510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

k120889

B. Purpose for Submission:

New assay

C. Measurand:

Anti-nuclear antibodies (ANA)

D. Type of Test:

Qualitative and/or semi-quantitative, indirect immunofluorescence

E. Applicant:

AESKU Diagnostics

F. Proprietary and Established Names:

AESKUSLIDES ANA-HEp-2

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5100 Antinuclear Antibody Immunological Test System

2. Classification:

   Class II

3. Product code:

   DHN – Antinuclear Antibody, Indirect Immunofluorescent, Antigen, Control

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use(s):

   AESKUSLIDES ANA-HEp-2 is an indirect fluorescent antibody assay utilizing HEp-2 tissue culture slides as a substrate for the qualitative and/or semi-quantitative determination of antinuclear antibodies (ANA) in human serum. AESKUSLIDES ANA-HEp-2 is intended for use as an aid in the diagnosis of systemic rheumatic diseases in conjunction with other clinical and laboratory findings.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   This device is for prescription use only

4. Special instrument requirements:

   Fluorescent microscope equipped with a mercury or tungsten-halogen light source, a 390 – 490 excitation filter and a 515 – 520 nm barrier filter, and optics to give a total magnification of 400x.

I. Device Description:

Each kit contains:

- Ten slides each containing 12 wells coated with ANA HEp-2 cells
- One 4.0 mL vial containing FITC-labeled goat anti-human IgG (heavy and light chain) conjugate in a solution of BSA and Evans Blue.
- One 0.5 mL vial of positive control (homogenous pattern) containing human serum and with <0.1% sodium azide as a preservative.
- One 0.5 ml vial of negative control containing diluted human serum with <0.1% sodium azide as a preservative.
- One 12 mL vial of mounting medium containing a solution of glycerol and PBS.
- One 100 mL bottle of sample/wash Buffer, a 10x solution containing BSA, PBS, and sodium azide as a preservative.

J. Substantial Equivalence Information:

1. Predicate device name(s) and Predicate K number(s):

   MBL Bion Antinuclear Antibody (ANA) Test Kit, k872845

2. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Detection of ANA	Same
Slides	12-well slides coated with antigen.	Same
Technology	Immunofluorescence	Same
Antigen	HEp-2 cells	Same
Conjugate	FITC-labeled goat anti-human IgG (heavy and light chain)	Same
Negative Control	Normal human serum negative for ANA	Same
Positive Control	ANA-positive human serum showing homogenous staining pattern	Same
Recommended Sample Dilution for Screen	1:40	Same
Matrix	Serum	Same
Mounting Medium	Phosphate buffered glycerol	Same

Differences		
Item	Device	Predicate
Wash buffer	PBS/BSA	PBS

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

Not applicable

L. Test Principle:

The AESKUSLIDES ANA-HEp-2 assay utilizes indirect immunofluorescent antibody assay techniques. Patient sera is diluted in wash/sample buffer and applied to a well on the slide. Anti-ANA antibodies, if present, will bind to antigens coated on the slide. After washing with wash/sample buffer, a conjugate specific for human IgG is applied which binds to the anti-ANA antibodies immobilized on the slide surface. After a final wash to remove excess conjugate, the slide is read as soon as possible using a fluorescence microscope. Fluorescence intensity, if present, is reported as a number between one and four:

1+	Very dim subdued fluorescence
2+	Definite but dull yellow-green fluorescence
3+	Less brilliant yellow-green fluorescence

4+	Maximal fluorescence; brilliant yellow-green

AESKU recommends a screening dilution of 1:40, followed by four-fold dilutions for semi-quantitative determinations (e.g. 1:40, 1:160, 1:640, 1:2,560, 1:10,240) but suggests each laboratory establish its own screening dilution and titration scheme based on its population and instrumentation.

Interpretation of test results: A serum dilution is considered negative for ANA antibodies if the cells exhibit < 1+ fluorescence and no discernible pattern. Cells appear reddish-orange due to the Evans Blue counterstain. Likewise, a serum dilution is considered positive for ANA antibodies if the cells exhibit ≥ 1+ fluorescence and a discernible pattern. A sample is considered positive for ANA antibodies if it exhibits exhibit ≥ 1+ fluorescence and a discernible pattern at a sample dilution of 1:40 or greater. Technicians should report all titers and patterns seen.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   The reproducibility of the assay was assessed using positive clinical samples and 20 normal samples. Clinical samples (50) were sourced from a primary rheumatology referral center in Germany where patients are clinically evaluated for rheumatological disorders. These patients had a variety of rheumatic diseases. Samples from apparently healthy people (20) were sourced from a health fair. Samples were manually diluted in two-fold steps from a starting dilution of 1:40. Each sample was tested with a slide from two separate assay lots of the then read by two technicians. All samples' identities were coded, and multiple readers were blinded to the other's interpretation. Slides were read to a 1+ end-point, and the final titer and ANA pattern(s) were recorded and analyzed.

   Lot-to-lot reproducibility: Two different lots of the reagents were tested with the samples described above. Each sample/lot combination was read by Technician 1 and Technician 2. None of the readings between lots was more than one titer level apart in either reader. Likewise, all pattern interpretations agreed between lots and between readers.

   Intra-assay reproducibility: Ten samples, eight positive and two negative, from those described above were each tested in triplicate in two different assay lots. Each replicate was read by Technician 1 and Technician 2. Each replicate within a sample/reader/lot combination was consistent. Within each sample, none of the readings was more than one titer level apart and all pattern interpretations agreed.

Inter-assay reproducibility: Five samples (one negative at < 1:40, two weak positive at 1:40 – 1:80, and two moderate positive at 1:160 – 1:320) were tested five times each for five days. Each replicate was read by two technicians blinded to their status. Only one lot was tested. Within each sample, none of the readings was more than one titer level apart and all pattern interpretations agreed.

Between-technician reproducibility: Sixty-eight (68) samples representing a range of rheumatic diseases were manually diluted in two-fold steps from a starting dilution of 1:40. Each sample read by three readers. All slides were read to a 1+ end-point, and the final titer and ANA pattern(s) were recorded. None of the titer readings between readers was more than one titer level apart. All pattern interpretations agreed between readers.

b. Linearity/assay reportable range:

US Centers for Disease Control and Prevention (CDC) ANA reference panel members #1, 4, 6, and 7 were serially diluted and then tested on the AESKUSLIDES ANA HEp-2 slides. Each sample/dilution combination was tested and read twice, and the fluorescence intensity and pattern were recorded. Each sample showed a decrease in fluorescence intensity as the samples were diluted. The pattern of the samples did not change with dilution, and any differences between the technicians reading the slides did not exceed the acceptable deviation of fluorescence intensity of ± 1 intensity level.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability: No recognized reference materials are available.

Controls: Negative and positive controls are included in the kit. The negative control should exhibit less than 1+ fluorescence and appear reddish-orange from the counter-stain. The positive control should exhibit a homogenous staining pattern with a fluorescence intensity of 3+ to 4+. The homogenous pattern of the positive control was confirmed by testing for anti-dsDNA antibodies by validated test methods. AESKU recommends using the positive and negative controls undiluted for the screening protocol; the positive control should be diluted as suggested for the semi-quantitative protocol.

Stability: Real-time stability was assessed with three different kit lots stored at 2 – 8°C and periodically tested with pre-specified positive and negative samples. All kit lots met their pre-determined acceptance criteria throughout testing, supporting a stability claim of 18 months.

d. Detection limit:

Not applicable.

e. Analytical specificity:

The specificity of the assays was evaluated by testing samples from the CDC ANA serum panel. See below for the known specificity of each sample. The table below lists the staining pattern observed; all staining patterns observed are consistent with published reports.

CDC ANA Human Reference Panel		
Sample	Target	Pattern Observed
CDC 1	dsDNA	Homogenous
CDC 2	SS-B/La	Speckled
CDC 3	U1RNP	Speckled
CDC 4	U1RNP	Speckled
CDC 5	Sm	Speckled
CDC 6	U3-RNP (anti-fibrillarin)	Nucleolar
CDC 7	SS-A/Ro	Speckled
CDC 8	CENP	Centromeric
CDC 9	Scl-70	Speckled
CDC 10	Jo-1	Cytoplasmic, Speckled
CDC 11	PM/SCL	Nucleolar

Endogenous interferents: The effect of interfering substances on assay results were tested by spiking nine clinical samples with hemoglobin (5 g/dL), bilirubin (5 mg/dL), and triglycerides(250 mg/dL). The samples consisted of three negative (< 1:40), three weak positive samples (1:40 – 1:80), and three strong positives (1:640 – 1:1280) with varying pattern specificities. The effect of rheumatoid factor and heterophiles was tested by diluting these samples 1:2 with high-titer rheumatoid factor, or EB-IgM positive samples, and the endpoint results were adjusted for the 1:2 dilution. Two readers blinded to sample identity read each slide and reported the titer and pattern observed where the fluorescence was +1. Both readers reported the same pattern for each sample and all titer interpretations agreed within ± 1 dilution. The results indicated that hemoglobin, bilirubin, triglyceride, rheumatoid factor and heterophiles at the concentration indicated above have no effect on assay results.

f. Assay cut-off:

The recommended starting dilution is 1:40. The manufacturer suggests performing four-fold dilutions but recommends that each laboratory establish its own titering protocol. The titers of 1:40 and 1:80 are considered low titers, 1:160 and 1:320 are considered medium titers, and 1:640 and greater are

considered high titers.

2. Comparison studies:

   a. *Method comparison with predicate device:*

   A comparison of the new assay and the predicate was performed using 138 clinical samples from patients being evaluated for rheumatological diseases. The clinical samples represented a range of rheumatic diseases including systemic lupus erythematous (SLE), anti-phospholipid syndrome, Sjögren's syndrome, systemic sclerosis, scleroderma, mixed connective tissue disease (MCTD), CREST syndrome, rheumatoid arthritis, polymyositis/dermatomyositis, osteoarthritis, etc. Twenty (20) of the samples were recruited from donors that did not evidence symptoms of autoimmune disease.

   The age range of the samples was 16 – 78 years. As per assay instructions, samples that had ≥ 1+ fluorescence at 1:40 were considered positive. Sample patterns were recorded and compared.

		Predicate		
		Positive	Negative	Total
AESKUSLIDES ANA HEp-2	Positive	116	0	116
	Negative	0	22	22
	Total	116	22	138

Positive % Agreement = 116/116 = 100% (95% CI: 96.8 ─ 100%)
Negative % Agreement = 22/22 = 100% (95% CI: 85.1 ─ 100%)

Pattern agreement is tabulated below. Some samples had more than one pattern; each was reported individually:

		Observed Pattern	
Pattern	n	Predicate	AESKUSLIDES
Homogenous	32	32	32
Speckled	82	82	82
Nucleolar	20	20	20
Centromere	6	6	6
Peripheral	3	3	3
Nuclear Membrane	1	1	1

Overall Agreement = 100%

Comparison of reciprocal titers between the predicate and the new assay is shown below. If multiple patterns were reported, those titers are reported

separately:

		Predicate: reciprocal titer										
		<40	40	80	160	320	640	1280	2560	5120	10240	Total
AESKU	<40	22										22
	40		10									10
	80			4								4
	160			1	19							20
	320				1	9	1					11
	640					3	17	1				21
	1280						2	12				14
	2560							1	22			23
	5120								1	7		8
	10240										11	11
	Total	22	10	5	20	12	20	14	23	7	11	**144**

b. Matrix comparison:

Not applicable.

3. Clinical studies:

a. Clinical Sensitivity and specificity:

Not applicable.

4. Clinical cut-off:

See assay cut-off.

5. Expected values/Reference range:

Positive ANA results are commonly seen in normal populations. However, the prevalence varies with age, sex, and titer tested. One study reported the ANA prevalence in the US population ages 12 years and older was 13.8% at a 1:80 dilution. ANA prevalence increased with age, and ANAs were more prevalent among females than males (17.8% versus 9.6%)[1]. Another study of a normal population showed ANA tests were positive in 31.7% of individuals at a 1:40 serum dilution, in 13.3% at 1:80, in 5.0% at 1:160, and 3.3% at 1:320[2].

1. Satoh, M., et al., Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. Arthritis & Rheumatism, 64: 2319–2327, 2012

2. Tan, E. M. (1997), Range of antinuclear antibodies in "healthy" individuals. Arthritis & Rheumatism, 40: 1601–1611, 1997

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.