

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

**A. 510(k) Number:**

k041753

**B. Purpose for Submission:**

New device

**C. Analyte:**

Anti-ENA antibodies (SS-B, SS-A (52 and 60 kDa), Scl-70, Jo-1, snRNP/Sm, Sm)

**D. Type of Test:**

Qualitative, EIA

**E. Applicant:**

AESKU, Inc.

**F. Proprietary and Established Names:**

AESKULISA<sup>®</sup> ENA 6S

**G. Regulatory Information:**

1. Regulation section:  
21 CFR §866.5100, Antinuclear Antibody Immunological Test System
2. Classification:  
Class II
3. Product Code:  
LLL, Extractable antinuclear antibody, antigen and control
4. Panel:  
Immunology (82)

**H. Intended Use:**

AESKULISA<sup>®</sup> ENA 6S is a solid phase enzyme immunoassay for the combined qualitative detection of IgG antibodies against six cellular and nuclear antigens in human serum. Each well is coated with recombinant SS-B, SS-A 52 kDa, Scl-70, Jo-1 and purified native human snRNP/Sm, Sm and SS-A 60 kDa. The assay is a tool in the diagnosis of certain systemic rheumatic diseases 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:  
Microplate plate reader 450 nm reading filter and optional 620 nm reference filter.

**I. Device Description:**

The device consists of 1) 12x8 antigen-coated well strips, 2) horseradish peroxidase conjugated anti-human IgG, 3) TMB substrate, 4) cut-off control, 5) positive control 6) negative control, 7) washing buffer concentrate (50x), 8) sample buffer concentrate (5x) and 9) stop solution. The positive, negative and cut-off controls are diluted human sera.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
ORGENTEC ENAScreen ELISA Assay
2. Predicate K number(s):  
k955134
3. Comparison with predicate:

DEVICE	PREDICATE
<b>A. Similarities</b>	
<b>Intended Use.</b> For the combined qualitative detection of IgG antibodies against six cellular and nuclear antigens in human serum. Each well is coated with recombinant SS-B, SS-A 52 kDa, Scl-70, Jo-1 and highly purified native human snRNP/Sm, Sm and SS-A 60 kDa. The assay is a tool in the diagnosis of certain systemic rheumatic diseases and should be used in conjunction with other serological tests and clinical findings.  <b>Assay type</b> – ELISA <b>Analytes</b> – anti- SS-A, SS-B, Sm, RNP/Sm, Scl-70 and Jo-1 antibodies <b>Assay Format</b> – Qualitative <b>Reporter conjugate</b> - Horseradish peroxidase <b>Substrate</b> – TMB	For the in vitro qualitative screening of IgG class autoantibodies against the extractable nuclear antigens, SS-A(Ro), SS-B(La), Sm, RNP/Sm, Scl-70 and Jo-1 in human serum or plasma. The assay is intended for the in vitro diagnostic use as an aid in the diagnosis of rheumatic diseases such as systemic lupus erythematosus (SLE), Sjögren's syndrome, scleroderma and mixed connective tissue disease.  Same Same  Same Same Same
<b>B. Differences</b>	
<b>Source of Antigens</b> Recombinant - SS-B, SS-A 52, Scl-70 and Jo-1 Human cell line - snRNP/Sm, Sm and SS-A 60 <b>Sample Type</b> – Serum <b>Cut-off Values</b> – Negative = Index Ratio <1.0 Positive = Index Ratio >1.0	Unknown   Serum and plasma Negative = <OD of Cut-off Control Positive = >OD of Cut-off Control

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

None referenced.

**L. Test Principle:**

The AESKULISA<sup>®</sup> ENA 6S Assay is an enzyme-linked immunoassay. The antigens are pooled prior to coating the microplate wells. Diluted patient serum is added to the microtiter well and if specific antibodies are present, they will bind to the immobilized antigens to form antigen/antibody complexes. Unbound material is washed away and an enzyme labeled anti-human IgG antibody (conjugate) is added to each well. The enzyme conjugate binds to the antigen/antibody complex. After washing away any unbound enzyme conjugates, the chromogenic substrate is added. The color intensity in the wells is proportional to the amount of autoantibodies in the sample.

**M. Performance Characteristics (if/when applicable):**1. Analytical performance:a. *Precision/Reproducibility:*

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 OD Ratio	CV (%)	Sample	Mean OD Ratio	CV (%)
1	3.1	0.8	1	3.1	0.7
2	2.3	1.0	2	2.2	0.5
3	1.5	1.1	3	1.4	1.4

b. *Linearity/assay reportable range:*

A high and a low positive serum were serially diluted to 1:100, 1:200, 1:400 and 1:800. Percent recovery for the high concentration sample ranged from 91.4% to 107.9% and for the low concentration sample, 93.8% to 100%.

c. *Traceability (controls, calibrators, or method):*

Controls are traceable to CDC reference ANA sera. A negative, a positive and a cut-off control are included in each device.

d. *Detection limit (functional sensitivity):*

No applicable

e. *Analytical specificity:*

To test for cross-reactivity, 11 sera positive for other autoantibodies (tissue transglutaminase, thyroglobulin, thyroid peroxidase, gliadin, proteinase 3 and RF) were assayed. 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 ENA positive patient serum are tested in triplicates. The OD<sub>450</sub> 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 CDC reference sera. The selected cut-off is equivalent to an OD of 0.5 to 0.6 of that of the reference sera. The cut-off values were validated by testing 80 healthy subjects from two hospitals. All subjects had OD values < 0.5.

**Result interpretation:**

OD<sub>Patient</sub> < OD<sub>cut-off</sub>                      Negative

OD<sub>Patient</sub> > OD<sub>cut-off</sub>                      Positive

Or

Index Value (OD<sub>Patient</sub>/OD<sub>cut-off</sub>)

Index Value < 1.0                      Negative

Index Value > 1.0                      Positive

**2. Comparison studies:**

*a. Method comparison with predicate device:*

Sixty-five clinically defined patient samples were analyzed on the new device and the predicate device. These samples consisted of 48 SLE, 7 Sjögren's syndrome, 7 polymyositis and 3 scleroderma. Fifty-six of the 65 sera were from female patients and 9 from male patients. Twenty-eight percent of the patients were < 26y, 32% were 26y to 45y and 35% were ≥46y. Results are summarized in the following tables.

		ORGENTEC ENAScreen		
		+	-	Total
AESKULISA ENA 6S	+	28	12*	40
	-	0	25	25
	Total	28	37	65

\*10 SLE and 2 polymyositis

% positive agreement = 100%

% negative agreement = 67.6% (95% CI 52.5% to 82.7%)

% total agreement = 81.5% (95% CI 76.7% to 86.3%)

Of the 10 SLE discrepant samples, 3 samples had a homogeneous pattern and 7 had a mixed homogeneous/speckle pattern by IFA.

The two polymyositis samples were anti-Jo-1 antibody positive by IFA.

*b. Matrix comparison:*

Not applicable since both the predicate and the new device use serum samples.

3. Clinical studies:

*a. Clinical sensitivity:*

Patient samples used in the method comparison study were used for determination of the clinical sensitivity of the AESKULISA ENA 6S assay. The clinical sensitivity was 61.5% (40/65). The following table shows distribution in the 4 disease groups tested.

Disease Group	N	AESKULISA ENA 6S	
		Positive	% Total
SLE	48	29	60.4
Sjögrens Syndrome	7	4	57
Polymyositis	7	5	71.4
Scleroderma	3	2	33

*b. Clinical specificity:*

The clinical specificity of the AESKULISA ENA 6S assay is 100% based on results of the 80 normal subjects.

4. Clinical cut-off:

Same as assay cut-off.

5. Expected values/Reference range:

The expected value for the normal population is negative. The frequency distribution of ENA autoantibodies in the various autoimmune disease cohorts according to published literature is depicted below:

Disease	% Positive					
	SS-A	SS-B	Sm	RNP/Sm	Scl-70	Jo-1
SLE	10-30	30-50	10-30	10-30		
Connective Tissue Disease				>90		
Sjogren syndrome	>90	>90				
Scleroderma	10-30				>90	
Polymyositis/Dermatomyositis						50-90

**N. Conclusion:**

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