510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k041628

B. Purpose for Submission:
New device

C. Analyte:
Anti-DNA Antibodies

D. Type of Test:
ELISA, Qualitative and Quantitative

E. Applicant:
AESKU INC.

F. Proprietary and Established Names:
AESKULISA®dsDNA G Test

G. Regulatory Information:
1. <u>Regulation section:</u>
   21 CFR § 866.5100, Antinuclear Antibody Immunological Test System
2. <u>Classification:</u>
   Class II
3. <u>Product Code:</u>
   LSW, Anti-DNA Antibody, Antigen and Control
4. <u>Panel:</u>
   82 Immunology

H. Intended Use:
1. <u>Intended use(s):</u>
   AESKULISA®dsDNA-G is a solid phase enzyme immunoassay with human recombinant double-stranded DNA (dsDNA) for the quantitative and qualitative detection of IgG antibodies against dsDNA in human serum. The assay is a tool in the diagnosis of systemic lupus erythematosus (SLE) and should be used in conjunction with other serological tests and clinical findings.
2. <u>Indication(s) for use:</u>
   The assay is a tool in the diagnosis of systemic lupus erythematosus (SLE) and should be used in conjunction with other serological tests and clinical findings.
3. <u>Special condition for use statement(s):</u>
   The device is for prescription use only.

4. Special instrument Requirements:
   Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm)
   Microplate washing device (multichannel pipette or automated system)

I. Device Description:

AESKULISA®dsDNA-G is a solid phase enzyme immunoassay with human recombinant double-stranded DNA (dsDNA) for the quantitative and qualitative detection of IgG antibodies against dsDNA in human serum. The assay components include antigen coated microtiter plate, negative, positive and cut-off controls, wash buffer concentrate, sample buffer concentrate, anti-human IgG HRP horseradish peroxidase conjugate, 3,3',5,5' tetramethylbenzidine (TMB) substrate and 1M HCl stop solution. The positive and cut-off controls are composed of human serum of a specific dilution in standard buffer. The negative control contains standard buffer without any reactive component.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Varelisa dsDNA ELISA
2. Predicate K number(s):
   k930421
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	AESKULISA dsDNA-G	Varelisa dsDNA ELISA
Intended Use	Quantitative and qualitative detection of IgG antibodies against dsDNA in human serum to aid in the diagnosis of SLE	Same
Assay Principle	Solid phase enzyme immunoassay	Same
Antigen	Recombinant plasmid DNA	Same
Differences		
Item	**Device**	**Predicate**
Sample Matrix	Serum	Serum and plasma
Recommended normal ranges	Normal ≤15 IU/mL Positive ≥15 IU/mL	Negative <35 IU/mL Equivocal 35-55 IU/mL Positive >55 IU/mL
Detection limit	3.0 IU/mL	1.0 IU/mL
Total incubation time	60 minutes	70 minutes

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

L. Test Principle:
Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient's antibodies, if present in the specimen, bind to the antigens. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the antibodies in the patient samples. The color formation is determined by measuring the OD value at 450 nm with a common photometer.

M. Performance Characteristics (if/when applicable):
    1. <u>Analytical performance:</u>
        a. Precision/Reproducibility:
        <u>Inter-assay</u> - Inter-assay precision (%CV) was determined by running 3 different sera (low, medium, and high) 18 times on three different microtiter plates for five days. The results are as follows:

Sample No.	Overall Mean (IU/mL)	Overall Mean %CV
Low	13.2	5.9
Med	151.6	2.3
High	463.3	2.6

        <u>Intra-assay</u> - To determine intra-assay variation, 3 different sera (low, medium, high) were tested 24 times on 3 microtiter plates. The results are as follows:

Sample No.	Overall Mean (IU/mL)	Overall Mean %CV
Low	12.71	5.91
Med	280.14	1.68
High	199.88	1.0

        b. Linearity/assay reportable range:
        Linearity of the assay was demonstrated by doing serial dilutions of 6 sera from 1:100 to 1:1800. Linearity was set at 3-300 IU/mL

Dilution .factor	dsDNA antibody Concentration (IU/mL)					
	Serum 1	Serum 2	Serum 3	Serum 4	Serum 5	Serum 6
1	331	73.4	775	153.1	42.9	179.4
0.5	139	31.1	375	71.18	21.6	86.4
0.25	48.16	12.32	120	28.87	10.8	41.8
0.125	20.24	5.73	43.37	12.18	5.4	19.8

| 0.0625 | 10.7 | 2.96 | 15.76 | 5.12 | ND | ND |

The regression analysis of the 6 sera gave the following results:

Regression variables	Serum 1	Serum 2	Serum 3	Serum 4	Serum 5	Serum 6
Intercept	-25.488	-4.428	-56.752	-7.877	0.091	-3.773
Slope	349.182	76.208	832.461	159.915	42.845	182.664
r^2	0.992	0.993	0.995	0.998	0.999	0.999

In addition, a recovery study was done at different dilution factors to see if the device will dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule. The results of this study are as follows:

Sample No.	Dilution Factor	Measured conc IU/mL	Expected Conc IU/mL	% Recovery
1	1/100	42.9	43.2	99.3
	1/200	20.4	21.6	99.4
	1/400	9.3	10.8	86.1
	1/800	4.9	5.4	90.78
2	1/100	179.4	176.0	101.9
	1/200	86.4	88.0	98.2
	1/400	41.8	44.0	95.2
	1/800	19.8	22.0	90.0

c. *Traceability (controls, calibrators, or method):*
   The **AESKULISA®dsDNA-G** calibrators are calibrated against the WHO/80 standard.
d. *Detection limit:*
   The value for the detection limit was calculated as the mean of the optical densities (OD) of the standard buffer plus 3 times the standard deviation (SD), expressed in IU/mL. The mean plus 3 SD was 2.326 IU/mL. The detection limit was set at 3 IU/mL.
e. *Analytical specificity:*
   Crossreactivity:
   Ten sera from patients with various autoimmune diseases were tested on the **AESKULISA®dsDNA-G** and were found negative.

disease	positive	negative
CREST	0	3
Polymyositis	0	1
Scleroderma	0	2
MCTD	0	1
Wegener's granulomatosis	0	1
Sjogrens Syndrome	0	1
Sicca Syndrome	0	1

Interference:

Interference study was not performed. However, a statement not to use icteric, hemolyzed, or bacterially contaminated samples was included in the product insert.

f. *Assay cut-off:*

80 healthy donors were used for this study. The healthy donors were divided in the following age groups:

Young (<26y)   46
Middle age (26y-45y) 21
Elderly (46y+)   13

The mean plus 3 times the standard deviation was chosen as the cut-off value which was 15 IU/mL.

2. Comparison studies:

a. *Method comparison with predicate device:*

Fifty four SLE patient sera were tested on the **AESKULISA®dsDNA-**and the **Varelisa® dsDNA**.

		Varelisa dsDNA		
		Pos	Neg	Total
AESKULISA dsDNA-2	Pos	34	13	47
	Neg	0	7	7
	Total	34	20	54

Negative agreement: 35.0%
Positive agreement: 72.3%
Total agreement   75.9%

The two devices gave conflicting results on 13 cases. All 13 cases are patients with SLE. The results are all positive on the AESKULISA but close to the cut-off value for the Varelisa. For this calculation, equivocal results for Varelisa were considered negative.

Because of the discrepancy in the method comparison with the predicate device, the sponsor was asked to make several dilutions of the WHO/80 standard and run them as unknowns on both devices. The results are as follows:

WHO/80 dilution	Conc IU/mL	Varelisa dsDNA			AESKULISA dsDNA-2		
		IU/mL	%CV	%recovery	IU/mL	%CV	%recovery
1:200	100	125	4	125	98	7	98
1:400	50	55	4	110	48	1	96
1:800	25	29	2	115	28	8	112
1:1600	12.5	13	3	101	9	6	72

Twenty healthy donors on both devices and linear regression analysis showed (AESKULISA) = 1.15 (Varelisa) + 6.52 with r^2 =

0.24. The correlation coefficient showed discrepancies at the low end but all healthy donors are below the respective cut-offs in both devices.

Twenty additional sera around the cut-off range of the devices were compared. The sera were from 19 female and 1 male, 2 belong to the group of <26 years; 10 to the group of 26-46 years and 8 to the group of 47+ years. A linear regression analyses was performed and the results are (AESKULISA) = 2.79 (Varelisa) – 18.75 with r^2 = 0.37

A histogram and a sigma plot with 95% confidence range were provided.

   b. *Matrix comparison:*
      Serum is the only recommended matrix.
3. Clinical studies:
   a. *Clinical sensitivity:*
      The **AESKULISA®dsDNA-G** detected 47 of 54 SLE patients who were positive for dsDNA antibodies. Clinical sensitivity is 87%.
   b. *Clinical specificity:*
      80 sera from healthy blood donors that were used in the cut-off study tested negative on the **AESKULISA®dsDNA-G.** Specificity was 100%
   c. *Other clinical supportive data (when a and b are not applicable):*
      Not applicable
4. Clinical cut-off:
   See assay cut-off.
5. Expected values/Reference range:
   The normal range for the assay is ≤ 15 IU/mL.

N. Conclusion:
The submitted material in this premarket notification is complete and supports a substantial equivalence decision.