

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k102425

B. Purpose for Submission:

New device

C. Measurand:

Human anti-cardiolipin IgG, IgM, and IgA antibodies

D. Type of Test:

Qualitative immunoassay

E. Applicant:

ZEUS Scientific, Inc.

F. Proprietary and Established Names:

ZEUS ELISA Cardiolipin IgG/IgM/IgA Test System

G. Regulatory Information:

1. Regulation section:
21 CFR §866.5660, Multiple autoantibodies immunological test system
2. Classification:
Class II
3. Product code:
MID – System, Test, Anti-Cardiolipin Immunological
4. Panel:
Immunology (82)

H. Intended Use:

1. Intended use(s):
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.
2. Indication(s) for use:
See intended use above.
3. Special conditions for use statement(s):
For prescription use only.
4. Special instrument requirements:
ELISA microwell reader capable of reading at wavelength 450 nm.

I. Device Description:

The ZEUS ELISA Cardiolipin IgG/IgM/IgA Test System contains components in sufficient quantities to perform the number of tests indicated on the packaging label. The components are as follows: a 96-well plate configured in twelve 1x8 well strips coated with cardiolipin antigen from bovine heart; one 15-mL ready-to-use vial of conjugated (horseradish peroxidase, HRP) goat anti-human IgG/IgM/IgA; one 0.35-mL vial of positive control (human serum); one 0.5-mL vial of calibrator (human

serum); one 0.35mL-vial of negative control (human serum); one ready-to-use 30-mL bottle of SAVe diluent (sample diluent) containing Tween 20, bovine serum albumin and phosphate buffered saline (pH 7.2 ± 0.2); one ready-to-use 15-mL bottle of 3,3',5,5', tetramethylbenzidine (TMB); one ready-to-use 15-mL bottle of stop solution containing 1M H₂SO₄ and 0.7M HCl and wash buffer concentrate.

J. Substantial Equivalence Information:

1. Predicate device name(s) and Predicate k number(s):
 ZEUS Scientific Cardiolipin IgA ELISA Reagents k973196
 ZEUS Scientific Anti-Cardiolipin IgG ELISA Test System, k981014
 ZEUS Scientific Anti-Cardiolipin IgM ELISA Test System, k981021
2. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	In vitro measurement of antibodies to cardiolipin	Same
Assay	Colorimetric Immunoassay	Same
Antigen	Cardiolipin antigen from bovine heart	Same
Sample Matrix	Serum	Same
Substrate	TMB	Same
Controls	One Positive Control, one Negative Control	Same
Calibration	Includes Calibrator	Same

Differences		
Item	Device	Predicate
Indications for 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.	The Zeus Scientific, Inc. Cardiolipin Antibody Test Systems are enzyme-linked immunosorbent assays (ELISA) designed for the semi-quantitative measurement of circulating IgA (IgM or IgG) autoantibodies to cardiolipin.
Assay type	Qualitative	Semi-quantitative

Differences		
Item	Device	Predicate
Analyte	Human IgA, IgM and IgG antibodies to cardiolipin	Human IgA, IgM or IgG antibodies to cardiolipin (depending on specific kit used)
Conjugate	HRP goat anti-human IgA, IgM, and IgG	HRP goat anti-human IgA, IgM, or IgG

K. Standard/Guidance Document Referenced (if applicable):

None

L. Test Principle:

The ZEUS ELISA Cardiolipin IgG/IgM/IgA is designed to detect IgG/IgM/IgA class antibodies to Cardiolipin in human sera. Wells of plastic microwell strips are coated by passive absorption 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.

Assay results are calculated as an Index Value (IV), a ratio of the sample optical density to the optical density of the Calibrator multiplied by a correction factor determined by the manufacturer. The correction factor is determined for each lot of kit components and is printed on the Component List located in the kit box. An IV of ≥ 1.0 indicates a positive assay.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Reproducibility of the assay was evaluated at the sponsor's laboratory and at two external clinical sites. Twelve samples were chosen to represent a range of sample IVs from negative to high positives, including a sample near the cut-off.

Each original serum sample was diluted 1:21 (as per assay directions) separately twice and then tested in triplicate. A second technologist repeated this process. Therefore, 12 results were obtained per day and the samples were tested for five days so that the total number of results was 60. Results are presented below by site:

Site	Sample	Mean IV	Result Expected	% Positive	% Negative	Result Range (IV)	
						Low	High
1	1	5.43	Positive	100%	0%	4.70	6.11
	2	5.58	Positive	100%	0%	4.66	6.27
	3	2.49	Positive	100%	0%	2.07	2.80
	4	2.32	Positive	100%	0%	1.90	2.85
	5	1.45	Positive	100%	0%	1.12	1.77
	6	1.38	Positive	100%	0%	1.09	1.64
	7	0.97	Negative	36.7%	63.3%	0.73	1.15
	8	0.86	Negative	2%	98.3%	0.69	1.02
	9	0.54	Negative	0%	100.0%	0.43	0.91
	10	0.60	Negative	0%	100%	0.43	0.77
	11	0.23	Negative	0%	100%	0.15	0.42
	12	0.30	Negative	0%	100%	0.21	0.58
2	1	5.48	Positive	100%	0%	4.54	6.48
	2	5.54	Positive	100%	0%	4.71	6.38
	3	2.50	Positive	100%	0%	2.10	2.95
	4	2.40	Positive	100%	0%	2.00	3.27
	5	1.53	Positive	100%	0%	1.28	2.27
	6	1.46	Positive	100%	0%	1.06	1.72
	7	1.06	Positive	73.3%	26.7%	0.89	1.18
	8	0.96	Negative	75%	25.0%	0.80	1.10
	9	0.64	Negative	0%	100%	0.48	0.82
	10	0.64	Negative	0%	100%	0.48	0.83
	11	0.23	Negative	0%	100%	0.17	0.29
	12	0.31	Negative	0%	100%	0.24	0.53
3	1	5.30	Positive	100%	0%	4.50	6.29
	2	5.20	Positive	100%	0%	4.53	5.73
	3	2.29	Positive	100%	0%	1.84	2.57
	4	2.23	Positive	100%	0%	1.82	2.88
	5	1.28	Positive	100%	0%	1.07	1.61
	6	1.32	Positive	100%	0%	1.04	1.56
	7	0.94	Negative	25.0%	75.0%	0.77	1.12
	8	0.84	Negative	2%	98.3%	0.70	1.02
	9	0.56	Negative	0%	100%	0.45	0.67
	10	0.55	Negative	0%	100%	0.42	0.68
	11	0.19	Negative	0%	100%	0.11	0.24
	12	0.24	Negative	0%	100%	0.18	0.31

Lot-to-Lot Reproducibility:

To assess the reproducibility of the assay across manufacturing lots, 90 clinical samples were obtained and tested once with each lot. The samples were chosen as follows: 30 negative samples (IV: ≤ 0.7), 30 positive samples (IV: ≥ 1.3), and 30 samples around the assay cut-off (target IV to range from 0.8 to 1.2). All positive samples were tested with all three lots on one day, all negative samples were tested with all three lots on another day, and all

samples around the cut-off were tested with all three lots on a third day to minimize non-lot-to-lot factors.

Group	Sample IV range (all lots)	Consensus*		
		Lot 1 and Lot 2	Lot 1 and Lot 3	Lot 2 and Lot 3
Negative	0.01 – 0.63	30/30	30/30	30/30
Positive	1.52 – 10.59	30/30	30/30	30/30
Near Cut-Off	0.74 – 1.55	22/30	22/30	24/30

*Consensus = agreement between results (e.g., positive and positive)

- b. *Linearity/assay reportable range:*
Not applicable.
- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
An international reference standard for anti-cardiolipin antibodies is not available.

Calibrators and Controls:

Calibrators and Controls are manufactured by diluting human serum containing anti-cardiolipin antibodies into a serum diluent. A target concentration is achieved through trial dilutions on a small scale. Once a dilution is selected, the control is bulked, tested, and adjusted, and the final value is determined.

Unopened kit stability:

Ongoing real-time stability testing currently supports a 12 month shelf-life claim. A kit is opened and tested for a baseline reading on the controls, calibrators and a positive sample and a negative sample. Every three months an unopened kit with the same lot number and an aliquot of the samples are re-tested. Mean and drift are calculated. The sponsor’s acceptance criteria were met at all time points.

Opened kit stability:

A stability study supports the claim that the antigen-coated well strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator strip on the desiccant pouch remains blue. A kit is opened and tested for a baseline reading on the controls and calibrators. After a minimum of 60 days, the same kit and an aliquot of the samples are re-tested. Drift is calculated. The sponsor’s acceptance criteria were met at all time points. Other components are stable until the stated expiration date if stored per the instructions.

Sample stability:

The package insert claim that serum samples should be stored at 2° – 8°C for no longer than 8 hours and refrigerated for no longer than 48 hours is based on CLSI Document H18-A2 “Procedures for the Handling and Processing of Blood Specimens”.

- d. *Detection limit:*
Not applicable for a qualitative assay.
- e. *Analytical specificity:*

Endogenous interferents:

Interfering Substances: The effect of potentially interfering substances on test results generated using the ZEUS ELISA Cardiolipin IgG/IgM/IgA Test System was evaluated with the following possible interfering substances: albumin, bilirubin, cholesterol, hemoglobin, triglycerides and intralipids. The level of each potentially interfering substance is as follows:

- Bilirubin: 1 mg/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)

The possible interfering substances were added to three samples (1 positive, 1 borderline and 1 negative) and then tested according to the instructions. All positive samples showed a change of signal less than 20% compared to the unspiked sample. All borderline samples showed a change of signal less than 20% with the exception of the high spike of hemoglobin (25.2%). The negative sample showed a change of signal (>20%) with the high and low spikes of albumin, hemoglobin, intralipid, bilirubin, cholesterol, and triglycerides, but the negative sample results in each instance stayed below the cut-off and the change in signal did not affect the qualitative result.

Reactivity to samples with known antibody status:

Ten samples each that were known to be seropositive for the following antibodies were tested with the ZEUS ELISA Cardiolipin IgG/IgM/IgA Test System: ANA, dsDNA, RF, Rubella, HSV 1, HSV 2, VZV, Measles, Mumps, HCV and syphilis. None of the samples tested positive (Index Value range 0.02 – 0.72).

f. Assay cut-off:

The assay cut-off is 1.0.

2. Comparison studies:

a. Method comparison with predicate device:

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 comparative study consisted of 806 serum samples, including 303 samples from patients for which cardiolipin antibody testing was requested, and 503 samples from patients with various autoimmune diseases (e.g., thrombocytopenia, pre-eclampsia and sera requested to be cardiolipin antibody positive but not otherwise defined). Testing was performed at three sites; each site tested a third of the samples from each population group:

		Predicate Assay		
		Positive	Negative	Total
ZEUS ELISA Cardiolipin IgG/IgM/IgA	Positive	78	20	98
	Negative	12	696	708
	Total	90	716	806

Positive agreement = 86.7% (95% C.I. = 77.9 – 92.9%)
 Negative agreement = 97.2% (95% C.I. = 95.7– 98.3%)

- b. *Matrix comparison:*
 Not applicable.

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

A total of 922 clinically defined samples were available for an analysis of the clinical sensitivity of PAPS and an analysis of the clinical sensitivity of SAPS. Both analyses included 303 samples from patients for which cardiolipin antibody testing was requested and 496 samples from patients with various autoimmune diseases (e.g., thrombocytopenia, pre-eclampsia). 98 samples from patients with clinically diagnosed PAPS were included in the analysis of PAPS, and 25 samples from patients with clinically diagnosed SAPS were included in the analysis of SAPS. The PAPS samples were not included in the SAPS analysis and vice versa. Results from the ZEUS ELISA Cardiolipin IgG/IgM/IgA Test System were compared to the clinical diagnosis:

PAPS samples (SAPS not included):

		Diagnosis		
		PAPS	non-PAPS	Total
ZEUS ELISA Cardiolipin IgG/IgM/IgA	Positive	83	90	173
	Negative	15	709	724
	Total	98	799	897

Sensitivity = 84.7% (95% C.I. = 74.0 – 91.2%)

Specificity = 88.7% (95% C.I. = 86.4 – 90.8%)

SAPS samples (PAPS not included):

		Diagnosis		
		SAPS	non-SAPS	Total
ZEUS ELISA Cardiolipin IgG/IgM/IgA	Positive	23	90	113
	Negative	2	709	711
	Total	25	799	824

Sensitivity = 92.0% (95% C.I. = 74.0 – 99.0%)

Specificity = 88.7% (95% C.I. = 86.4 – 90.8%)

The results for non-APS subjects are shown below:

Population	ZEUS ELISA Cardiolipin IgG/IgM/IgA Results			Observed % Prevalence
	Positive	Negative	total	Cardiolipin Antibodies
Sera Submitted for Cardiolipin Antibody Testing	47	256	303	15.5%
Sera from Healthy Population of Blood Donors	10	284	294	3.4%

Sera from Other Autoimmune Diseases	43	453	496	8.7%
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%
Sjorgen's Syndrome	0	11	11	0.0%
SLE	13	30	43	30.2%
Vasculitis	0	17	17	0.0%

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

The expected value in the normal population is negative.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.