG/IgM/IgA	**Clinically Defined Samples**				
	Positive	**Negative**	**Site Total**	**PPA/NPA**	**95% CI**
Positive	33	18	51	82.5%	67.2 - 92.7
Negative	7	445	452	96.1%	93.9 - 97.7
Site Total	40	463	503		
	Samples Submitted for Cardiolipin Antibody Testing				
	Positive	**Negative**	**Site Total**	**NPA**	**95% CI**
Positive	45	2	47	90.0%	78.2 - 96.7
Negative	5	251	256	99.2%	97.2 - 100
Site Total	50	253	303		

Note: samples that were equivocal by the predicate methodology were treated as negative in the calculations

Table 7: Analytical Specificity (Evaluated at three Sites)

Population	Number	Negative	Positive	% Positivity*
Blood Donors	294	284	10	3.4

*% positivity with the predicate was found to be: 4.4%

2. Reproducibility:

Precision and reproducibility were evaluated internally and at two external clinical sites. The study was conducted as follows: 12 samples were identified and/or prepared for use in the study based upon their activity on the assay. Two samples each were selected that were high positive (1 & 2), moderate positive (3 & 4) low positive (5 & 6), near cutoff (7 & 8), high negative (9& 10), and low negative (11 & 12). The Negative and Positive Controls and Calibrator from the Test System were also used to assess precision and reproducibility. On each day of testing, each sample was diluted twice and each dilution was run in triplicate. This process was repeated by a second technologist, resulting in twelve results per day. This was repeated for five days at each site and the resulting data used to assess reproducibility.

Table 8: Summary of Precision and Reproducibility

			Site One					Site Two					Site Three				
Sample	**N**	**Expected Result**	**Mean IV**	**% Positive**	**% Negative**	**Result Range (IV)**		**Mean IV**	**% Positive**	**% Negative**	**Result Range (IV)**		**Mean IV**	**% Positive**	**% Negative**	**Result Range (IV)**	
						Low	**High**				**Low**	**High**				**Low**	**High**
1	60	Positive	5.43	100	0	4.700	6.110	5.48	100	0	4.540	6.480	5.30	100	0	4.5	6.29
2	60	Positive	5.58	100	0	4.660	6.270	5.54	100	0	4.710	6.380	5.20	100	0	4.53	5.73
3	60	Positive	2.49	100	0	2.066	2.799	2.50	100	0	2.103	2.946	2.29	100	0	1.839	2.57
4	60	Positive	2.32	100	0	1.905	2.846	2.40	100	0	2.004	3.274	2.23	100	0	1.815	2.88
5	60	Positive	1.45	100	0	1.123	1.767	1.53	100	0	1.275	2.272	1.28	100	0	1.066	1.612
6	60	Positive	1.38	100	0	1.086	1.638	1.46	100	0	1.057	1.725	1.32	100	0	1.036	1.556
7	60	Negative	0.97	36.7	63.3	0.728	1.150	1.06	73.3	26.7	0.887	1.179	0.94	25.0	75.0	0.771	1.124
8	60	Negative	0.86	2	98.3	0.694	1.020	0.96	75	25.0	0.801	1.099	0.84	2	98.3	0.705	1.025
9	60	Negative	0.54	0	100.0	0.426	0.912	0.64	0	100	0.475	0.817	0.56	0	100	0.45	0.671
10	60	Negative	0.60	0	100	0.431	0.766	0.64	0	100	0.482	0.833	0.55	0	100	0.423	0.685
11	60	Negative	0.23	0	100	0.151	0.417	0.23	0	100	0.169	0.289	0.19	0	100	0.113	0.238
12	60	Negative	0.30	0	100	0.208	0.580	0.31	0	100	0.235	0.531	0.24	0	100	0.175	0.314
NC	60	Negative	0.10	0	100	0.064	0.138	0.09	0	100	0.058	0.169	0.06	0	100	0.026	0.09
Cal.	60	Positive	3.26	100	0	2.655	3.655	3.27	100	0	2.748	4.888	3.25	100	0	2.859	3.52
Cal.	60	Positive	3.26	100	0	2.831	3.741	3.26	100	0	2.833	4.65	3.22	100	0	2.735	3.456
Cal.	60	Positive	3.19	100	0	2.756	3.611	3.25	100	0	2.759	3.736	3.28	100	0	2.995	3.576
PC	60	Positive	6.15	100	0	5.375	6.650	5.98	100	0	5.241	6.724	6.11	100	0	5.435	7.001

3. Cross Reactivity:

Studies were performed at the manufacturing facility to assess cross reactivity with the ZEUS ELISA IgG/IgM/IgA Screen Test System using samples that were sero-positive to ANA, dsDNA, RF, Rubella, HSV 1, HSV 2, VZV, Measles, Mumps, HCV and syphilis. AtheNA Multi-Lyte and ELISA immunoassay test systems manufactured for commercial distribution were used to determine the sero-positivity of the samples. Ten samples for each possible cross-reactant were tested. The results presented were obtained by testing the analytes against high concentrations of possible cross reactants and summarized in Table 9.

Table 9: Cross Reactivity

Cross Reactivity Study - ZEUS ELISA Cardiolipin IgG/IgM/IgA Test System	
Analyte	Positive/Tested
ANA	0/10
DNA	0/10
HSV 1	0/10
HSV 2	0/10
Measles	0/10
Mumps	0/10
RF IgM	0/10
Rubella	0/10
Syphilis	0/10
HCV	0/10
VZV	0/10

4. Interfering Substances:

The effect of potential interfering substances on sample results generated using the assay was evaluated with the following possible interfering substances: albumin, bilirubin, cholesterol, hemoglobin, triglycerides and intralipids. The quantity of analyte in each interfering substance is as follows:

Bilirubin: 1mg/dL (low), 15 mg/dL (high)
Albumin: 3.5 g/dL (low), 5 g/dL (high)
Cholesterol: 150 mg/dL (low), 250 mg/dL (high)
Triglycerides: 150 mg/dL (low), 500 mg/dL (high)

Hemoglobin: 10 g/dL (low), 20 g/dL (high)
Intralipid: 300 mg/dL (low), 750 mg/dL (high)

All positive samples showed a change of signal less than 20%. The borderline samples showed a change of signal less than 20% of with the exception of the high spike of albumin (28.3%), intralipid (31.4%) and cholesterol (35.7%). The negative sample showed a reduction of signal >20% with the high spike of hemoglobin (-21.2%) and an increase of signal with the high spikes of cholesterol (32.5%) and triglyceride (44.9%) and low spike of cholesterol (68.3%). The negative sample results in each instance stayed below the cut-off and the change in signal did not affect the qualitative result.

Table 10: ZEUS ELISA Cardiolipin IgG/IgM/IgA Interfering Substances Study

	Positive Sample			Borderline Sample			Negative Sample		
Spiked Level	Index Value	Result Outcome	% Positive Signal	Index Value	Result Outcome	% Positive Signal	Index Value	Result Outcome	% Positive Signal
Neat Control	8.60	Positive		1.20	Positive		0.36	Negative	
Albumin 3.5g/dL	9.70	Positive	112.8	1.54	Positive	128.3	0.33	Negative	93.0
Albumin 5g/dL	9.79	Positive	113.8	1.47	Positive	122.0	0.36	Negative	100.8
Hemoglobin 10g/dL	8.25	Positive	96.0	0.99	Negative	82.5	0.28	Negative	78.8
Hemoglobin 20g/dL	8.54	Positive	99.2	0.98	Negative	81.5	0.30	Negative	83.3
Intralipid 300mg/dL	9.45	Positive	109.8	1.58	Positive	131.4	0.30	Negative	84.7
Intralipid 750mg/dL	9.80	Positive	114.0	1.40	Positive	116.1	0.36	Negative	101.4
PBS Control	9.30	Positive		1.36	Positive		0.32	Negative	
Bilirubin 1mg/dL	8.82	Positive	94.9	1.41	Positive	103.8	0.29	Negative	91.9
Bilirubin 15mg/dL	8.93	Positive	96.0	1.32	Positive	97.6	0.26	Negative	80.3
Ethanol Control	8.95	Positive		1.14	Positive		0.24	Negative	
Cholesterol 150mg/dL	9.40	Positive	104.9	1.55	Positive	135.7	0.32	Negative	132.5
Cholesterol 250mg/dL	8.80	Positive	98.2	1.06	Positive	92.7	0.41	Negative	168.3
Triglycerides 150mg/dL	9.23	Positive	103.1	1.19	Positive	104.4	0.35	Negative	144.9
Triglycerides 500mg/dL	8.23	Positive	91.9	0.97	Negative	85.1	0.27	Negative	110.7

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