

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE**

A. 510(k) Number:

k040953

B. Purpose for Submission:

New device

C. Analyte:

Antinuclear antibody

D. Type of Test:

Qualitative ELISA

E. Applicant:

AESKU INC

F. Proprietary and Established Names:

AESKULISA[®] ANA Hep-2 test

G. Regulatory Information:

1. Regulation section:
21CFR § 866.5100, Antinuclear Antibody Immunological Test System
2. Classification:
Class II
3. Product Code:
LKJ, Antinuclear Antibody, Antigen, Control
4. Panel:
82 Immunology

H. Intended Use:

1. Intended use(s):
AESKULISA ANA-Hep2 is a solid phase enzyme immunoassay for the combined qualitative detection of IgG antibodies against Hep2 cells in human serum. Each well is coated with lysed Hep2 cells and specific antigens. The test collectively detects, in one well, total ANAs against double stranded DNA (dsDNA), histones, SS-A (Ro), SS-B (La), Sm, snRNP/Sm, Scl-70, Jo-1 and centromeric antigens along with sera positive for Hep2 immunofluorescence test (IFT).
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.
2. Indication(s) for use:

Same as intended use.

3. Special condition for use statement(s):
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:

The assay components of the AESKULISA ANA-Hep2 include antigen coated microtiter plate, negative, positive and cut-off controls, wash buffer concentrate, sample buffer concentrate, anti-human IgG horseradish peroxidase (HRP) 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):
HELIX Diagnostics Antinuclear Antibody Screening
2. Predicate K number(s):
k954723
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	AESKULISA ANA Hep-2	Helix ANA Screening Test
Assay principle	Indirect, non-competitive qualitative ELISA test using microtiter technology.	Same
Indications for Use	Aid in the diagnosis of systemic rheumatic diseases and should be used in conjunction with other serological tests and clinical findings.	Aid in the diagnosis of certain systemic rheumatic diseases.
Sample matrix	Serum	Same
Analyte	Antinuclear antibodies	Same
Differences		
Item	Device	Predicate
Sample buffer	To be diluted (1:5)	Ready to use
Wash buffer	To be diluted (1:50)	To be diluted (1:16.6)
Cut-off control, positive and negative controls	Ready to Use	Positive and negative controls only To be diluted (1:40)
Incubation times	30, 15 plus 15 minutes	30, 30 plus 30 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 ANA antigens. ANA antibodies, if present in the patient specimen, bind to the antigens forming antigen/antibody complexes. The unbound fraction is washed off. The anti-human IgG HRP conjugate is added and reacts with the antigen/antibody complexes 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 is a function of the amount of conjugate bound to the antigen/antibody complexes and 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.

M. Performance Characteristics (if/when applicable)

1. Analytical performance:

a. *Precision/Reproducibility:*

Intra-assay

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 OD Ratio	Overall Mean %CV
Low	4.6	1.5
Med	2.8	2.0
High	1.4	1.8

Inter-assay

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 OD Ratio	Overall Mean %CV
Low	4.7	3.1
Med	3.0	2.5
High	1.2	2.4

b. *Linearity/assay reportable range:*

A dilution/recovery study was performed using two fold serial dilutions of two positive samples. The results of this study are as follows:

Sample No.	Dilution Factor	Measured conc (OD ratio)	Expected Conc (OD ratio)	% Recovery
1	1/100	4.10	4.20	97.6
	1/200	2.10	2.10	100

	1/400	1.00	1.05	95.2
	1/800	0.55	0.53	103.8
2	1/100	6.10	6.20	98.4
	1/200	3.00	3.10	96.8
	1/400	1.59	1.55	102.6
	1/800	0.79	0.78	102.0

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

Positive controls are the CDC-ANA reference human sera or WHO standards for the respective antigen.

d. *Detection limit:*

Not applicable

e. *Analytical specificity:*

To demonstrate assay specificity, 57 characterized patient sera from various autoimmune diseases (SLE, MCTD, CREST and Sjogren syndrome) obtained from major hospitals which were positive on IFA Hep2 ANA were all found positive in the AESKULISA ANA-Hep2.

disease	# of tested sera
SLE	39
MCTD	3
CREST	4
Sjogrens Syndrome	4
Various AI diseases	7

In addition, 11 sera positive for the following antigens: tissue transglutaminase (3), Proteinase (3), thyroglobulin (2), gliadin (2) and thyroid peroxidase (2) and 80 sera from a control group were tested and found negative on the AESKULISA ANA Hep-2. The healthy donors consisted of 54% female and 46% male. Forty-one percent of the cohort was young adults (n=33), 53% middle aged (n=42) and 6% (n=5) elderly.

f. *Assay cut-off:*

To determine the cut-off value, serial dilutions (1:3) of an antibody specific patient serum are tested in triplicates. The OD at 450nm is determined and plotted against the dilution factor to determine a linear range. The dilution in the linear range with an OD of approximately 2.0 is defined as calibrator F and is assigned 300 U/mL. Calibrator F is diluted to a concentration of 15 U/mL and calibrated to the respective CDC ANA reference serum. The selected cut-off is equivalent to an OD of 0.5 to 0.6 of that of the reference serum. To validate the cut-off, samples from the control group used in the specificity study were tested. The cut-off value is an OD ratio of 1.0

2. Comparison studies:

a. *Method comparison with predicate device:*

Fifty nine patient sera were tested on the AESKULISA and the HELIX device. The samples consisted of 39 SLE, 7 suspected reactive Arthritis, 4 CREST Syndrome, 3 Sjögren's Syndrome, 2 Mixed Connective Tissue Disease (MCTD), one each of SLE/Sjögren overlap, MCTD/Sicca Symptoms, anti-Jo-1 Syndrome and Rheumatoid Arthritis. Total agreement between the two devices was 100% with 57 sera positive and two sera negative on both devices.

		HELIX		
		Pos	Neg	Total
AeskuLisa ANA Hep-2	Pos	57	0	57
	Neg	0	2	2
	Total	57	2	

b. *Matrix comparison:*

Serum is the only recommended matrix.

3. Clinical studies:

a. *Clinical sensitivity:*

Not provided

b. *Clinical specificity:*

Not provided

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 expected value in the normal population is negative.

N. Conclusion:

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