510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
   k040463

B. Purpose for Submission:
   New devices

C. Analyte:
   Anti-cardiolipin antibodies

D. Type of Test:
   Qualitative and semi-quantitative, EIA

E. Applicant:
   AESKU, Inc.

F. Proprietary and Established Names:
   AESKULISA® Cardiolipin AGM
   AESKULISA® Cardiolipin A
   AESKULISA® Cardiolipin GM
   AESKULISA® Cardiolipin Check

G. Regulatory Information:
   1. <u>Regulation section:</u>
      21 CFR §866.5660, Multiple Autoantibodies Immunological Test System
   2. <u>Classification:</u>
      Class II
   3. <u>Product Code:</u>
      MID, System Test, Anti-cardiolipin Immunological
   4. <u>Panel:</u>
      Immunology (82)

H. Intended Use:
   <u>AESKULISA Cardiolipin AGM</u> is a solid phase enzyme immunoassay employing highly purified cardiolipin plus native human β2-glycoprotein I (β2-GPI) for the semiquantitative and qualitative detection of IgA, IgG and/or IgM antibodies against cardiolipin in human serum. Anti-cardiolipin antibodies mainly recognize specific epitopes on a complex composed of cardiolipin and β2-GPI which are only expressed when β2-GPI interacts with cardiolipin. The assay is an aid in the diagnosis of systemic lupus erythematosus (SLE), primary and secondary anti-phospholipid syndrome (APS) and should be used in conjunction with other serological tests and clinical findings.

AESKULISA Cardiolipin GM is a solid phase enzyme immunoassay employing highly purified cardiolipin plus native human β2-glycoprotein I (β2-GPI) for the semiquantitative and qualitative detection of IG and/or IgM antibodies against cardiolipin in human serum. Anti-cardiolipin antibodies mainly recognize specific epitopes on a complex composed of cardiolipin and β2-GPI which are only expressed when β2-GPI interacts with cardiolipin. The assay is an aid in the diagnosis of systemic lupus erythematosus (SLE), primary and secondary anti-phospholipid syndrome (APS) and should be used in conjunction with other serological tests and clinical findings.

AESKULISA Cardiolipin A is a solid phase enzyme immunoassay employing highly purified cardiolipin plus native human β2-glycoprotein I (β2-GPI) for the semiquantitative and qualitative detection of IgA antibodies against cardiolipin in human serum. Anti-cardiolipin antibodies mainly recognize specific epitopes on a complex composed of cardiolipin and β2-GPI which are only expressed when β2-GPI interacts with cardiolipin. The assay is an aid in the diagnosis of systemic lupus erythematosus (SLE), primary and secondary anti-phospholipid syndrome (APS) and should be used in conjunction with other serological tests and clinical findings.

AESKULISA Cardiolipin Check is a solid phase enzyme immunoassay employing highly purified cardiolipin plus native human β2-glycoprotein I (β2-GPI) for the combined semiquantitative and qualitative detection of IgA, IgG and IgM antibodies against cardiolipin in human serum. Anti-cardiolipin antibodies mainly recognize specific epitopes on a complex composed of cardiolipin and β2-GPI which are only expressed when β2-GPI interacts with cardiolipin. The assay is an aid in the diagnosis of systemic lupus erythematosus (SLE), primary and secondary anti-phospholipid syndrome (APS) and should be used in conjunction with other serological tests and clinical findings.

1. Indication(s) for use:
   Same as Intended Use.
2. Special condition for use statement(s):
   The devices are for prescription use only.
3. Special instrument Requirements:
   None

I. Device Description:
Each device consists of 1) an antigen coated 96 well microtiter plate, 2) individual horseradish peroxidase conjugated anti-human IgA, IgG and IgM (for AESKULISA Cardiolipin Check, it is a mixture of anti-human IgA/IgG/IgM), 3) TMB substrate, 4) cut-off control, 5) positive control (not included in AESKULISA Cardiolipin Check), 6) negative control, 7) calibrators A to F, 8) washing buffer concentrate (50x), 9) sample buffer concentrate (5x) and 10) stop solution. The calibrators and the positive and cut-off controls are diluted human sera. The negative control is BSA/PBS.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Varelisa Cardiolipin Antibodies IgA, Varelisa Cardiolipin Antibodies IgG, Varelisa Cardiolipin Antibodies IgM and Varelisa Cardiolipin Antibodies Screen.

2. Predicate K number(s):
   k020757, k020752, k020758, k023433

3. Comparison with predicate:

DEVICE	PREDICATE
A. Similarities	
Intended Use. For the determination of antibodies against cardiolipin in human serum. The assay is an aid in the diagnosis of systemic lupus erythematosus (SLE), primary and secondary anti-phospholipid syndrome (APS) and should be used in conjunction with other serological tests and clinical findings. (Note: thrombotic risk evaluation was not stated in the claim)	For the determination of cardiolipin antibodies in serum or plasma to aid in the diagnosis of antiphospholipid syndrome (APS), and to evaluate the thrombotic risk in patients with Systemic lupus erythematosus (SLE)
Assay type – ELISA	Same
Analytes – Anti-cardiolipin antibodies	Same
Assay Format – Qualitative and semi-quantitative	Same
Capture Antigens - cardiolipin and β2-GPI	Same
Reporter conjugate - Horseradish peroxidase	Same
Substrate – TMB	Same
B. Differences	
Source of β2-GPI -human	Bovine
Sample Type – Serum	Serum and plasma
Cut-off Values – 15 PL/mL for IgA, IgG and IgM 20 U/mL for screening assay	1.2 (ratio) with an equivocal zone of 1.0 to 1.2

K. Standard/Guidance Document Referenced (if applicable):
None referenced.

L. Test Principle:
The AESKULISA devices are enzyme-linked immunoassays. Purified antigens are coated separately in wells of microtiter plates. Diluted patient serum is added to the microtiter well and antibody specific to the antigen will bind to the immobilized antigen if present. Unbound sample is washed away and an enzyme labeled anti-human IgA or IgG or IgM antibody (except for AESKILISA Cardiolipin Check which uses a mixture if IgA/IgG/IgM) is added to each well and bind to the

antigen/antibody complex. After washing away any unbound enzyme conjugate, the chromogenic substrate is added. The color intensity in the wells is proportional to the amount of each autoantibody in the sample.

M. Performance Characteristics (if/when applicable):
1. Analytical performance:
   a. *Precision/Reproducibility:*
   AESKULISA Cardiolipin GAM, A and GM assays – To determine intra-assay reproducibility 3 serum samples with high, medium and low antibody reactivity were assayed 24 times on one plate. For inter-assay reproducibility, three different sera were tested for 18 times on three plates on different days. The mean concentrations and %CV of the intra-assay and inter-assay results are summarized.

	Intra-Assay			Inter-Assay	
Sample	Mean Concentrations (GPL)	CV (%)	Sample	Mean Concentrations (GPL)	CV (%)
1	586.2	1.5	1	499.8	0.9
2	67.4	3.4	2	68.9	1.7
3	34.5	7.6	3	40.7	4.6
Sample	Mean Concentrations (APL)	CV (%)	Sample	Mean Concentrations (APL)	CV (%)
1	63.9	3.8	1	78.2	3.2
2	42.0	6.1	2	37.6	3.1
3	22.5	6.9	3	24.9	3.3
Sample	Mean Concentrations (MPL)	CV (%)	Sample	Mean Concentrations (MPL)	CV (%)
1	156.2	0.4	1	110.2	0.7
2	37.0	0.8	2	24.0	0.7
3	11.4	2.6	3	11.8	2.7

AESKULISA Cardiolipin Check – To determine the intra-assay reproducibility, three sera with high, medium and low antibody reactivity were tested 24 times on one plate with IgA/G/M mixed-conjugate. For inter-assay reproducibility, three different sera were tested for 18 times on three plates on different days. The mean concentrations and %CV of the intra-assay and inter-assay results are summarized.

	Intra-Assay			Inter-Assay	
Sample	Mean Concentrations (GPL)	CV (%)	Sample	Mean Concentrations (GPL)	CV (%)
1	20.5	3.6	1	18.4	9.5
2	43.6	5.5	2	47.5	5.5
3	111.3	4.1	3	120.3	8.7

   b. *Linearity/assay reportable range:*

For anti-cardiolipin IgG assay, two serum samples with antibody concentrations of 680 MPL/mL and 1418 MPL/mL were serially diluted to 1:100, 1:200, 1:400 and 1:800. Percent recovery for the higher concentration sample ranged from 93.5% to 101.1% and for the moderate concentration sample, 93.0% to 105.9%.

For anti-cardiolipin IgA assay, two serum samples with antibody concentrations of 240 APL/mL and 340 APL/mL were diluted to 1:100, 1:200, 1:400 and 1:800. Percent recovery for the higher concentration sample ranged from 95.3% to 105.9% and for the lower concentration sample, 93.3% to 103.3%.

For anti-cardiolipin IgM assay, two serum samples were antibody concentrations of 3629 MPL/mL and 295 MPL/mL were serially diluted to 1:100, 1:200, 1:400 and 1:800. Percent recovery for the higher concentration sample ranged from 84.0% to 106.1% and for the lower concentration sample, 89.7% to 101.7%.

For the AESKULISA Cardiolipin Check, two serum samples with antibody concentrations of 761 U/mL and 1011 U/mL were diluted to 1:100, 1:200, 1:400 and 1:800. Percent recovery for the higher concentration sample ranged from 90.1% to 108.4% and for the lower concentration sample, 91.5% to 108.7%.

c. *Traceability (controls, calibrators, or method):*
Calibrators are traceable to the reference sera from E.N. Harris and are purchased from a commercial source. Calibrators are supplied in 6 concentrations (0, 3, 10, 30, 100 and 300 U/mL). A negative, a positive and a cut-off control are included in each device. The cut-off controls are calibrated to the reference sera.

d. *Detection limit (functional sensitivity):*
The analytical sensitivity has been determined to be 1.0 APL/mL, GPL/mL or MPL/mL.

e. *Analytical specificity:*
To test for cross-reactivity, 16 sera from patients with other autoimmune diseases (Wegener's Granulomatosis, reactive arthritis, RA, MCTD, Jo-1 Syndrome, CREST, thyroid disease, celiac disease and vasculitis) were assayed on the AESKULISA Cardiolipin Check. All results were negative. Interference testing with hemolyzed, lipemic or icteric samples were not performed but the package insert specified that these types of samples should not be used.

f. *Assay cut-off:*
To determine the cut-off value, three fold serial dilutions of an antibody specific patient serum are tested in triplicates. The OD_{450} value for each dilution is plotted (linear-log with a 4 parameter

fitting) against the dilution factor to determine the linear range. The dilution in the linear range with an OD value of ~2.0 is defined as Calibrator F and assigned an arbitrary unit of 300 U/mL. Calibrator F is diluted and calibrated to the respective reference serum. The selected cut-off is equivalent to an OD of 0.5 to 0.6 of that of the reference serum. The cut-off values were validated by testing 40 healthy subjects from two hospitals. For the AESKULISA Cardiolipin GAM, A and GM assays, the cut-off value for the IgA, IgG and IgM anti-cardiolipin antibodies is 15 PL/mL and for the AESKULISA Cardiolipin Check, the cut-off value for the combined IgGAM antibodies is 20 U/mL.

2. Comparison studies:
    a. *Method comparison with predicate device:*
        In the original submission, there were very few samples for primary and secondary APS, the sponsor was asked to provide data on additional positive samples and the sponsor supplemented data from 13 primary and 13 secondary APS patients.

        i) AESKULISA Cardiolipin GM
           One hundred and eleven clinically defined patient samples were tested on the new device and the predicate devices Varelisa Cardiolipin Antibodies IgG, Varelisa Cardiolipin Antibodies IgM. These samples consisted of 28 SLE, 36 RA, 19 primary APS, 15 secondary APS, 3 connective tissue disease, 3 polyneuropathy and one each of cerebral vasculitis, p-ANCA positive vasculitis, Sjogren Syndrome, prolonged reversible ischemic neurological deficiency, fibromyalgia, polyangiitis and hypertension. Twenty-four of the 111 sera were from male patients and 87 from female patients. Forty-six percent of the patients were between 16y to 44y, 32% were 45y to 64y and 22% were ≥65y. Results are summarized in the following tables.

		Varelisa IgG		
		+	-	Total
AESKULISA IgG	+	42	1	43
	-	4	64	68
	Total	46	65	111

% positive agreement = 91.3% (95% CI 83.2% to 99.4%)
% negative agreement = 98.5% (95% CI 95.5% to 101.5%)
% total agreement = 95.5% (95% CI 91.6% to 99.4%)

        The overall agreement between the AESKULISA IgG and the Varelisa IgG assays was 95.5%. Three of the six Varelisa[+]/AESKULISA[-] discrepant samples were from patients

with other disorders (polyneuropathy, vasculitis and RA) and the other three from patients with primary APS. The two Varelisa$^-$/AESKULISA$^+$ discrepant samples were a fibromyalgia sample and a secondary APS sample.

		Varelisa IgM		
		+	-	Total
AESKULISA IgM	+	18	1	19
	-	22	70	92
	Total	40	71	111

% positive agreement = 45% (95% CI 29.6% to 60.4%)
% negative agreement = 98.6% (95% CI 95.9% to 101.3%)
% total agreement = 79.3% (95% CI 71.8% to 86.8%)

The overall agreement between the AESKULISA IgM and the Varelisa IgM assays was 79.3%. The discrepant samples included 5 RA, 5 SLE, 6 primary APS, 3 secondary APS and 4 with other disorders (MCTD, polyangiitis, vasculitis and cerebral vasculitis). The samples were from patients with RA and other disorders suggesting that the results for the predicate device were false positives. The discrepancies observed with the IgM positive SLE and APS samples were not resolved.

ii) AESKULISA Cardiolipin A and AESKULISA Cardiolipin Check

Seventy-nine clinically defined patient samples and three normal donor samples were tested on the new device and the predicate devices Varelisa Cardiolipin Antibodies IgA, Varelisa Cardiolipin Antibodies Screen. These samples consisted of 25 SLE, 17 primary APS, 16 secondary APS, 6 RA, 4 suspected reactive arthritis, 2 CREST, 2 Sjogren Syndrome and one each of inflammatory bowel disease, microsomia, MCTD, chronic heart disease/hypertension, apthous oral infection and Wegener's Granulomatosis. Results are summarized in the following tables.

		Varelisa IgA			
		+	-	Equiv	Total
AESKULISA IgA	+	22	5	2	29
	-	4	46	2	52
	Total	26	51	4	81

% positive agreement = 84.6% (95% CI 35.5% to 95.6%)
% negative agreement = 90.2% (95% CI 82.0% to 98.4%)
% total agreement = 86.1% (95% CI 78.5% to 93.7%)

Excluding the equivalent samples, the overall agreement between the AESKULISA IgA and the Varelisa IgA assays was 86.1%. The five Varelisa$^-$/AESKULISA$^+$ samples were from 3 SLE and 2 primary APS patients. Two of the SLE patients were known to have anti-cardiolipin antibodies. The four Varelisa$^+$/AESKULISA$^-$ sample were from 3 primary APS patients and one SLE patient with unknown anti-cardiolipin antibody status. For the four Varelisa IgA equivalent samples, two AESKULISA positives were from SLE patients and the two AESKULISA negative were from an APS patient and a SLE patient.

		Varelisa Screen			
		+	-	Equiv	Total
AESKULISA Check	+	50	8	1	59
	-	0	21	1	22
	Total	50	29	2	81

% positive agreement = 100% (50/50)
% negative agreement = 72.4% (95% CI 27.6% to 87.3%)
% total agreement = 89.9% (95% CI 83.3% to 96.5%)

Excluding the equivalent samples, the overall agreement between the AESKULISA Check and the Varelisa Screen was 89.9%. Based on the clinical diagnosis, 6 of 8 discrepant samples i.e. AESKULISA Check$^+$/Varelisa Screen$^-$ were from patients known to have anti-cardiolipin antibodies, therefore suggesting that the predicate results were false negatives.

b. *Matrix comparison:*
Both the predicate and the new device use serum samples.

3. Clinical studies:
    a. *Clinical sensitivity:*
    Not applicable.
    b. *Clinical specificity:*
    Not applicable
4. Clinical cut-off:
Not applicable.
5. Expected values/Reference range:
The expected value for the normal population is negative. The frequency distribution of anti-cardiolipin antibodies in the various disease cohorts was determined using the same study samples from the correlation studies.

Anti-cardiolipin IgG and IgM:

Study Population	N	% IgG Positive	% IgM Positive
Primary APS	19	78.9% (15/19)	31.6% (6/19)
Secondary APS	17	93.3% (14/15)	41.2% (7/17)
SLE	28	32.1% (9/28)	10.7% (3/28)
RA	36	0% (0/36)	0% (0/36)
Other	13	30.8% (4/13)	23.1% (3/13)

Anti-cardiolipin IgA and Check:

Study Population	N	% IgA Positive	% IGAM Positive
Primary APS	17	23.5% (4/17)	100% (17/17)
Secondary APS	16	81.3% (13/16)	100% (16/17)
SLE	25	40% (10/25)	76% (19/25)
RA	6	0% (0/6)	33% (2/6)
Other	14	7.1% (1/14)	42.9% (6/14)

The sponsor provided expected values from published literature which showed the frequency for total anti-cardiolipin antibodies in SLE ranged from 16% to 86%, in primary and secondary APS 80% to 90% and in normals 2% to 5%. Frequency of anti-cardiolipin antibody for each allotype was not provided. In general, IgG and IgM anti-cardiolipin antibodies are considered to be of higher significance than IgA antibodies.

N. Conclusion:

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