510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K172348

B. Purpose for Submission:

New Device

C. Measurand:

Anti-DNA Antibodies, IgG

D. Type of Test:

Indirect Immunofluorescence (IFA)

E. Applicant:

Aesku.Diagnostics GmbH & Co. KG

F. Proprietary and Established Names:

AESKUSLIDES nDNA (Crithidia luciliae)

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.5100 – Antinuclear antibody immunological test system

2. Classification:

   Class II

3. Product codes:

   LSW, Anti-DNA Antibody, Antigen and Control

   PIV, automated indirect immunofluorescence microscope and software-assisted system for clinical use

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use:

   AESKUSLIDES nDNA (Crithidia luciliae) is an indirect immunofluorescence assay utilizing Crithidia luciliae coated slides as a substrate for the qualitative and/or semi-quantitative determination of antibodies to native double stranded DNA (dsDNA) in human serum. This in vitro diagnostic assay is used as an aid for the diagnosis of Systemic Lupus Erythematosus (SLE) in conjunction with other clinical and laboratory findings. The assay can be processed manually and analyzed at the microscope or processed and analyzed with HELIOS AUTOMATED IFA SYSTEM. All suggested results obtained with the HELIOS AUTOMATED IFA SYSTEM must be confirmed by trained personnel.

2. Indication for use:

   Same as the intended use.

3. Special conditions for use statements:

   - For prescription use only
   - The HELIOS AUTOMATED IFA SYSTEM is only for use with reagents that are indicated for use with that system
   - This device is for use by a trained operator in a clinical laboratory setting
   - All software-aided results must be confirmed by the trained operator
   - For use only by manual microsocopy or with HELIOS AUTOMATED IFA SYSTEM

4. Special instrument requirements:

   Not applicable

I. Device Description:

AESKUSLIDES nDNA (Crithidia luciliae) is an indirect immunofluorescence assay utilizing Crithidia luciliae coated slides as a substrate for the qualitative and/or semi-quantitative determination of antibodies to native double stranded DNA (dsDNA) in human serum.

Each kit contains (Quantity depends on product variant):

- Slides, each containing 10 wells coated with Crithidia Luciliae cells
- 4.0 ml vial containing Fluorescein (FITC) labelled Anti-human Antibody IgG conjugate in a solution of BSA, ready for use

- 0.5 ml vial of positive control containing human serum (diluted), ready for use
- 0.5 ml vial of negative control containing diluted human serum, ready for use
- 8.0 ml vial of mounting medium containing a solution of glycerol and PBS, ready for use
- 70 ml bottle of sample buffer, containing BSA, PBS and ready for use
- 100 ml bottle of wash buffer, concentrated buffer 1:10 in distilled water, containing BSA, PBS

J. Substantial Equivalence Information:

1. Predicate device name:

   NOVA Lite dsDNA Crithidia luciliae

2. Predicate 510(k) number:

   K880742

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	AESKUSLIDES nDNA (Crithidia luciliae) is an indirect immunofluorescence assay utilizing Crithidia luciliae coated slides as a substrate for the qualitative and/or semi-quantitative determination of antibodies to native double stranded DNA (dsDNA) in human serum. This in vitro diagnostic assay is used as an aid for the diagnosis of Systemic Lupus Erythematosus (SLE) in conjunction with other clinical and laboratory findings. The assay can be processed manually and analyzed at the microscope or processed and analyzed with HELIOS® AUTOMATED IFA SYSTEM. All suggested results obtained with the HELIOS® AUTOMATED IFA SYSTEM must be confirmed by trained personnel.	NOVA Lite dsDNA Crithidia luciliae is an indirect immunofluorescent assay for the screening and semi-quantitative determination of anti-double stranded DNA (dsDNA) in human serum. The presence of anti-double stranded DNA can be used in conjunction with other serological tests and clinical findings to aid in the diagnosis of systemic lupus erythematosus (SLE)

Similarities		
Item	Device	Predicate
Methodology	Indirect Immunofluoresence assay (IFA)	Same
Results	Qualitative and semi-quantitative titer	Same
Sample Matrix	Serum	Same
Fluorescence Marker	FITC	Same
Controls	One positive control, one negative control	Same
Screening dilution	1:10	Same
Shelf life	24 months (2–8ºC)	Same

Differences		
Item	Device	Predicate
Manual Interpretation of result	Manual fluorescense microscopy or with HELIOS w/ trained operator verification	Manual fluorescense microscopy
Automated interpretation of results	HELIOS w/ trained operator verification	Not Applicable
Slides	12 wells coated with antigen	10 wells coated with antigen

K. Standard/Guidance Document Referenced:

CLSI EP07-A2: Interference Testing in Clinical Chemistry

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures

CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents

CLSI EP28-A3c: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory

CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures A Statistical Approach

GP44-A4: Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline-Fourth Edition

Guidance for Industry and FDA Staff: Recommendations for Anti-Nuclear Antibody (ANA) Test System Premarket (510k) Submissions

Guidance for Industry and Staff: Factors to Consider Regarding Benefit-Risk in Medical

Device Product Availability, Compliance, and Enforcement Decisions

Gudidance for Industry and Food and Drug Administration Staff: Applying Human Factors and Usability Engineering to Medical Devices

L. Test Principle:

The AESKUSLIDES nDNA assay utilizes indirect immunofluorescent antibody assay techniques. Patient sera are diluted in wash/sample buffer at a recommend screening dilution of 1:10 and applied to a well on the slide. nDNA 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 nDNA antibodies immobilized on the slide surface. After a final wash to remove excess conjugate, the slide is mounted and read as soon as possible using a fluorescence microscope for manual microscopy or HELIOS AUTOMATED IFA SYSTEM. The HELIOS AUTOMATED IFA SYSTEM will dilute samples, process slides, scans the slides, generates the image, and provides a positive/negative pre-classification suggestion that must be confirmed by trained personel.

Interpretation of results

Manual interpretation of tests results

AESKU recommends a screening dilution of 1:10, followed by serial dilution for semiquantitative determinations and suggests each laboratory establish its own screening dilution and titration scheme based on its population and instrumentation.

The fluorescence intensity level is the intensity of the specific fluorescence expressed as a numeric value. These values, if present, are reported as a number between “0” (no specific flurescence) and “4+” (very strong visible reaction).

Qualitative evaluation

A serum dilution is considered negative for nDNA antibodies if the cells exhibit < 1+ fluorescence of the kinetoplast. A sample is considered positive for nDNA antibodies if it exhibits exhibit ≥ 1+ fluorescence of the kinetoplast at a sample dilution of 1:10 or greater. Operators should report all titers and specific fluorescence staining seen.

Semi-quantitative evaluation

The endpoint titer is defined as the highest sample dilution factor for which a specific pattern is identifiable. The titers are classified as in the table below:

Dilution	Titer
1:10	Low
1:20 and 1:40	Medium
1:80 and greater	High

Automated instrument interpretation of results

The HELIOS DEVICE SOFTWARE examines the fluorescence intensity and uses an analysis algorithm which takes exposure and pixel frequency into account. It examines relevant regions of the captured images, such as the fluorescence cell area and subtracts the background, to provide an assessment of positive or negative results.

Trained operators must confirm all assessments made by the HELIOS DEVICE SOFTWARE. Intensity and End Point Titers are identified by the HELIOS DEVICE SOFTWARE and pattern assessmentst are made by the HELIOS PATTERN RECOGNITION tool.

Intensity	Interpretation
+	nDNA positive
-	nDNA negative

AESKU recommends a screening dilution of 1:10, followed by serial dilution for semiquantitative determinations and suggests each laboratory establish its own screening dilution and titration scheme based on its population.

M. Performance Characteristics:

Nomenclature and acronyms used in the studies:

The HELIOS Microscope and Software system includes a slide processing module (the HELMED). The system allows for automated imaging or manual imaging by the microscope. All studies were evaluated by comparing the four possible reading methods (Method A, B, C and D) as shown in the table below; these modes are consistent throughout this document. All results generated by the HELIOS software must be confirmed by a trained operator.

Modes of operations that were evaluated in the study:

Method	Sample Processing	Imaging	Reading/Evaluation of slide	Alternate name of Method
A	Automated	Automated	Automated (software interpretation)	HELIOS
B	Automated	Automated	Manual (read of digital image)	HELIOS User Evaluation
C	Manual	Manual	Manual (read of microscope field)	Manual AESKUSLIDES nDNA
D	Manual	Manual	Manual (read of microscope field)	Manual NOVA Lite dsDNA (Predicate)

Fluorescence Intertpretation for Manual Processing, Imaging, and Reading of slide (Method C):

Intensity	Interpretation	
4+	high positive	maximal fluorescence, very strong visible reaction; brilliant yellow-green
3+	positive	strong visible reaction; less brilliant as 4+; yellow-green fluorescence
2+	positive	moderate visible reaction; definite but dull yellow-green fluorescence
1+	positive	weak visible reaction, very dim subdued fluorescence
0	negative	no specific fluorescence

Names of diseases present in samples and their abbreviations:

Disease name	Abbreviations
Systemic lupus erythematosus	SLE
Antiphospholipid Syndrome	APS
Mixed Connective Tissue Disease	MCTD
Undifferentiated connective tissue disease	UCTD
Systemic sclerosis	SSc
Polymyositis	PM
Dermatomyositis	DM
Rheumatoid Arthritis	RA
Autoimmune Hepatitis	AIH
Primary biliary cholangitis	PBC
Primary sclerosing cholangitis	PSC
ANCA-associated vasculitides	AAV
Hepatitis C Virus	HCV
Hepatitis B Virus	HBV
Epstein–Barr virus	EBV

1. Analytical performance:

   All results met the Manufacturer's pre-determined acceptance criteria.

   a. *Precision/Reproducibility:*

      Repeatibility

      Eleven samples were assayed on five days, two runs per day, and three replicates per sample per run, resulting in 30 data points for each sample for Method A. The same samples were assayed using the same protocol for Method B and C and the results were analyzed by two independent readers, for a total of 60 replicates per sample. All runs have been performed according to the respective instructions for use. One positive and one negative control (kit controls) were included in each run. Samples were tested at 1:10 dilution. The samples and results are summarized in the following table.

Samples used for repeatability:

Sample ID	AESKUSLIDES nDNA	
	Result	Grading
1	Pos	borderline
2	Pos	low postive
3	Pos	low postive
4	Pos	medium positive
5	Pos	medium positive
6	Pos	medium positive
7	Pos	high positive
8	Pos	high positive
9	Pos	high positive
10	Neg	negative
11	Neg	negative

Results for repeatbility:

Sample ID	N=30		N=60			
	Method A		Method B		Method C	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
1	100	0	60	40	15	85
2	33.3	66.7	0	100	0	100
3	56.7	43.3	5	95	0	100
4	46.7	53.3	8.3	91.7	0	100
5	0	100	0	100	0	100
6	6.7	93.3	0	100	0	100
7	0	100	0	100	0	100
8	0	100	0	100	0	100
9	3.3	96.7	0	100	0	100
10	83.3	16.7	100	0	98.3	1.7
11	70	30	100	0	100	0

Reproducibility

Between-Lab

Eleven samples were assayed over five days, two runs per day, three replicates per sample per run, at three different study sites. Two study sites were in the U.S., one study site was in Germany, with one HELIOS instrument at each study site. Results were analyzed by two different readers per study site, resulting in a total of 90 data

points per sample per reader. Positive/negative classification was recorded for each sample in each run and for each Method. Positive agreement was caculated as, across all positive samples, the number of correctly found samples divided by the number of total positive samples. Negative agreement was calculated as, across all negative samples, the number of correctly found samples divided by the number of total negative samples. Overall agreement was calculated as, across both positive and negative samples, the number of correctly found samples divided by the number of total samples. Fluorescence intensity (FI) was recorded for samples prepared and processed using Method C. Fluorescence intensity agreement was calculated as, across all samples processed by Method C, the number of correctly found samples divided by the number of total samples. The results are summarized in the following tables:

Method A

Agreement (95% CI)	Site 1 vs Site 2	Site 2 vs Site 3	Site 1 vs Site 3	All Sites
Positive Agreement	63.1 (59.0–67.1)	52.2 (48.0–56.4)	59.4 (55.3–63.5)	58.3 (54.8–61.6)
Negative Agreement	87.5 (80.4–92.3)	95.0 (89.5–97.7)	87.5 (80.4–92.3)	90.0 (84.7–93.6)
Overall Agreement	67.6 (63.9–71.0)	60.0 (56.2 – 63.7)	64.5 (60.8 – 68.1)	64.0 (61.0–67.0)
FI Agreement	N/A	N/A	N/A	N/A

Method B

Agreement (95% CI)	Site 1 vs Site 2	Site 2 vs Site 3	Site 1 vs Site 3	All Sites
Positive Agreement	92.1 (90.4–93.6)	84.4 (82.2–86.5)	84.2 (81.9–86.2)	86.9 (85.2–88.85)
Negative Agreement	99.2 (97.0–99.8)	99.2 (97.0–99.8)	100 (98.4–100)	99.4 (98.0–99.8)
Overall Agreement	93.4 (91.9–94.6)	87.1 (85.2–88.8)	87 (85.1–88.8)	89.2 (87.7–90.5)
FI Agreement	N/A	N/A	N/A	N/A

Method C

Agreement (95% CI)	Site 1 vs Site 2	Site 2 vs Site3	Site 1 vs Site 3	All Sites
Positive Agreement	99.2 (98.4–99.6)	99.0 (98.2–99.4)	98.1 (97.2–98.8)	98.8 (98.1–99.2)
Negative Agreement	99.6 (97.7–99.9)	100 (98.4 –100)	99.6 (97.7–99.9)	99.7 (98.4–100)
Overall Agreement	99.2 (98.6–99.6)	99.2 (98.5–99.5)	98.4 (97.6–99.0)	98.9 (98.4–99.3)
FI Agreement	98.7 (97.9–99.2)	97.7 (96.7–98.3)	97.4 (96.4–98.2)	97.9 (97.2–98.5)

Between-Operator

Agreement within a site was calculated by determining the total number of samples identified correctly, either positive or negative, divided by the expected number of samples that were pre-determined to be classified as positive or negative.

Method B

Agreement (95% CI)	Site 1	Site 2	Site 3
Positive Agreement	91.9 (89.2–93.9)	92.4 (89.9–94.4)	76.5 (72.7–79.9)
Negative Agreement	100 (96.9–100)	98.3 (94.1–99.5)	100 (96.9–100)
Overall Agreement	93.3 (91.2–95.0)	93.5 (91.3–95.1)	80.8 (77.6–83.6)
FI Agreement	N/A	N/A	N/A

Method C

Agreement (95% CI)	Site 1	Site 2	Site 3
Positive Agreement	98.3 (96.9–99.1)	100 (99.3–100)	98 (96.4–98.9)
Negative Agreement	99.2 (95.4–99.9)	100 (96.9 – 100)	100 (96.9–100)
Overall Agreement	98.5 (97.2–99.2)	100 (99.4–100)	98.3 (97–99.1)
FI Agreement	98.5 (97.2–99.2)	98.9 (97.8–99.5)	96.4 (94.6–97.5)

Single Operator

Assay Methods B and C were performed by two trained readers at three different study sites. The assay was performed according to the instructions for use. Agreement for each method was calculated as the number of samples classified correctly divided by the expected number of positive or negative samples, which were determined prior to the study.

Method B

Agreement (95% CI)	Site 1		Site 2		Site 3	
	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6
Positive Agreement	87.4 (82.9–90.8)	96.3 (93.3–98.0)	95.2 (91.9–97.2)	89.6 (85.4–92.7)	77.4 (72.1–82.0)	75.6 (70.1–80.3)
Negative Agreement	100 (94.0–100)	100 (94.0–100)	100 (94.0–100)	96.7 (88.6–99.1)	100 (94.0–100)	100 (94.0–100)
Overall Agreement	89.7 (85.9–92.5)	97.0 (94.5–98.3)	96.1 (93.4–97.7)	90.9 (87.3–93.6)	81.5 (77.0–85.3)	80.0 (75.4–84.0)
FI Agreement	N/A	N/A	N/A	N/A	N/A	N/A

Method C

Agreement (95% CI)	Site 1		Site 2		Site 3	
	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6
Positive Agreement	98.5 (96.3–99.4)	98.1 (95.7–99.2)	100 (98.6–100)	100 (98.6–100)	98.5 (96.3–99.4)	97.4 (94.7–98.7)
Negative Agreement	98.3 (91.1–99.7)	100 (94.0–100)	100 (94.0–100	100 (94.0–100)	100 (94.0–100)	100 (94.0–100)
Overall Agreement	98.5 (96.5–99.4)	98.5 (96.5–99.4)	100 (98.8–100)	100 (98.8–100)	98.8 (96.9–99.5)	97.9 (95.7–99)
FI Agreement	99.7 (98.3–99.9)	97.3 (94.9–98.6)	99.7 (98.3–99.9)	98.2 (96.1–99.2)	97.6 (95.3–98.8)	95.2 (92.3–97.0)

Between-Instrument

Imprecision due to instrument for Method A was evaluated at three different study sites. The assay was performed according to the instructions for use. Agreement was calculated as the number of positive or negative samples found correctly by the automated reader, i.e., HELIOS, divded by the expected number of positive or negative samples.

Method A

% Agreement (95% CI)	Site 1	Site 2	Site 3
Postive Agreement	70.4 (64.7–75.5)	55.9 (50–61.7)	48.5 (42.6–54.5)
Negative Agreement	80.0 (68.2–88.2)	95.0 (86.3–98.3)	95.0 (86.3–98.3)
Overall Agreement	72.1 (67.0– 76.7)	63.0 (57.7–68.1)	57.0 (51.6–62.2)
FI Agreement	N/A	N/A	N/A

Between-Lot

Imprecision due to reagent lot was performed using three reagent lots each for AESKUSLIDES nDNA. Eleven serum samples were assayed 10 times on each reagent lot to give a total of 30 replicates per serum sample. Slides were processed manually according to the instructions for use and subsequently analyzed at the microscope by two independent readers. The results demonstrated one hundred percent agreement (i.e., positive, negative, overall, and FI agreement) between for all three reagent lots tested.

Endpoint titer determination

Within-Lab Precision

The imprecision associated with reporting endpoint titer was performed at three different sites. Five serum samples with endpoint titers between 1:10 and 1:320 (10, 20,40, 160, 320) were diluted serially, ranging from 1:10 up to 1:1280. For each sample, a minimum of four different dilutions around the expected endpoint titer were made. Serum dilutions were assayed on AESKUSLIDES nDNA and analyzed on HELIOS (Method B) and manually at the microscope (Method C) by two independent readers. For each of the two methods, each serum dilution was assayed on five days, with two runs per day and three replicates per run, resulting in 30 repetitions per serum dilution per reader. For each replicate of the dilution series the endpoint titer was reported as the reciprocal of the highest dilution at which the sample was classified as positive. The percentage of samples that differ a maximum +/-1 titer level from each other and percentage of samples that are > +/-1 titer level away from each other were calculated. The following table represents the samples used in the the study:

The results of within-lab precision for Method B and Method C are summarized in the following tables:

Method B

AESKUSLIDES nDNA		N	Percentage of samples within +/- 1 titer level	Percentage of samples > +/- 1 titer level
Site 1	Reader 1	150	92.0	8.0
	Reader 2	150	98.0	2.0
Site 2	Reader 1	150	91.0	9.0
	Reader 2	150	91.0	9.0
Site 3	Reader 1	150	95.0	5.0
	Reader 2	150	90.0	10.0
All readers combined		900	93.0	7.0

Method C

AESKUSLIDES nDNA		N	Percentage of samples within +/- 1 titer level	Percentage of samples > +/- 1 titer level
Site 1	Reader 1	150	99.0	1.0
	Reader 2	150	94.0	6.0
Site 2	Reader 1	150	93.0	7.0
	Reader 2	150	93.0	7.0
Site 3	Reader 1	150	93.0	7.0
	Reader 2	150	92.0	8.0
All readers combined		900	94.0	8.0

Between-Site Imprecision

The imprecision associated with endpoint titer determination between sites was evaluated by calculating agreement between titers determined at three sites (site 1 vs site 2, site 2 vs site 3, site 1 vs site 3) for each Methods B and C.

% Titer Agreement (95% CI)	Site 1 vs. Site 2	Site 2 vs. Site 3	Site 2 vs. Site 3
Method B	88.7 (84.6–91.8)	80.3 (75.5–84.4)	85.7 (81.2–89.2)
Method C	94.3 (91.1–96.4)	83.0 (78.3–86.8)	86.7 (82.4–90.1)

b. Linearity/assay reportable range:

Five serum samples were assayed in duplicates and analyzed on HELIOS (Method B, Reader Confirmation) and manually at the microscope (Method C) by two independent readers. The reciprocal of the highest dilution in which both replicates were reported as positive was defined as the endpoint titer of the sample.

Sample ID	AESUSLIDES nDNA					
	Pos/Neg	Expected Endpoint Titer	Method B		Method C	
			Reader 1	Reader 2	Reader 1	Reader 2
1	Pos	1280	1280	1280	1280	1280
2	Pos	640	640	1280	1280	1280
3	Pos	40	80	80	40	40
4	Pos	320	160	160	320	320
5	Pos	80	80	80	40	80

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

Traceability:

The Positive Control is manufactured by diluting human serum that contains high titter of anti-dsDNA antibodies with protein-based diluent solution, containing preservative. The positive control is a serum from a certified supplier.

Stability:

Shelf-life Stability:

Real-time shelf-life stability testing is ongoing. At the time of submission, stability of three different reagent lots of AESKUSLIDES nDNA stored at 2–8°C was demonstrated to be three months.

Accelerated Stability:

Three lots of complete kits (including slides, controls, conjugate, & sample buffer) of AESKUSLIDES nDNA were stored at 37°C for 6 weeks. At t=0, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, and 6 weeks, a kit was manually tested on a set of eight samples that included negative, low, medium, and high titers. Slides were processed manually according to the instructions for use and subsequently analyzed at the microscope by two independent readers. The data supported a shelf life of 24 months at 2–8°C.

Sample Freeze-Thaw Stability:

Serum stability following multiple freeze-thaw intervals was demonstrated using eight serum samples (seven positive, one negative). Low positive, medium positive, and high positive samples were tested. For each serum, one aliquot was prepared to serve as control (no freeze-thawing). A second aliquot for each serum was prepared that underwent four freeze-thaw cycles. All samples and controls were analyzed on AESKUSLIDES nDNA according to the instructions for use. Slides were read by two independent readers. Each freeze/thaw sample was compared to the respective control

sample. Freezing and thawing up to four times did not affect serum stability.

Long-Term stability

Seven serum samples (four positive and three negative) were aliquoted and stored at −20°C. Frozen aliquots of the different sera were thawed and assayed on AESKUSLIDES nDNA at 4, 8 10, 12, and 14 months. All studies were performed using Method C according to the device's instruction for use. Stability was mainted over the entire 14 months for all samples tested.

d. Detection limit:

Not applicable

e. Analytical specificity:

Interference

The effect of interfering substances was evaluated by spiking eight different substances, listed in the table below, at two different concentrations into eight different serum samples. Controls were prepared for each serum sample by spiking in only the respective amount of diluent without interfering substances. Spiked serum samples and controls were tested in triplicates for each concentration of interferent with AESKUSLIDES nDNA. All tests were performed manually according to the instruction for use. Results were analyzed by two independent readers at the microscope. No significant interference was observed for samples containing each interferernce up to the following concentration:

Interferring Substance	Highest Concentration Tested
Bilirubin conjugated	0.4 mg/ml
Bilirubin unconjugated	0.4 mg/ml
Hemoglobin	5.0 mg/mL
Triglycerides	20 mg/mL
RF IgM	400 U/mL
Rituximab	up to 2.0 mg/mL
Methylprednisolone	0.8 mg/mL
Cyclophosphamide	4.0 mg/mL
Methotrexate	0.1 mg/mL
Azathioprine	0.03 mg/mL
Belimumab	8 mg/ml
Hydroxychloroquine	0.024 mg/mL
Mycophenolat	0.048 mg/mL
Ibuprofen	2 mg/mL
Naproxen	0.2 mg/mL

Carry-over

A study was performed to demonstrate that the HELIOS instrument does not carry over a positive sample to a negative well. Three high positive samples were run on HELIOS in alternate with a negative serum sample. Tests were performed in accordance with the respective instructions for use. Results were confirmed by a reader. No carry over was observed from well to well.

f. Assay cut-off:

The manufacturer recommends a screening dilution of 1:10 followed by serial dilutions for semi-quantitative determinations. Each laboratory should establish its own screening dilution and titration scheme based on its patient population.

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison with the predicate device was performed using the same 776 serum samples (comprising 297 serum samples from patients with SLE and 479 samples from patients with other diseases) tested in the clinical study (see table below). Method comparison was performed with AESKUSLIDES nDNA and the predicate assay (K880742). For each assay, the sample set was processed manually and analyzed (positive/negative classification) at the microscope (Methods C and D). All assays were performed according to the respective instructions for use. Samples were screened at a dilution of 1:10.

Samples used for method comparison and clinical sensitivity and specificity:

Diagnosis			N
Target Disease		Systemic Lupus Erythematosus	297
Control Diseases	Other Rhuematic Diseases	Antiphospholipid Syndrome	12
		Sjoegren´s Syndrome	30
		Systemic Sclerosis	44
		Dermatomyositis/Polymyositis	31
		Rheumatoid Arthritis	58
		Mixed Connective Tissue Diseases	24
		Undifferentiated Connective Tissue Diseases	21
	Autoimmune Liver Diseases	Autoimmune Hepatitis	21
		Primary Biliary Cirrhosis	20
		Primary Sclerosing Cholangitis	10
	Vasculitis	ANCA associated Vasculitis	44
	Infectious Diseases	Epstein-Barr Virus Infection	25
		Hepatitis B Virus Infection	34
		Hepatitis C Virus Infection	44
	Leukemia	Lymphoma	31
	Other	Fibromyalgia	30
Total			776

From the results of both assays, the positive, negative and overall agreements were calculated and are presented in the tables below:

Method Comparison		Predicate		
		Pos	Neg	Total
AESKUSLIDES nDNA	Pos	80	13	93
	Neg	98	585	683
	Total	178	598	776

Predicate vs. AESKUSLIDES nDNA	% Agreement (95% CI)
Positive Agreement	44.9 (37.8–52.3)
Negative Agreement	97.8 (96.3–98.7)
Overall Agreement	85.7 (83.1–88.0)

Comparison of method A, B, and C:

Comparison of methods was performed using the same 776 serum samples at 3 different study sites. All assays were performed according to the instructions for uses.

Method comparison at each site.

% Agreement (95% CI)		Method C vs B	Method B vs. A	Method C vs A
Site 1	Positive Agreement	85.3 (79.0–89.9)	51.2 (43.7–58.7)	49.7 (42.1–57.3)
	Negative Agreement	98.1 (97.2–98.7)	84.2 (82.2–86.0)	83.9 (81.9–85.8)
	Total Agreement	96.7 (95.7–97.5)	80.7 (78.6–82.6)	80.3 (78.3–82.2)
Site 2	Positive Agreement	91.0 (85.4–94.5)	55.4 (47.8–62.8)	54.2 (46.3–61.8)
	Negative Agreement	98.2 (97.4–98.8)	86.3 (84.4–88.0)	85.8 (83.9–87.6)
	Total Agreement	97.5 (96.6–98.2)	83.0 (81.0–84.8)	82.7 (80.7–84.5)
Site 3	Positive Agreement	86.2 (79.7–90.9)	47.7 (39.8–55.6)	44.8 (37.0–53.0)
	Negative Agreement	98.3 (97.5–98.9)	90.5 (88.9–91.9)	90.1 (88.5–91.6)
	Total Agreement	97.2 (96.2–97.9)	86.4 (84.6–88.0)	85.9 (84.1–87.5)

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

A clinical study using 776 serum samples was performed with AESKUSLIDES nDNA (Method C) and the predicate assay (Method D), comparing the result of each device to the clinical diagnosis. The samples were the same as those used in the method comparison (see table above). The study was performed at two U.S. sites and one German site. The results are presented in the tables below:

Method C

		Diagnosis		
		Pos	Neg	Total
AESKUSLIDES nDNA	Pos	73	25	98
	Neg	224	454	678
	Total	297	479	776

Method D

		Diagnosis		
		Pos	Neg	Total
Predicate Assay	Pos	110	68	178
	Neg	187	411	598
	Total	297	479	776

	% Sensitivity (95% CI)	% Specificity (95% CI)	PPV	NPV
	SLE (n=297)	Other Diseases (n= 479)		
AESKUSLIDES nDNA	24.6 (20.0–29.8)	94.8 (92.4–96.4)	74.5	67.0
Predicate Assay	37.0 (31.7–42.7)	85.8 (82.4–88.6)	61.8	68.7

Clinical Sensitivity and Specificity by Site and Method

% Diagnostic Sensitivity & Specificity			% Sensitivity (95% CI)	Specificity (95% CI)		% Sensitivity (95% CI)	% Specificity (95% CI)
			SLE (n= 297)	Other Diseases (n=479)		SLE (n= 297)	Other Diseases (n=479)
Site 1	Manual	Reader 1	20.9 (16.6–25.9)	94.8 (92.4–96.4)	Combined Readers	20.4 (17.3–23.8)	95.6 (94.1–96.7)
		Reader 2	19.9 (15.7–24.8)	96.5 (94.4–97.8)			
	Reader Confirmation	Reader 1	21.2 (16.9–26.2)	95.4 (93.1–96.9)	Combined Readers	20.7 (17.6–24.1)	95.5 (94.0–96.7)
		Reader 2	20.2 (16.0–25.1)	95.6 (93.4–97.1)			
	HELIOS		20.5 (16.3-25.5)	81.0 (77.2–84.3)			
Site 2	Manual	Reader 3	22.6 (18.2–27.6)	97.3 (95.4–98.4)	Combined Readers	21.7 (18.6–25.2)	97.3 (96.1–98.1)
		Reader 4	20.9 (16.6–25.9)	97.3 (95.4–98.4)			
	Reader Confirmation	Reader 3	21.2 (16.9–26.2)	95.4 (93.1–96.9)	Combined Readers	21.9 (18.7–25.4)	96.1 (94.7–97.2)
		Reader 4	22.6 (18.2–27.6)	96.9 (94.9–98.1)			
	HELIOS		21.9 (17.6–26.9)	84.1 (80.6–87.1)			
Site 3	Manual	Reader 5	21.9 (17.6–26.9)	97.7 (95.9–98.7)	Combined Readers	20.4 (17.3–23.8)	97.5 (96.3–98.3)
		Reader 6	18.9 (14.8–23.7)	97.3 (95.4–98.4)			
	Reader Confirmation	Reader 5	19.2 (15.1–24.1)	97.3 (95.4–98.4)	Combined Readers	19.2 (16.2–22.6)	96.9 (95.6–97.8)
		Reader 6	19.2 (15.1–24.1)	96.5 (94.4–97.8)			
	HELIOS		15.2 (11.5–19.7)	88.1 (84.9–90.7)			

Cross Reactivity:

Site 1

Cross Reactivity	N	Method C		Method B		Method A
		Reader 1	Reader 2	Reader 1	Reader 2	
		% Pos	% Pos	% Pos	% Pos	% Pos
Systemic Lupus Erythematosus	297	20.9	19.9	21.2	20.2	20.5
Antiphospholipid Syndrome	12	0.0	0.0	8.3	0.0	41.7
Sjoegren´s Syndrome	30	0.0	0.0	3.3	3.3	33.3
Systemic Sclerosis	44	2.3	0.0	0.0	0.0	13.6
Dermatomyositis/Polymyositis	31	3.2	3.2	3.2	3.2	19.4
Rheumatoid Arthritis	58	13.8	12.1	12.1	13.8	25.9
Mixed Connective Tissue Diseases	24	8.3	8.3	12.5	8.3	41.7
Undifferentiated Connective Tissue Diseases	21	0.0	0.0	0.0	0.0	9.5
Autoimmune Hepatitis	21	9.5	9.5	9.5	9.5	23.8
Primary Biliary Cirrhosis	20	27.3	0.0	38.4	27.3	58.6
Primary Sclerosing Cholangitis	10	0.0	0.0	0.0	0.0	0.0
ANCA associated Vasculitis	44	11.4	11.4	4.5	4.5	25.0
Epstein-Barr Virus Infection	25	0.0	0.0	0.0	0.0	12.0
Hepatitis B Virus Infection	34	5.9	0.0	2.9	2.9	2.9
Hepatitis C Virus Infection	44	0.0	0.0	0.0	0.0	6.8
Lymphoma	31	3.2	0.0	0.0	3.2	16.1
Fibromyalgia	30	0.0	0.0	0.0	0.0	10.0
Total	776					

Site 2

Cross Reactivity	N	Manual		Reader Confirmation		HELIOS
		Reader 1	Reader 2	Reader 1	Reader 2	
		% Pos	% Pos	% Pos	% Pos	% Pos
Systemic Lupus Erythematosus	297	22.6	20.9	21.2	22.6	21.9
Antiphospholipid Syndrome	12	0.0	0.0	8.3	0.0	41.7
Sjoegren´s Syndrome	30	0.0	0.0	3.3	3.3	33.3
Systemic Sclerosis	44	0.0	0.0	3.3	0.0	13.3
Dermatomyositis/Polymyositis	31	3.2	3.2	6.5	12.9	15.9
Rheumatoid Arthritis	58	10.3	10.3	12.1	10.3	20.7
Mixed Connective Tissue Diseases	24	8.3	8.3	12.5	8.3	33.3
Undifferentiated Connective Tissue Diseases	21	0.0	0.0	0.0	0.0	14.3
Autoimmune Hepatitis	21	9.5	9.5	9.5	4.8	14.3
Primary Biliary Cirrhosis	20	0.0	0.0	38.4	20.2	47.5
Primary Sclerosing Cholangitis	10	0.0	0.0	0.0	0.0	30.0

Cross Reactivity	N	Manual		Reader Confirmation		HELIOS
		Reader 1	Reader 2	Reader 1	Reader 2	
		% Pos	% Pos	% Pos	% Pos	% Pos
ANCA associated Vasculitis	44	2.3	2.3	4.5	2.3	9.1
Epstein-Barr Virus Infection	25	0.0	0.0	0.0	0.0	4.0
Hepatitis B Virus Infection	34	2.9	2.9	2.9	2.9	11.8
Hepatitis C Virus Infection	44	0.0	0.0	0.0	0.0	11.4
Lymphoma	31	0.0	0.0	0.0	0.0	25.8
Fibromyalgia	30	0.0	0.0	0.0	0.0	0.0
Total	776					

Site 3

Cross Reactivity	N	Manual		Reader Confirmation		HELIOS
		Reader 1	Reader 2	Reader 1	Reader 2	
		% Pos	% Pos	% Pos	% Pos	% Pos
Systemic Lupus Erythematosus	297	21.9	18.9	19.2	19.2	15.2
Antiphospholipid Syndrome	12	0.0	0.0	0.0	0.0	0.0
Sjoegren´s Syndrome	30	0.0	0.0	3.3	3.3	13.3
Systemic Sclerosis	44	0.0	0.0	2.2	2.2	15.9
Dermatomyositis/Polymyositis	31	0.0	0.0	0.0	0.0	3.2
Rheumatoid Arthritis	58	8.6	13.8	12.1	10.3	12.1
Mixed Connective Tissue Diseases	24	8.3	4.2	8.3	8.3	16.7
Undifferentiated Connective Tissue Diseases	21	0.0	0.0	0.0	0.0	9.5
Autoimmune Hepatitis	21	4.8	0.0	9.5	9.5	14.3
Primary Biliary Cirrhosis	20	0.0	11.1	0.0	11.1	20.2
Primary Sclerosing Cholangitis	10	0.0	0.0	0.0	0.0	0.0
ANCA associated Vasculitis	44	4.5	2.3	4.5	4.5	13.6
Epstein-Barr Virus Infection	25	0.0	0.0	0.0	0.0	12.0
Hepatitis B Virus Infection	34	2.9	0.0	5.9	2.9	14.7
Hepatitis C Virus Infection	44	0.0	0.0	0.0	0.0	4.5
Lymphoma	31	0.0	3.2	3.2	3.2	22.6
Fibromyalgia	30	0.0	0.0	0.0	0.0	13.3
Total	776					

b. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off**:**

   Please see assay cut-off for this information.

5. Expected values/Reference range:

   Expected values for AESKUSLIDES nDNA (Crithidia luciliae) were analyzed with a panel of 164 sera from healthy donors from two different sites:114 from site 1 (63 females, 51 males, mean age 32 years, range of 18–58 years) and 50 from site 2 (17 females, 33 males, mean age 34 years, range of 19–62 years). Slides were processed manually according to the device instructions for use and analyzed at the microscope by two independent readers.

	Positive (%)	Negative (%)	Total
Reader 1	1 (0.6)	163 (99.4)	164
Reader 2	0 (0)	164 (100)	164

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