510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

K153117

B. Purpose for Submission:

Assay and instrument

C. Measurand:

Anti-nuclear antibodies (ANA)

D. Type of Test:

Qualitative and semi-quantitative, indirect immunofluorescence

E. Applicant:

AESKU SYSTEMS GmbH & Co.KG (instrument and software)

AESKU DIAGNOSTICS GmbH & Co.KG (assay)

F. Proprietary and Established Names:

HELIOS® AUTOMATED IFA SYSTEM

AESKUSLIDES® ANA HEp-2-Gamma

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

PIV − Automated indirect immunofluorescence microscope and software-assisted system for clinical use

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

Instrument:
The HELIOS® AUTOMATED IFA SYSTEM is an automated system for immunofluorescence processing with an integrated fluorescence microscope and software for routine laboratory use by professional users under controlled environmental conditions. All suggested results obtained with the HELIOS® AUTOMATED IFA SYSTEM must be confirmed by trained operator.

Assay:
AESKUSLIDES® ANA HEp-2-Gamma is an indirect fluorescent antibody assay utilizing HEp-2 cell coated slides as a substrate for the qualitative and/or semi-quantitative determination of antinuclear antibodies (ANA) in human serum by manual microscopy or with HELIOS® AUTOMATED IFA SYSTEM. This *in vitro* diagnostic assay is used as an aid in the diagnosis of systemic rheumatic diseases in conjunction with other clinical and laboratory findings. All suggested results obtained with the HELIOS® AUTOMATED IFA SYSTEM instrument must be confirmed by trained operator.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):
   - For prescription use only
   - This device is only for use with reagents that are indicated for use with the device.
   - The device is for use by a trained operator in a clinical laboratory setting.
   - All software-aided results must be confirmed by a trained operator
   - Special instrument requirement: for use only with the HELIOS AUTOMATED IFA SYSTEM

I. Device Description:

Assay kit components:
- Slides, each containing 12 wells coated with ANA HEp-2 cells, package of 2, 10, 50 or 100
- One 4.0 ml vial containing Fluorescein (FITC) labeled goat anti-human IgG (Gamma Chain) conjugate in a solution of BSA and Evans Blue, ready for use

- One 0.5 ml vial of positive control (homogenous pattern) containing human serum, ready for use
- One 0.5 ml vial of negative control containing diluted human serum, ready for use
- One 7.0 ml vial of mounting medium containing a solution of glycerol and PBS, ready for use
- One 70 ml bottle of sample buffer, containing BSA, PBS, ready for use
- One 100 ml bottle of wash buffer, concentrated buffer 1:10 in distilled water, containing BSA, PBS

J. Substantial Equivalence Information:

1. Predicate device name(s):
   AESKUSLIDES® ANA HEp-2

2. Predicate 510(k) number(s):
   K120889

3. Comparison with predicate:

Similarities		
Item	Device AESKUSLIDES® ANA HEp-2-Gamma	Predicate AESKUSLIDES® ANA HEp-2 (K120889)
Intended Use	Qualitative and/or semi-quantitative determination of antinuclear antibodies (ANA) in human serum. This in vitro diagnostic assay is used as an aid in the diagnosis of systemic rheumatic diseases in conjunction with other clinical and laboratory findings.	Same
Methodology	Immunofluorescence assay (IFA)	Same
Procedure	Standard IFA technique	Same
Results	Pattern and titer; qualitative; semi-quantitative titer	Same
Sample Matrix	Serum	Same
Antigen	HEp-2 cells	Same
Slides	12-well coated with antigen	Same
Fluorescence Marker	Fluorescein isothiocyanate (FITC)	Same
Controls	One positive control	Same

Similarities		
Item	Device AESKUSLIDES® ANA HEp-2-Gamma	Predicate AESKUSLIDES® ANA HEp-2 (K120889)
	(homogenous pattern) and one negative control	
Storage	2°C–8°C	Same
Counterstain	Evans Blue	Same

Differences		
Item	Device AESKUSLIDES® ANA HEp-2-Gamma	Predicate AESKUSLIDES® ANA HEp-2
Shelf-Life-Stability	24 months	18 months
Initial Screening Dilution	1:40 or 1:80	1:40
Analyte	ANA (Fc Gamma, IgG isotype)	ANA (H+L, IgG isotype)
Conjugate	Fluorescein (FITC) labelled goat anti-human IgG (Fc Gamma Chain)	Fluorescein (FITC) labelled goat anti-human IgG (H+L Chain)
Interpretation of Results	Manual fluorescence microscopy or HELIOS AUTOMATED IFA SYSTEM with trained operator verification	Manual fluorescence microscopy

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

- 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 CLSI Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory
- CLSI EP06-A CLSI Evaluation of the Linearity of Quantitative Measurement Procedures A Statistical Approach
- ISO 14971 - Medical Devices - Application of risk management to medical devices, Second Edition
- IEC 62366 - Medical Devices; Part 1: Application Of Usability Engineering To Medical Devices, Edition 1.0
- ISO15223-1 - Medical Devices - Symbols To Be Used With Medical Device Labels, Labelling, And Information To Be Supplied - Part 1: General requirements, Second Edition
- Guidance for Industry and FDA Staff: Recommendations for Anti-Nuclear Antibody (ANA) Test System Premarket (510k) Submissions (January 22, 2009)

- Guidance for the Content of Premarket submission for Software Contained in Medical Devices (May 11, 2005)

L. Test Principle:

The AESKUSLIDES ANA HEp-2-Gamma assay is an indirect immunofluorescent antibody assay technique. Patient sera is diluted in wash/sample buffer and applied to a well on the slide. 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 that binds to the ANA antibodies immobilized on the slide surface. After a final wash to remove excess conjugate, the slide is mounted and read immediately at 400–800 x total magnification with a fluorescent microscope (490 nm excitation filter, 510 nm barrier filter). If not read immediately, the slide(s) is covered (to protect from light) and stored at 2–8°C/35–46°F.

Interpretation of results:

Manual interpretation of test results:
AESKU recommends a screening dilution of 1:40 or 1:80, followed by serial dilution for semi-quantitative determination and suggests each laboratory establish its own screening dilution and titration scheme based on its population and instrumentation.

The fluorescence intensity (FI) 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 fluorescence) and "4+" (very strong visible reaction).

A sample is considered negative for ANA antibodies if the cells exhibit < 1+ fluorescence and no discernible pattern at the chosen screening (1:40 or 1:80) and all greater dilutions. A sample is considered positive for ANA antibodies if it exhibits ≥ 1+ fluorescence and a discernible pattern at a sample dilution of 1:40 or greater. Operators should report all titers and patterns seen. Note: Pattern refers to the predefined immunofluorescent patterns that are described in section M below.

Qualitative evaluation
A titer of 1:40 or greater that has results in a discernable pattern is considered positive.

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
1:40 and 1:80	Low
1:160 and 1:320	Medium
1:640 and greater	High

Instrument interpretation of test results:
The HELIOS DEVICE SOFTWARE provides positive or negative results, and can produce estimated end point titers and pattern suggestions for the positive results. All software

suggested results must be confirmed by a trained operator (OP).

Additional instrument and software information is in sections N and O.

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, D and E) 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	Processing	Imaging	Reading/Evaluation of Slides
A	Automated	Automated	Automated (software interpretation)
B	Automated	Automated	Manual (read of digital image)
D	Manual	Manual	Manual (read of microscope field)
E	Manual (Predicate)	Manual	Manual (read of microscope field)

Method E is the predicate device/assay, AESKUSLIDES ANA HEp-2 assay.
Methods A, B, and D are performed with AESKUSLIDES ANA HEp-2-Gamma assay.
Methods A and B use the HELMED slide processing module.
Note that there is no Method C.

The HELIOS software identifies the following immunofluorescence (IF) patterns:

Abbreviation	Pattern
hom or ho	homogeneous
sp	speckled (granular)
nuc or nu	nucleolar
cen or ce	centromere
num or nm	nuclear membrane
nud or nudo	nuclear dots
cyt	cytoplasmic

Names of diseases present in samples and their abbreviations:

Disease Group/Diagnosis	Abbreviations
Group 0: Healthy	
Normal Controls	n/a
Group 1: Rheumatic Diseases associated with ANA positivity Connective Tissue Disease) + Autoimmune Liver disease	CTD + AIL
Autoimmune Hepatitis	n/a
Calcinosis, Raynaud phenomenon, Esophageal dysmotility, Sclerodactyly, and Telangiectasia	CREST
Mixed Connective Tissue Disease	MCTD

Myositis	n/a
Scleroderma	n/a
Sjoegren`s Syndrome	n/a
Systemic Lupus Erythematosus	SLE
Undifferentiated Collagenosis	Undif.
Group 2: Other diseases not associated with ANA positivity	
Antiphospholipid Syndrome	APS
Celiac Disease	n/a
Diverse I (Fibromyalgia, Type 1 Diabetes, & Lumbago)	n/a
Hepatitis B Virus	HBV
Hepatitis C Virus	HCV
Rheumatoid Arthritis	RA
Spondyloarthritis	SpA
Vasculitis	n/a
Group 3: Diverse samples (selected due to ANA positivity or rare patterns)	
Diverse II (unknown disease with rare ANA pattern)	n/a
Total	

Interpretation of Fluorescent Intensity (FI):

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

1. Analytical performance:

   The Manufacturer's acceptance criteria were met for all studies.

   a. *Precision/Reproducibility:*

      The AESKUSLIDES ANA HEp-2-Gamma kit and the predicate AESKUSLIDES ANA HEp-2 kit contain the same materials with the exception that the AESKUSLIDES ANA HEp-2-Gamma kit incorporates an IgG Fc-gamma-specific FITC conjugate whereas the predicate incorporates an IgG H+L specific FITC conjugate.

      Reproducibility/Inter-assay

      All studies were performed according to the Instructions for Use.

      Method D vs. E:
      Reproducibility was evaluated with 12 samples (10 positive, 2 negative) representing

the seven patterns at two dilutions each. Samples were run in duplicate over five days, morning and evening for a total of 40 replicates per sample/per dilution, 480 total per dilution. Samples were evaluated at 1:40 and 1:80 dilutions, by two independent readers at each site, one instrument per site. Results for within-run, run-to-run and day-to-day precision were analyzed and are summarized below. There was a 100% positive/negative agreement at 1:40. At 1:80 there were discrepancies due to three sera that are low positive with an end point titer of 1:40; therefore, these would be expected to be negative at a 1:80 dilution. There was 100% pattern and FI agreement.

Method D vs. E reproducibility agreement:

% Agreement (95% CI)	1:40 Dilution Method D vs. E	1:80 Dilution Method D vs. E
Positive Agreement	100 (99.5–100)	100 (99.3–100)
Negative Agreement	100 (97.7–100)	89.0 (85.6–91.7)
Overall Agreement	100 (99.6–100)	95.4 (93.9–96.6)

Pattern Agreement	100 (99.5–100.0)	100 (99.3–100.0)
F.I. Agreement	100 (99.6–100)	100 (99.6–100)

Methods A, B and D vs. E:
Reproducibility was evaluated with 15 samples (10 positive, 5 negative). For Methods A and B, 10 replicates were tested per sample per run, a total of 100 replicates per sample per dilution (at 1:40 and 1:80 dilutions) for a total of 1500 total readings per dilution. For Method D, two replicates were tested per sample per run for a total of 20 replicates per sample per dilution, for a total of 300 total readings, using sample dilutions of 1:40 and 1:80.

Methods A, B, and D vs. E reproducibility agreements

% Agreement (95% CI)	1:40 Dilution	1:80 Dilution
	Method A vs. E	
Positive Agreement	100 (99.6–100)	99.2 (98.4–99.6)
Negative Agreement	99.6 (98.6–99.9)	100 (99.2–100)
Overall Agreement	99.9 (99.5–100)	99.5 (99.0–99.7)
Total Pattern Agreement	98.8 (97.9–99.3)	99.1 (98.3–99.5)
	Method B vs. E	
Positive Agreement	100 (99.6–100)	100 (99.6–100)
Negative Agreement	100 (99.2–100)	100 (99.2–100)
Overall Agreement	100 (99.7–100)	100 (99.7–100)
Total Pattern Agreement	100 (99.6–100)	100 (99.6–100)
	Method D vs. E	
Positive Agreement	100 (98.1–100)	100 (98.1–100)
Negative Agreement	100 (96.3–100)	100 (96.3–100)
Overall Agreement	100 (98.7–100)	100 (98.7–100)
Total Pattern Agreement	100 (98.1–100)	100 (98.1–100)

Repeatability/Intra-assay

Method D vs. E**:**
12 samples (10 positive, 2 negative) were tested within the same run, with 20 replicates per sample, per dilution, and evaluated by two independent, blinded readers for each Method, for a total of 40 replicates per dilution and 480 total readings per dilution, using sample dilutions at 1:40 and 1:80.

Method D vs. E repeatability agreements:

% Agreement (95% CI)	1:40 Dilution Method D vs. E	1:80 Dilution Method D vs. E
Positive Agreement	100 (99.5–100)	99.6 (98.7–99.9)
Negative Agreement	100 (97.7–100)	94.5 (91.8–96.3)
Overall Agreement	100 (99.6–100)	97.5 (96.3–98.3)
Pattern Agreement	100 (99.5–100)	100 (99.3–100)
FI Agreement	100 (99.6–100)	100 (99.6–100)

Methods A, B and D vs. E repeatability was tested with eight characterized samples (seven positive representing the seven different patterns and one negative) were tested in 30 replicates per dilution (1:40 and 1:80), and evaluated by two readers (for Method B and D) for a total of 240 samples analyzed per dilution. Tests were performed, using sample dilutions of 1:40 and 1:80.

Methods A, B and D vs. E repeatability agreement:

% Agreement (95% CI)	1:40 Dilution	1:80 Dilution
	Method A vs. E	
Positive Agreement	100 (98.2–100)	99 (96.6–99.7)
Negative Agreement	100 (88.6–100)	100 (88.6–100)
Overall Agreement	100 (98.4–100)	99.2 (97–99.8)
Total Pattern Agreement	96.7 (93.3–98.4)	96.2 (92.7–98.1)
	Method B vs. E	
Positive Agreement	100 (99.1–100)	100 (99.1–100)
Negative Agreement	100 (94–100)	100 (94–100)
Overall Agreement	100 (99.2–100)	100 (99.2–100)
Total Pattern Agreement	100 (99.1–100)	100 (99.1–100)
	Method D vs. E	
Positive Agreement	100 (99.1–100)	100 (99.1–100)
Negative Agreement	100 (94–100)	100 (94–100)
Overall Agreement	100 (99.2–100)	100 (99.2–100)
Total Pattern Agreement	100 (99.1–100)	100 (99.1–100)

Lot-to-Lot

Method D vs. E:
Three lots of complete AESKUSLIDES ANA HEp-2-Gamma kits and three lots of the Predicate Device were processed and evaluated manually. Each was assayed

using 12 serum samples (10 positive and 2 negative) for a total of 144 total replicates per dilution. Tests were performed 1:40 and 1:80 in duplicate. Slides were evaluated by two independent readers. There were two low positive samples at 1:40 dilution (FI = 1+) that were either negative or low positive (FI = 1+) at 1:80 dilution across the three lots and both readers. The discrepancies were attributed to the low titer samples at 1:40 dilution and were not immunofluorescent pattern-specific.

Method D vs. E Lot-to-Lot agreement:

% Agreement (95% CI)	1:40 Dilution Method D vs. E	1:80 Dilution Method D vs. E
Positive Agreement	99.6 (97.7–99.9)	100 (97.8–100.0)
Negative Agreement	100 (92.6–100.0)	81.7 (73.8–87.6)
Overall Agreement	99.7 (98.1–99.9)	92.4(88.7–94.9)

Pattern Agreement	100 (98.4–100.0)	100 (97.8–100.0)
F.I. Agreement	100 (98.7–100.0)	100 (98.7–100.0)

Instrument-to-Instrument Precision

Methods A and B vs. E:
Instrument-to-Instrument reproducibility was evaluated with 15 characterized samples that were tested in 10 replicates in a single run on three HELIOS instruments with AESKUSLIDES ANA HEp-2-Gamma. The samples were evaluated by Method A and Method B and compared separately with Method E at 1:40 and 1:80 dilutions. Positive, Negative, Overall, Single Pattern and Total Pattern Agreement were ≥ 90%. Results are summarized in the table below:

	Method A vs. E	
% Agreement (95% CI)	1:40 Dilution	1:80 Dilution
Positive Agreement	100 (98.7–100)	100 (98.7–100)
Negative Agreement	99.3 (96.3–99.9)	100 (97.5–100)
Overall Agreement	99.8 (98.8–100)	100 (99.2–100)
Total Pattern Agreement	98.3 (96.2– 99.3)	98.7 (96.6–99.5)
	Method B vs. E	
% Agreement (95% CI)	1:40 Dilution n = 450	1:80 Dilution n = 450
Positive Agreement	100 (98.7–100)	100 (98.7–100)
Negative Agreement	100 (97.5–100)	100 (97.5–100)
Overall Agreement	100 (99.2–100)	100 (99.2–100)
Total Pattern Agreement	100 (98.7–100)	100 (98.7–100)

b. *Linearity/assay reportable range:*

Methods A, B, D and E:

To determine linearity, 10 high positive samples (End-point-Titer higher than 1:640) were serially diluted and evaluated. The sera were tested starting at 1:40 dilution and titrated through 1:10,240 dilutions. Slides were evaluated on one instrument with Methods A and B (samples were run in duplicates and evaluated by one reader) and with Methods D and E with two independent readers. Results are reported as the pattern detected and the highest titer at which a pattern is recognized (e.g., hom 640 indicated homogeneous pattern at 1:640). Patterns did not change when the samples were diluted. All results were within ± 1 expected titer level.

Linearity Results for Methods D and E:

Expected Result	Method D		Method E	
	Reader 1	Reader 2	Reader 1	Reader 2
ho 640	hom 640	hom 640	hom 640	hom 640
ho 640	hom 640	hom 640	hom 640	hom 640
sp 2560	spe 2560	spe 2560	spe 1280	spe 2560
sp 5120	spe 5120	spe 5120	spe 2560	spe 2560
ce 2560	cen 2560	cen 2560	cen 1280	cen 1280
ce 1280	cen 1280	cen 1280	cen 640	cen 1280
nu 2560	nuc 2560	nuc 2560	nuc 1280	nuc 1280
nm 640	num 640	num 640	num 640	num 640
nud 5120	nud 5120	nud 5120	nud 2560	nud 2560
cyt 5120	cyt 5120	cyt 5120	cyt 2560	cyt 5120

Linearity Results for Methods A and B:

Expected Result	Method A		Method B	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
ho 640	ho 1280	ho 1280	ho 1280	ho 1280
ho 640	ho 1280	ho 1280	ho 1280	ho 1280
sp 2560	sp 2560	sp 5120	sp 5120	sp 5120
sp 5120	sp 2560	sp 2560	sp 5120	sp 5120
ce 2560	ce 1280	ce 2560	ce 2560	ce 2560
ce 1280	ce 2560	ce 1280	ce 2560	ce 2560
nu 2560	nu 1280	nu 2560	nu 2560	nu 2560
nm 640	nm 320	nm 320	nm 320	nm 320
nud 5120	nud 2560	nud 2560	nud 2560	nud 2560
cyt 5120	cyt 2560	cyt 2560	cyt 2560	cyt 2560

The upper limit of the measuring range is 1:10,240.

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

Traceability:

There is no recognized standard for anti-nuclear antibodies.

Stability:

Stability Studies: Method D
The reagents for this assay are the same as those in the predicate device, except for changes to the conjugate; therefore, an accelerated stability study was performed to verify that there was no change to the stability of the conjugate. Shelf life stability is extended from 18 to 24 months. Open bottle stability is currently seven months when stored at 4°C. Real time stability testing is ongoing for Shelf life (closed bottle) and open bottle stability and will continue for two years. Open-bottle stability for Wash Buffer is one week. The results are summarized in the table below:

Type of stability	Stability claim
Shelf life stability	2 years at 2–8°C
Transport stability	6 weeks at up to 37°C
Open bottle (ready to use reagents)	7 months at 2–8°C
Open bottle (Wash Buffer)	1 week at 2–8°C
Freeze/thaw	4 cycles

Controls:
Positive and negative controls are included in the AESKUSLIDES ANA HEp-2-Gamma kit. They are the same as in the predicate device. The positive control is ready-to-use and consists of pre-diluted human serum selected for the presence of anti-nuclear antibodies exhibiting a strong homogenous staining in the nucleus. The negative control consists of ready-to-use pre-diluted human serum selected for the absence of autoantibodies. AESKU recommends use of both positive and negative controls in each run. Negative Control: the cells should exhibit less than 1+ fluorescence and appear green. Positive Control: the cells should exhibit a homogeneous staining pattern with a fluorescent intensity of 3+.

d. *Detection limit:*

Not applicable

e. *Analytical specificity:*

Interfering substances:
The effects of interfering substances on assay results were tested by spiking 10 serum samples with Bilirubin, Hemoglobin and Triglycerides. Serum samples spiked and tested by Method D and E by the same technician on the same day at 1:40 and 1:80 dilutions. The results are summarized below:

Interferents Tested	Patterns Tested	No interference up to concentrations
Hemoglobin	ho, sp, ce, nu, nm, cyt, nd, neg	500 mg/dL
Triglycerides	ho, sp, ce, nu, nm, cyt, nd, neg	2000 mg/dL
Conjugated Bilirubin	ho, sp, ce, nu, nm, cyt, nd, neg	40 mg/dL

Unconjugated Bilirubin	ho, sp, ce, nu, nm, cyt, nd, neg	40 mg/dL
RF	ho, sp, ce, nu, nm, cyt, nd, neg	500 IU/mL

Cross-reactivity:
To determine analytical cross-reactivity of the conjugate in non-ANA samples, 16 CDC ANA reference samples were evaluated manually (Method D and E) on both devices. In addition, clinically defined samples for celiac disease (20), ANCA-associated vasculitis (18), Crohn's disease (10) and infectious disease (20) were tested. These samples were tested at dilutions of 1:40 and 1:80. Slides were evaluated by two independent readers. The sponsor stated that no cross reactivity was observed for either assay, at both 1:40 and 1:80 dilutions.

Carry over study:

A study was done to show that the HELIOS does not carry over a positive sample to a negative well. Seven high positive samples (≥ 1:1280 representing the seven different patterns) were run in alternate with seven negative samples and were evaluated by Methods A, B and D at 1:40 and 1:80 dilutions. There was no carry-over seen. All positive samples were identified as positive. All negative samples were identified as negative.

f. Assay cut-off:

AESKU recommends a screening dilution of 1:40 or 1:80, followed by serial dilutions for semi-quantitative determinations, but suggests each laboratory establish its own screening dilution and titration scheme based on its population. 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:

The samples from two separate Clinical Studies were used for the Method Comparison Evaluation. In the first study, 288 Clinical samples were evaluated at three different sites (two U.S. and one German), comparing Methods A, B, D, and E. An additional 268 clinical samples (for a total of 556 samples) were evaluated at the same two U.S. sites, comparing Methods A, B, and D. The results are summarized in the tables below.

Method D vs. Method E, Manual to Manual Comparison

288 samples were assayed with the predicate device (Method E) and the new manual device mode (Method D) at three sites at 1:40 and 1:80 dilutions. The results were evaluated by two independent readers at each site, six readers total. Different numbers of each pattern were evaluated at the different sites per the table below.

The following samples were tested:

Definition	Total	Diagnosis	Total
Group 0: Healthy	30	Normal Controls	30
Group 1: Rheumatic Diseases associated with ANA positivity	104	Autoimmune Hepatitis	1
		CREST	1
		MCTD	3
		Myositis	12
		Scleroderma	10
		Sjoegren`s Syndrome	31
		SLE	40
		undiff. Collagenosis	6
Group 2: Other diseases not associated with ANA positivity	143	APS	5
		Celiac Disease	20
		Diverse I (fibromyalgia, type I diabetes, lumbargo)	5
		RA	60
		SpA	35
		Vasculitis	18
Group 3 Diverse samples I (selected due to ANA positivity or rare patterns)	11	Diverse II (unknown disease with rare ANA pattern)	11
Total	288		288

The results are summarized in the tables below:

Method D vs. E at 1:40 dilution, two readers per site:

% Agreement (95% CI)	Site A (D vs. E) n = 288	Site B (D vs. E) n = 288	Site C (D vs. E) n = 288	All Sites
Positive Agreement	97.5 (95.2–98.7)	98.1 (96–99.1)	98.1 (95.9–99.1)	97.9 (96.8–98.6)
Negative Agreement	96.1 (92.9–97.8)	96.9 (94–98.4)	96.9 (94–98.4)	96.6 (95.1– 97.7)
Overall Agreement	96.9 (95.1–98)	97.6 (96–98.5)	97.6 (96–98.5)	97.3 (96.5–98.0)

Total Pattern Agreement	97.2 (94.8-98.5)	96.9 (94.4-98.3)	97.2 (94.8-98.5)	97.1 (95.8-98.0)
Endpoint Titer Agreement	96.6 (94–98.1)	98.1 (96–99.1)	94.7 (91.7–96.7)	96.5 (95.1–97.5)

Method D vs. E at 1:80 dilution, two readers per site:

% Agreement (95% CI)	Site A (D vs. E) n = 288	Site B (D vs. E) n = 288	Site C (D vs. E) n = 288	All Sites
Positive	96.1	92.2	91.5	93.4

Agreement	(93.3–97.8)	(88.5–94.8)	(87.6–94.3)	(91.5–94.9)
Negative Agreement	95.5 (92.4–97.4)	94.9 (91.8–96.9)	91.8 (88.1–94.4)	94.0 (92.2–95.4)
Overall Agreement	95.8 (93.9–97.2)	93.6 (91.3–95.3)	91.7 (89.1–93.7)	93.7 (92.4–94.7)

Total Pattern Agreement	93.5 (90.2-95.7)	88.5 (84.6-91.5)	85.7 (81.5-89.1)	89.2 (87.1-91.0)
Endpoint Titer Agreement	96.9 (94.4–98.3)	98.4 (96.4–99.3)	95.3 (92.5–97.2)	96.9 (95.6–97.8)

Method D and E: Individual patterns identified at three sites at 1:40 dilution:

Patterns 1:40	Site A				Site B				Site C			
	Method D		Method E		Method D		Method E		Method D		Method E	
	n/556		n/566		n/566		n/288		n/288		n/288	
	OP 1	OP 2	OP 1	OP 2	OP 1	OP 2	OP 1	OP 2	OP 1	OP 2	OP 1	OP 2
	n	n	n	n	n	n	n	n	N	n	N	n
hom	74	74	75	75	74	75	40	39	40	40	40	39
spe	130	131	132	132	128	127	61	59	60	59	61	58
cen	35	35	35	35	34	35	10	11	10	11	10	11
nuc	30	30	30	30	29	29	6	6	6	6	6	6
num	7	6	7	7	7	7	6	6	6	6	6	5
nud	7	7	7	7	6	6	6	6	6	6	6	6
cyt	45	45	46	44	44	45	26	24	24	25	26	24
undef	20	19	20	20	20	19	5	6	6	6	6	6
neg	203	203	203	203	203	203	127	127	127	127	127	127
Total	556	556	556	556	556	556	288	288	288	288	288	288
False negative	5	6	1	3	11	10	1	4	3	2	0	6

Method D and E: Individual patterns identified across three sites at 1:80 dilution:

Patterns 1:80	Site A				Site B				Site C			
	Method D		Method E		Method D		Method E		Method D		Method E	
	n/556		n/556		n/556		n/288		n/288		n/288	
	OP 1	OP 2	OP 1	OP 2	OP 1	OP 2	OP 1	OP 2	OP 1	OP 2	OP 1	OP 2
	n	n	n	n	n	n	n	n	n	n	n	n
hom	73	71	71	69	66	70	36	33	36	33	35	32
spe	104	103	104	103	106	104	56	54	54	52	54	49
cen	35	35	35	35	33	35	10	11	9	11	10	10
nuc	29	29	29	29	26	27	5	4	3	4	5	4
num	6	6	6	6	5	5	5	4	4	5	4	3
nud	5	5	5	5	5	5	5	5	5	4	5	5
cyt	34	35	35	34	33	34	18	18	18	18	16	19
undef	11	10	10	10	9	9	4	5	5	5	5	5
neegative	259	259	259	259	297	297	143	143	143	143	143	143
Total	556	556	556	556	556	556	288	288	288	288	288	288
False negative	0	3	2	6	14	8	6	11	11	13	11	18

<u>Method A vs. Method B vs. Method D Method Comparison</u>**:**
The comparison was done by analyzing 556 clinical serum samples (288 samples from study above and 268 additional samples) at 1:40 and 1:80 screening dilutions at two different U.S. locations with two independent, blinded readers and one HELIOS instrument at each site. The samples are described in the table below:

Disease Group: Diagnosis	Total
Group 0: Healthy	
Normal Controls	80
Group 1: Rheumatic Diseases associated with ANA positivity	
Autoimmune Hepatitis	18
Calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia	4
Mixed Connective Tissue Disease	8
Myositis	35
Scleroderma	39
Sjögren's Syndrome	64
Systemic Lupus Erythematosus	90
Undifferentiated Collagenosis	6
Group 2: Other diseases not associated with ANA positivity	
Antiphospholipid Syndrome	5
Celiac Disease	20
Diverse I (Fibromyalgia, Type 1 Diabetes, and Lumbago)	5
Hepatitis B Virus	30
Hepatitis C Virus	28
Rheumatoid Arthritis	60
Spondyloarthritis	35
Vasculitis	18
Group 3: Diverse samples (selected due to ANA positivity or rare patterns)	
Diverse II (unknown disease with rare ANA pattern)	11
Total	556

The results are summarized in the tables below:

Methods A, B, D, Sites A, B, C, two readers per site total agreement

Method	Positive/negative total agreement 1:40 dilution		
	% Total Agreement (95% CI)		
	Site A (n = 556)	Site B (n = 556)	Site C (n = 288)
D vs. A	96.0 (94.6–97)	96.0 (94.6–97)	98.3 (96.8–99.1)
D vs. B	95.7 (94.5–96.6)	96.9 (95.8–97.8)	98.1 (96.6–98.9)
B vs. A	96.2 (94.9–97.2)	97.1 (96.0–98.0)	98.8 (97.5–99.4)
	Positive/negative total agreement 1:80 dilution		
D vs. A	93.6 (92.2–94.7)	95.7 (94.3–96.7)	93.1 (90.7–94.9)
D vs. B	95.2 (93.8–96.3)	95.4 (94–96.5)	93.6 (91.3–95.3)
B vs. A	97.7 (96.7–98.3)	95.4 (94.1–96.4)	96.7 (94.9–97.9)

Methods A, B, D, sites A, B, C at 1:40 Pattern Agreement

Method	Pattern agreement 1:40 dilution		
	% Total Agreement (95% CI)		
	Site A (n = 556)	Site B (n = 556)	Site C (n = 288)
D vs. A	94.2 (92.2–95.7)	92.6 (90.5–94.3)	97.5 (95.2–98.7)
D vs. B	96.0 (94.3–97.2)	94.8 (92.9–96.2)	97.5 (95.2–98.7)
B vs. A	95.0 (93.2–96.4)	94.1 (92.1–95.6)	98.8 (96.8–99.5)
	Pattern agreement 1:80 dilution		
D vs. A	93.6 (91.3–95.3)	91.8 (89.3–93.7)	91.7 (88–94.4)
D vs. B	95.5 (93.5–96.9)	92.4 (90–94.3)	90.7 (86.8–93.5)
B vs. A	96.1 (94.3–97.4)	92.1 (89.6–94)	95.9 (92.9–97.6)

Method A, B, D: Individual patterns identified for each site and each method at 1:40: compared to Method E:

Patterns 1:40 (n)	Method A		Method B				Method D			
	Site A	Site B	Site A		Site B		Site A		Site B	
	Instr. 1	Instr. 2	OP 1	OP 2	OP 1	OP 2	OP 1	OP 2	OP 1	OP 2
	n	n	n	n	n	n	n	n	n	n
hom (75)*	74	75	73	72	73	75	74	74	74	75
spe (132)	130	125	134	132	132	125	130	131	128	127
cen (35)	32	33	35	35	35	35	35	35	34	35
nuc (30)	26	26	27	27	27	27	30	30	29	29
num (7)	7	7	7	7	6	7	7	6	7	7
nud (7)	7	6	7	7	7	6	7	7	6	6
cyt (46)	45	50	43	43	47	41	45	45	44	45
undef (20)	19	18	19	19	17	20	20	19	20	19
neg (203)	203	203	203	203	203	203	203	203	203	203
Total (556)	556	556	556	556	556	556	556	556	556	556
False negative	13	13	8	11	9	17	5	6	11	10

*The number of samples associated with each pattern is the number detected by Method E (predicate)

Methods A, B, D: Individual patterns identified for each site and each method at 1:80

Patterns 1:80 (n)	Method A		Method B				Method D			
	Site A	Site B	Site A		Site B		Site A		Site B	
	Instr.1	Instr.2	OP 1	OP 2	OP 3	OP 4	OP 1	OP 2	OP 3	OP 4
	n	n	n	n	n	n	n	n	n	n
hom (75)*	72	77	71	71	75	74	73	71	66	70
spe (103)	101	97	105	103	102	98	104	103	106	104
cen (35)	34	31	36	35	35	36	35	35	33	35
nuc (30)	27	27	27	27	29	26	29	29	26	27
num (6)	5	6	5	5	6	6	6	6	5	5
nud (5)	5	5	5	5	5	5	5	5	5	5
cyt (35)	33	34	31	32	32	32	34	35	33	34
undef (10)	9	9	11	11	10	9	11	10	9	9
neg (259)	259	259	259	259	259	259	259	259	259	259

total (556)	556	556	556	556	556	556	556	556	556	556
False negative	11	11	6	8	3	11	0	3	14	8

*The number of samples associated with each pattern is the number detected by Method E (Predicate)

b. *Matrix comparison:*
Not applicable

3. Clinical studies:

To determine clinical sensitivity and specificity (Se/Sp), a cohort of 288 clinically characterized samples were tested at the three sites (A, B, and C). 268 additional samples were tested at sites A and B only. Sensitivity and Specificity of the assay was analyzed for each site with 460 samples (normal controls and "diverse" samples used in the method comparison study were excluded from this analysis). The number and distribution of the samples are shown below:

Samples (Site A and B):

Disease Group	Diagnosis	Total	Total
Group 1 Rheumatic Diseases associated with ANA positivity (CTD + AIL)	SLE	90	264
	Autoimmune Hepatitis	18	
	CREST	4	
	MCTD	8	
	Myositis	35	
	Scleroderma	39	
	Sjögren's Syndrome	64	
	undiff. Collagenosis	6	
Group 2 Other diseases not associated with ANA positivity	APS	5	196
	Celiac Disease	20	
	HBV	30	
	HCV	28	
	RA	60	
	SpA	35	
	Vasculitis	18	
Total		460	460

Samples (Site C).

Disease Group	Diagnosis	Total	Total
Group 1 Rheumatic Diseases associated with ANA positivity (CTD + AIL)	Autoimmune Hepatitis	1	104
	CREST	1	
	MCTD	3	
	Myositis	12	
	Scleroderma	10	
	Sjögren's Syndrome	31	
	SLE	40	
	Undiff. Collagenosis	6	
Group 2 Other diseases not associated with ANA positivity	APS	5	143
	Celiac Disease	20	
	Diverse	5	

	RA	60	
	SpA	35	
	Vasculitis	18	
Total		247	247

The results are summarized in the tables below:

Se/Sp Summary, Methods A, B, D and E at 1:40 dilution

Site OP	% Sensitivity at 1:40								% Specificity at 1:40			
	SLE				CTD+ AIL				Non-Healthy Controls			
	Method				Method				Method			
	A	B	D	E	A	B	D	E	A	B	D	E
A1	88.9	88.9	90.0	87.8	86.0	86.0	86.7	86.4	58.2	58.2	56.6	56.1
A2		88.9	88.9	87.8		85.6	86.0	86.0		59.2	54.1	56.6
B1	87.8	87.8	87.8	80.0	85.6	86.0	85.6	80.8	57.1	57.1	57.1	58.7
B2		88.9	87.8	80.0		85.2	85.6	80.8		56.6	55.6	59.4
C1	82.5	80.0	82.5	80.0	82.7	81.7	83.7	81.7	58.7	58.7	60.1	58.7
C2		80.0	80.0	80.0		81.7	81.7	78.9		59.4	60.9	60.9

Se/Sp Summary, Methods A,B, D and E at 1:80 dilution:

Site OP	% Sensitivity at 1:80								% Specificity at 1:80			
	SLE				CTD+AIL				Non-Healthy Controls			
	Method				Method				Method			
	A	B	D	E	A	B	D	E	A	B	D	E
A1	78.9	80.0	82.2	82.2	78.8	79.2	82.6	82.2	70.9	69.4	68.9	67.9
A2		80.0	83.3	82.2		78.8	82.2	81.4		68.9	67.9	70.9
B1	77.8	80.0	81.1	77.5	79.2	80.3	79.5	78.8	70.9	68.4	71.4	66.7
B2		75.6	81.1	80.0		77.7	79.2	78.8		68.9	70.9	67.4
C1	77.5	77.5	77.5	75.0	76.9	77.9	74.0	76.9	63.0	63.8	65.2	67.4
C2		77.5	70.0	72.5		79.8	77.9	78.9		65.2	68.8	65.9

Se/Sp Summary, Methods A, B, D and E, combined for sites A, B, C at 1:40 Dilution

Method	SLE	CTD + AIL	Non-Healthy Controls
	% Sensitivity (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)
A	86.4 (76.1–92.7)	84.8 (78.8–89.3)	58 (50.6–65.1)
B	85.7 (75.4–92.3)	84.4 (78.4–88.9)	58.2 (50.8–65.3)
D	86.2 (75.8–92.6)	84.9 (79–89.4)	57.4 (50–64.5)
E	82.6 (70–90.7)	82.4 (74–88.5)	58.4 (50.5–65.9)

Se/Sp Summary, Methods A, B, D and E, combined for sites A, B, C at 1:80 Dilution

Method	SLE	CTD + AIL	Non-Healthy Controls
	% Sensitivity (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)
A	78.1 (66.7–86.3)	78.3 (71.8–83.6)	68.3 (61.1–74.8)
B	78.4 (67.1–86.6)	78.9 (72.5–84.2)	67.4(60.1–74)
D	79.2 (68–87.3)	79.2 (72.8–84.5)	68.9 (61.6–75.3)
E	78.2 (65.1–87.4)	79.5 (70.9–86)	67.7 (60.0–74.6)

More detailed Se/Sp results are summarized in the tables below:

Se/Sp for Methods A and B at 1:40 Dilution:

1:40 Dilution		SLE	CTD + AIL	Non-Healthy Controls
Site	Operator	% Sensitivity (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)

Method A (HELIOS) (1:40)				
		n = 90	n = 264	n = 196
Site A	Inst.1	88.9 (80.7–93.9)	86 (81.3–89.7)	58.2 (51.2–64.8)
Site B	Instr. 2	87.8 (79.4–93)	85.6 (80.9–89.3)	57.1 (50.1–63.9)
		n = 40	n = 104	n = 143
Site C	Instr. 3	82.5 (68–91.3)	82.7 (74.3–88.8)	58.7 (50.4–66.6)

Method B (HELIOS + operator evaluation) (1:40)				
		n = 90	n = 264	n = 196
Site A	OP 1	88.9 (80.7–93.9)	86 (81.3–89.7)	58.2 (51.2–64.8)
	OP2	88.9 (80.7–93.9)	85.6 (80.9–89.3)	59.2 (52.2–65.8)
Site B	OP 3	87.8 (79.4–93)	86 (81.3–89.7)	57.1 (50.1–63.9)
	OP 4	88.9 (80.7–93.9)	85.2 (80.4–89)	56.6 (49.6–63.4)
		N = 40	N = 104	N = 143
Site C	OP 5	80 (65.2–89.5)	81.7 (73.2–88)	58.7 (50.4–66.6)
	OP 6	80 (65.2–89.5)	81.7 (73.2–88)	59.4 (51.1–67.3)

Se/Sp for Methods D and E at 1:40 Dilution:

1:40 Dilution		SLE	CTD + AIL	Non-Healthy Controls
Site	Operator	% Sensitivity (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)

Method D (manual) (1:40)				
		n = 90	n = 264	n = 196
Site A	OP 1	90 (82.1–94.6)	86.7 (82.1–90.3)	56.6 (49.6–63.4)
	OP2	88.9 (80.7–93.9)	86 (81.3–89.7)	54.1 (47.1–60.9)
Site B	OP 3	87.8 (79.4 - 93)	85.6 (80.9–89.3)	57.1 (50.1–63.9)
	OP 4	87.8 (79.4 - 93)	85.6 (80.9 - 89.3)	55.6 (48.6–62.4)
		n = 40	n = 104	n = 143
Site C	OP 5	82.5 (68–91.3)	83.7 (75.4–89.5)	60.1 (51.8–67.9)
	OP 6	80 (65.2–89.5)	81.7 (73.2–88)	60.9 (52.5–68.6)

Method E (manual predicate) 1:40				
		N = 90	N = 264	N = 196
Site A	OP 1	87.8 (79.4–93)	86.4 (81.7–90)	56.1 (49.1–62.9)
	OP 2	87.8 (79.4–93)	86 (81.3–89.7)	56.6 (49.6–63.4)
		n = 40	n = 104	n = 143
Site B	OP 3	80 (65.2–89.5)	80.8 (72.2–87.2)	58.7 (50.4–66.6)
	OP 4	80 (65.2–89.5)	80.8 (72.2–87.2)	59.4 (51.1–67.3)

Site C	OP 5	80 (65.2–89.5)	81.7 (73.2–88)	58.7 (50.4–66.6)
	OP 6	80 (65.2–89.5)	78.9 (63.7–88.9)	60.9 (52.5–68.6)

Se/Sp for Methods A and B at 1:80 Dilution:

1:80 Dilution		SLE	CTD + AIL	Non-Healthy Controls
Site	Operator	% Sensitivity (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)
Method A (1:80)				
		n = 90	n = 264	n = 196
Site A	Instr. 1	78.9 (69.4–86)	78.8 (73.5–83.3)	70.9 (64.2–76.8)
Site B	Instr. 2	77.8 (68.2–85.1)	79.2 (73.9–83.6)	70.9 (64.2–76.8)
		n = 40	n = 104	n = 143
Site C	Instr. 3	77.5 (62.5–87.7)	76.9 (68.0–84.0)	63 (54.7–70.6)
Method B (1:80)				
		n = 90	n = 264	n = 196
Site A	OP 1	80 (70.6–87)	79.2 (73.9–83.6)	69.4 (62.6–75.4)
	OP 2	80 (70.6–87)	78.8 (73.5–83.3)	68.9 (62.1–74.9)
Site B	OP 3	80 (70.6–87)	80.3 (75.1–84.7)	68.4 (61.6–74.5)
	OP 4	75.6 (65.8–83.3)	77.7 (72.3–82.3)	68.9 (62.1–74.9)
		n = 40	n = 104	n = 143
Site C	OP 5	77.5 (62.5–87.7)	77.9 (69–84.8)	63.8 (55.5–71.3)
	OP 6	77.5 (62.5–87.7)	79.8 (71.1–86.4)	65.2 (57–72.7)

Se/Sp for Methods D and E at 1:80 Dilution:

1:80 Dilution		SLE	CTD + AIL	Non-Healthy Controls
Site	Operator	% Sensitivity (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)
Method D (1:80)				
		n = 90	n = 264	n = 196
Site A	OP 1	82.2 (73.1–88.8)	82.6 (77.5–86.7)	68.9 (62.1–74.9)
	OP 2	83.3 (74.3–89.6)	82.2 (77.1–86.3)	67.9 (61–74)
Site B	OP 3	81.1 (71.8–87.9)	79.5 (74.3–84)	71.4 (64.7–77.3)
	OP 4	81.1 (71.8–87.9)	79.2 (73.9–83.6)	70.9 (64.2–76.8)
		n = 40	n = 104	n = 143
Site C	OP 5	77.5 (62.5–87.7)	74.0 (64.9–81.5)	65.2 (57–72.7)
	OP 6	70 (54.6–81.9)	77.9 (69–84.8)	68.8 (60.7–76)
Method E (1:80)				
		n = 90	n = 264	n = 196
Site A	OP 1	82.2 (73.1–88.8)	82.2 (77.1–86.3)	67.9 (61–74)
	OP 2	82.2 (73.1– 88.8)	81.4 (76.3–85.7)	70.9 (64.2–76.8)
		n = 40	n = 104	n = 143
Site B	OP 3	77.5 (62.5–87.7)	78.8 (70–85.6)	66.7 (58.4–74)
	OP 4	80 (65.2–89.5)	78.8 (70–85.6)	67.4 (59.2–74.6)
Site C	OP 5	75 (59.8–85.8)	76.9 (68–84)	67.4 (59.2–74.6)

	OP 6	72.5 (57.2–83.9)	78.9 (63.7–88.9)	65.9 (57.7–73.3)

Percent positive samples per site per diagnosis:

Site A (Methods A, B, D, and E) at 1:40 dilution

Site A 1:40		% Positive samples with diagnosis (n = 556)						
Disease Group / Diagnosis	N	Method A	Method B		Method D		Method E	
		HELIOS Instr. 1	OP 1	OP 2	OP 1	OP 2	OP1	OP 2
Group 0: Healthy controls								
Normal Controls	80	33%	36%	35%	34%	34%	35%	34%
Group 1: Rheumatic Diseases associated with ANA positivity								
CREST	4	100%	100%	100%	100%	100%	100%	100%
MCTD	8	100%	100%	100%	100%	100%	100%	100%
Myositis	35	69%	71%	71%	74%	71%	74%	71%
Scleroderma	39	90%	90%	90%	90%	90%	90%	90%
SLE	90	89%	89%	89%	90%	89%	88%	88%
undiff. Collagenosis	6	100%	100%	100%	100%	100%	100%	100%
Sjögren's Syndrome	64	84%	83%	83%	84%	84%	84%	84%
Autoimmune Hepatitis	18	89%	89%	83%	83%	83%	89%	89%
Group 2: Other diseases not associated with ANA positivity								
APS	5	60%	60%	60%	60%	60%	60%	60%
Celiac Disease	20	25%	25%	25%	25%	25%	25%	25%
RA	60	53%	50%	52%	50%	53%	50%	50%
SpA	35	40%	43%	37%	37%	40%	40%	37%
Vasculitis	18	22%	22%	22%	22%	28%	28%	22%
HCV	28	43%	46%	46%	43%	46%	39%	39%
HBV	30	40%	40%	37%	60%	60%	60%	63%
Diverse I	5	20%	20%	20%	20%	20%	20%	20%
Group 3: Diverse samples (selected due to ANA positivity or rare patterns)								
Diverse II	11	91%	100%	100%	100%	100%	100%	100%
Total	556	62%	63%	62%	63%	64%	64%	63%

Site B (Methods A, B, D, and E) at 1:40 dilution

Site B 1:40		% Positive samples with diagnosis						
Disease group/ Diagnosis	N	Method A	Method B		Method D		Method E	
		n = 556					n = 288	
		HELIOS Instr. 1	OP 3	OP 4	OP3	OP 4	OP 3	OP 4
Group 0: Healthy controls								
Normal Controls	80	28%	33%	25%	28%	28%	27%	23%
Group 1: Rheumatic Diseases associated with ANA positivity								

CREST	4	100%	100%	100%	100%	100%	100%	100%
MCTD	8	88%	88%	100%	100%	100%	100%	100%
Myositis	35	71%	71%	66%	69%	69%	67%	67%
Scleroderma	39	92%	92%	90%	90%	90%	100%	100%
SLE	90	88%	88%	89%	88%	88%	80%	80%
Undiff. Collagenosis	6	100%	100%	100%	100%	100%	100%	100%
Sjögren's Syndrome	64	84%	84%	84%	84%	84%	74%	74%
Autoimmune Hepatitis	18	83%	89%	83%	89%	89%	100%	100%
Group 2: Other diseases not associated with ANA positivity								
APS	5	60%	60%	60%	60%	60%	60%	40%
Celiac Disease	20	30%	25%	25%	25%	25%	25%	25%
RA	60	50%	52%	50%	48%	50%	52%	50%
SpA	35	40%	40%	40%	40%	37%	40%	40%
Vasculitis	18	22%	22%	33%	22%	28%	22%	28%
HCV	28	39%	39%	39%	39%	39%	n/a	n/a
HBV	30	53%	53%	53%	60%	67%	n/a	n/a
Diverse I	5	20%	20%	20%	20%	20%	20%	20%
Group 3: Diverse samples (selected due to ANA positivity or rare patterns)								
Diverse II	11	100%	91%	82%	100%	100%	100%	91%
Total	556	62%	63%	61%	62%	62%	56%	55%

Site C (Methods A, B, D, and E) at 1:40 dilution

Site C 1:40		% Positive samples with diagnosis (n = 288)						
Disease group / Diagnosis	n	Method A	Method B		Method D		Method E	
		HELIOS Instr. 1	Op 5	Op 6	Op 5	Op 6	Op 5	Op 6
Group 0: Healthy controls								
Normal Controls	30	27%	27%	27%	27%	27%	27%	23%
Group 1: Rheumatic Diseases associated with ANA positivity								
CREST	1	100%	100%	100%	100%	100%	100%	100%
MCTD	3	100%	100%	100%	100%	100%	100%	100%
Myositis	12	67%	67%	67%	67%	67%	67%	67%
Scleroderma	10	100%	100%	100%	100%	100%	100%	100%
SLE	40	83%	80%	80%	83%	80%	80%	80%
Undiff. Collagenosis	6	100%	100%	100%	100%	100%	100%	83%
Sjögren's Syndrome	31	77%	77%	77%	81%	77%	77%	74%
Autoimmune Hepatitis	1	100%	100%	100%	100%	100%	100%	100%
Group 2: Other diseases not associated with ANA positivity								
APS	5	60%	60%	60%	60%	60%	60%	40%
Celiac Disease	20	25%	25%	25%	25%	25%	25%	25%
RA	60	52%	52%	50%	52%	48%	52%	50%
SpA	35	40%	40%	40%	37%	37%	40%	37%

Vasculitis	18	22%	22%	22%	17%	22%	22%	22%
HCV	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
HBV	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Diverse I	5	20%	20%	20%	20%	20%	20%	20%
Group 3: Diverse samples (selected due to ANA positivity or rare patterns)								
Diverse II	11	100%	100%	82%	91%	100%	100%	100%
Total	288	57%	56%	55%	56%	55%	56%	54%

Site A (Methods A, B, D, and E) at 1:80 dilution

Site A 1:80		Percent positive samples (n = 556)						
Disease Group/ Diagnosis	n	Method A	Method B		Method D		Method E	
		HELIOS Instr. 1	Op 1	Op 2	Op 1	Op 2	Op 1	Op 2
Group 0: Healthy controls								
Normal Controls	80	23%	23%	20%	21%	25%	23%	21%
Group 1: Rheumatic Diseases associated with ANA positivity								
CREST	4	100%	100%	100%	100%	100%	100%	100%
MCTD	8	100%	100%	100%	100%	100%	100%	100%
Myositis	35	66%	63%	63%	66%	66%	66%	66%
Scleroderma	39	82%	82%	82%	90%	90%	87%	87%
SLE	90	79%	80%	80%	82%	83%	82%	82%
Undiff. Collagenosis	6	100%	100%	100%	100%	100%	100%	100%
Sjögren’s Syndrome	64	77%	78%	77%	83%	81%	83%	81%
Autoimmune Hepatitis	18	83%	83%	83%	83%	78%	83%	78%
Group 2: Other diseases not associated with ANA positivity								
APS	5	40%	60%	60%	60%	60%	60%	60%
Celiac Disease	20	20%	20%	20%	25%	25%	25%	25%
RA	60	45%	47%	47%	45%	48%	47%	40%
SpA	35	34%	34%	34%	34%	34%	37%	37%
Vasculitis	18	11%	17%	17%	17%	22%	22%	17%
HCV	28	14%	14%	14%	14%	11%	14%	11%
HBV	30	20%	20%	23%	23%	23%	20%	20%
Diverse I	5	20%	20%	20%	20%	20%	20%	20%
Group 3: Diverse samples (selected due to ANA positivity or rare patterns)								
Diverse II	11	73%	82%	73%	91%	82%	100%	73%
Total	556	53%	53%	53%	55%	56%	56%	54%

Site B (Methods A, B, D, and E) at 1:80 dilution

Site B 1:80		Percent positive samples						
Diagnosis	n	Method A	Method B		Method D		Method E	
		n = 556					n = 228	
		Instr. 1	Op 3	Op 4	Op 3	Op 4	Op 3	Op 4
Group 0: Healthy controls								
Normal Controls	80	18%	21%	21%	16%	20%	20%	13%
Group 1: Rheumatic Diseases associated with ANA positivity								
CREST	4	100%	100%	100%	100%	100%	100%	100%
MCTD	8	100%	100%	100%	100%	100%	100%	100%

Myositis	35	66%	66%	66%	57%	57%	67%	58%
Scleroderma	39	87%	87%	82%	90%	90%	100%	100%
SLE	90	78%	80%	76%	81%	81%	78%	80%
Undiff. Collagenosis	6	100%	100%	100%	100%	100%	100%	100%
Sjögren’s Syndrome	64	77%	78%	78%	78%	77%	71%	71%
Autoimmune Hepatitis	18	83%	83%	78%	78%	78%	100%	100%
Group 2: Other diseases not associated with ANA positivity								
APS	5	60%	60%	60%	40%	60%	60%	40%
Celiac Disease	20	20%	25%	20%	25%	20%	20%	20%
RA	60	43%	48%	47%	42%	45%	42%	42%
SpA	35	34%	34%	37%	34%	34%	34%	31%
Vasculitis	18	11%	17%	17%	11%	6%	11%	17%
HCV	28	14%	14%	14%	14%	14%	n/a	n/a
HBV	30	20%	20%	20%	20%	20%	n/a	n/a
Diverse I	5	20%	20%	20%	0%	20%	20%	20%
Group 3: Diverse samples (selected due to ANA positivity or rare patterns)								
Diverse II	11	82%	91%	82%	73%	73%	73%	64%
Total	556	52%	54%	53%	52%	52%	50%	48%

Site C (Methods A, B, D and E) at 1:80 dilution

Site C 1:80		% Positive Samples at 1:80 (n = 288)						
Disease Group/ Diagnosis	n	Method A	Method B		Method D		Method E	
		HELIOS Instr.	Op 5	Op 6	Op 5	Op 6	Op 5	Op 6
Group 0: Healthy controls								
Normal Controls	30	17%	23%	20%	13%	10%	17%	17%
Group 1: Rheumatic Diseases associated with ANA positivity								
CREST	1	100%	100%	100%	100%	100%	100%	100%
MCTD	3	100%	100%	100%	100%	100%	100%	100%
Myositis	12	58%	58%	67%	42%	67%	58%	50%
Scleroderma	10	100%	100%	100%	100%	100%	100%	100%
SLE	40	78%	78%	78%	78%	70%	75%	73%
Undiff. Collagenosis	6	100%	100%	100%	100%	100%	100%	67%
Sjögren’s Syndrome	31	68%	71%	74%	65%	77%	71%	61%
Autoimmune Hepatitis	1	100%	100%	100%	100%	100%	100%	100%
Group 2: Other diseases not associated with ANA positivity								
APS	5	60%	60%	60%	40%	60%	60%	40%
Celiac Disease	20	20%	20%	20%	25%	20%	20%	25%
RA	60	48%	47%	45%	45%	40%	45%	40%
SpA	35	34%	34%	34%	34%	29%	29%	34%
Vasculitis	18	17%	17%	11%	11%	11%	6%	22%
HCV	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
HBV	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Diverse I	5	20%	20%	20%	20%	20%	20%	20%
Group 3: Diverse samples (selected due to ANA positivity or rare patterns)								
Diverse II	11	82%	82%	82%	73%	73%	64%	73%

Total	288	51%	51%	51%	48%	47%	48%	47%

The discrepant samples in this study had lower end-point titers. The discrepancy seen when comparing the Se/Sp for 1:40 dilution vs. 1:80 dilution is due to the number of low positive (low titer) samples analyzed in this study. At 1:40 dilution, these sera were identified as positive while at 1:80 dilution, these sera were identified as negative. This impacts the sensitivity calculation. Additionally, there is a known inverse relationship between sensitivity and specificity (Lalkhen AG, et. al. 2008). Thus, at 1:80 dilution compared to 1:40 dilution, sensitivity decreases while specificity increases.

b. *Clinical specificity:*

See clinical sensitivity

4. Clinical cut-off:

See assay cut-off

5. Expected values/Reference range:

To determine the prevalence of ANA in the general population 151 sera from healthy controls were tested with AESKUSLIDES ANA HEp-2-Gamma. Each serum was tested at dilutions of 1:40 and 1:80. Slides were processed manually according to the IFU and subsequently analyzed by two independent readers (medical technologists) that manually evaluated the IFA results using a microscope. In a healthy population, there may be some percentage of the population with true positive ANAs. The number of positive ANA samples found with AESKUSLIDES ANA HEp-2-Gamma (Method D) in this reference range study correlate well with numbers reported in the literature. The results are summarized in the table below:

Reference Range Study AESKUSLIDES ANA HEp-2 Fc Gamma								
Sample type	1:40				1:80			
	Reader 1		Reader 2		Reader 1		Reader 2	
	n	%	n	%	n	%	n	%
Negative	109	71.7	104	68.4	120	78.9	119	78.3
Positive	43	28.3	48	31.6	32	21.1	33	21.7
* Densely Fine Speckled (DFS) 70	9	5.9	11	7.2	9	5.9	11	7.2
anti-mitochondrial antibodies (AMA)	11	7.2	12	7.9	6	3.9	6	3.9
Cytoplasmic	2	1.3	4	2.6	0	0.0	0	0.0
Speckled	12	7.9	12	7.9	11	7.2	8	5.3
Nucleolar	3	2.0	3	2.0	1	0.7	3	2.0
homogeneous	3	2.0	3	2.0	2	1.3	2	1.3
undefined positive	3	2.0	3	2.0	3	2.0	3	2.0
Total	152	100.0	152	100.0	152	100.0	152	100.0

*The significance of DFS is not clear at this time. Published studies show DFS 70 has no clinical significance. However, there is an ongoing discussion among the experts in ANA testing about the DFS 70 pattern and if it is a characteristic pattern for healthy individuals (Mahler et al.; 2016).

Of the 151 tested sera, there were 43 (28.5%) sera positive at a dilution of 1:40 and 32 (21.2%) at a dilution of 1:80. In comparison, Tan et al. (1997) found 31.7% ANA positives at 1:40 and 13.3% at 1:80 in a total of 125 normal sera.

N. Instrument Name:
HELIOS AUTOMATED IFA SYSTEM

O. System Descriptions:

Instrument (main components):

- HELIOS
- HELIOS Device Software
- HELIOS Pattern Recognition
- All in One PC
- Sample Racks
- Reagent Rack

The HELIOS AUTOMATED IFA SYSTEM is an automated system including a pipetting unit with microscope and software that acquires, interprets, stores and displays digital images of stained indirect immunofluorescence slides. The HELIOS DEVICE SOFTWARE is designed to support input of results from the AESKUSLIDES into electronic laboratory data management systems. The HELIOS AUTOMATED IFA SYSTEM should only be used with AESKUSLIDES assays that are cleared or approved for use on the instrument. All suggested results obtained with the HELIOS DEVICE SOFTWARE must be confirmed by trained operator.

Automated assay on the HELIOS instrument:
The workflow of the HELIOS AUTOMATED IFA SYSTEM is based on the manual procedure of indirect immunofluorescent antibody assays. This method entails all protocols and analyses are performed by HELIOS system. Each serum is tested at 1:40 or 1:80 dilutions. From these screening runs HELIOS classifies each image as positive or negative. In addition estimated end point titer and pattern are suggested.

1. Modes of Operation:

Mode	Description
HELIOS	automated processing with automated imaging and automated reading
HELIOS User Evaluation	automated processing with automated imaging and manual reading of the image (read of the digital image)
Manual Mode	manual processing with manual imaging and manual reading (read of microscope field)

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes ___X_____ or No ________

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes _______ or No __X______

2. Software:
   General:
   The HELIOS AUTOMATED IFA SYSTEM is an automated system for immunofluorescence processing with an integrated camera with an optic (microscope) and software for routine laboratory use by professional users under controlled environmental conditions.

   The software consists of three modules (pipetting, image capturing and analysis documentation) and a separate tool for estimating patterns. The pipetting sequences are specified with the corresponding volumes and incubation times, as are the pipetting positions and their mechanical paths. Digital images are taken by a camera and stored on the computer system. The separate software tool recognizes the pattern of the captured image by using SVM technique (Support Vector Machine). After image pre-processing, feature extraction and classification the software tool delivers the results of determination to the analysis documentation module. The software performs a positive/negative and pattern classification of the cells. The software suggests the result as a qualitative result (positive, negative) and, for positive samples, suggests a pattern and titer. All suggested results obtained with the software must be confirmed within the documentation module by trained operator.

   Device and Software Description:
   Software: Version 3.1 R2

   Level of Concern:
   Level of concern was determined according to the "Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices" dated May 11, 2005. Following the recommendations of this standard, the HELIOS software is assigned moderate level of concern.

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X____ or No ________

3. Specimen Identification:

Manual sample identification and/or Barcode

4. Specimen Sampling and Handling:

   Not applicable

5. Calibration:

   There is no calibration of the instrument by the user.

6. Quality Control:

   Positive and negative controls are supplied with the assay reagents.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

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