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

A. 510(k) Number:
K172348

B. Purpose for Submission:
New Device

C. Measurand:
native double helix DNA (dsDNA)

D. Type of Test:
Qualitative and/or semi-quantitative, indirect immunofluorescence

E. Applicant:
AESKU.Diagnostics GmbH & Co. KG

F. Proprietary and Established Names:
AESKUSLIDES® nDNA (*Crithidia luciliae*)

G. Regulatory Information:

1. Regulation section:
§CFR 866.5100 - Antinuclear antibody immunological test system

2. Classification:
Class II

3. Product code:
LSW, Anti-DNA Antibody, Antigen and Control

4. Clinical use
Immunology (82)

H. Intended Use:

1. Intended use(s):

   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(s) for use:

   Same as intended use

3. Special conditions for use statement(s):

   1. For prescription use only
   2. This device is only for use with reagents that are indicated for use with the device.
   3. The device is for use by a trained operator in a clinical laboratory setting.
   4. All software-aided results must be confirmed by the trained operator.
   5.For use only by manual microscopy or with HELIOS® AUTOMATED IFA SYSTEM.

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.

Standard Ref.	Description	Tests
53.100.US	nDNA (Crithidia luciliae) (10 wells)	100
53.100.US.Demo	nDNA (Crithidia luciliae) (10 wells) Demo kit	20
53.100.US.Bulk5	nDNA (Crithidia luciliae) (10 wells) bulk kit x5	500
53.100.US.Bulk10	nDNA (Crithidia luciliae) (10 wells) bulk kit x10	1000

Not provided in the kit:

Ref.	Reagent	Quantity / Volume		Description	Ready to use
EBIFA	Evans Blue 0.2%	1x	3ml	Capped white: Blue coloured solution Containing: PBS, Evans Blue. Dilute the Evans Blue 0.2% 1:3000 in 1x WBIFA	NO

HELIOS AUTOMATED IFA SYSTEM (k153117) or equivalent manual microscope.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   NOVA Lite dsDNA Crithidia luciliae

2. Predicate 510(k) number(s):
   K880742

3. Comparison with predicate device:

Table 6: comparison with predicate device

	Item	Predicate NOVA Lite dsDNA Crithidia luciliae	AESKUSLIDES® nDNA (Crithidia luciliae)
Similarities	Intended Use	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)	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.
	Methology	Immunofluoreszenz assay (IFA)	same as Predicate
	Procedure	Standard IFA technique	same as Predicate
	Results	qualitative and semi-quantitative titer	same as Predicate
	Samples Matrix	Serum	same as Predicate
	Analyte	dsDNA	same as Predicate
	Antigen	dsDNA Crithidia luciliae cells	same as Predicate
	Flurescence Marker	FITC	same as Predicate
	Controls	one positive control, one negative control	same as Predicate
	conjugate	anti-human IgG	same as Predicate
	Screening dilution	1:10	same as Predicate
	Storage	2 - 8 °C	same as Predicate
	Shelf life	24 months	same as Predicate
Differences	Slides	12 wells coated with antigen	10 wells coated with antigen
	Manual Interpretation of result	Manual fluorescense microscopy	Manual fluorescense microscopy or with HELIOS w/ trained operator verification
	Automated interpretation of results	N/A	HELIOS w/ trained operator verification

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

Table 7: List of Standards / Guidance Documents

#	Standards Title	Version
1	ISO 14971 – Medical Devices – Application of risk management to medical devices	Second Edition
2	IEC 62366 - Medical Devices - Part 1: Application Of Usability Engineering To Medical Devices [Including CORRIGENDUM 1 (2016)]	Edition 1.0
3	15223-1 – Medical Devices – Symbols To Be Used With Medical Device Labels, Labelling, and Information to be supplied – Part 1: General Requirements	Second Edition
4	Interference Testing in Clinical Chemistry	EP07-A2
5	Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures	EP17-A2
6	Evaluation of Stability of In Vitro Diagnostic Reagents	EP25-A
7	Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory	EP28-A3c
8	Evaluation of the Linearity of Quantitative Measurement Procedures A Statistical Approach	EP06-A
9	Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline-Fourth Edition	GP44-A4
10	Guidance for Industry and FDA Staff: Recommendations for Anti-Nuclear Antibody (ANA) Test System Premarket (510k) Submissions	-
11	Factors to Consider Regarding Benefit-Risk in Medical Device Product Availability, Compliance, and Enforcement Decisions	-
12	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 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.

Manual interpretation of test results

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).

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 fluorescen

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.

Qualitative evaluation

A serum dilution is considered negative for nDNA antibodies if the cells exhibit < 1+ fluorescence of the kinetoplast. Likewise, a serum dilution is considered positive 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 specific fluorescence of the kinetoplast is identifiable.

The titers are classified as:

- 1:10 are considered low titers
- 1:20 and 1:40 are considered medium titers
- 1:80 and greater are considered high titers

Automated instrument interpretation of test 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.

Intensity	Interpretation
+	nDNA positive
-	nDNA negative

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

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:

1. Analytical performance

During the study 4 different methods have been applied:

Method	Processing	Imaging	Reading/Evaluation od 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 - Predicate NOVA Lite dsDNA

Method D is the predicate for method C

In the clinical study the method comparison was:
Method D vs Method C
Method C vs. Method A, B
Method B vs Method A
Nomenclature and acronyms used in studies

Table 8: Nomenclature and acronyms

Nomenclature	Description/ Also known as
AESKUSLIDES nDNA	nDNA
HELIOS AUTOMATED IFA SYSTEM	HELIOS
Manual or Manual Reading	Results obtained by the operator after reading and interpreting the slides with a traditional fluorescence microscope
Method A	HELIOS + AESKUSLIDES nDNA w/slides read by HELIOS
Method B	HELIOS + AESKUSLIDES nDNA w/slides read by User
Method C	Manual AESKUSLIDES nDNA w/slides read by User
Method D	Manual Nova Lite dsDNA w/slides read by User
Negative IFA score/result, negative	neg, negative
Positive IFA score/result	pos, psoitive
Diseases Abbreviation	**Diseases Name**
SLE	Systemic lupus erythematosus
APS	Antiphospholipid Syndrome
MCTD	Mixed Connective Tissue Disease
UCTD	Undifferentiated connective tissue disease
SSc	Systemic sclerosis
PM	Polymyositis
DM	Dermatomyositis
RA	Rheumatoid Arthritis
AIH	Autoimmune Hepatitis
PBC	Primary biliary cholangitis
PSC	Primary sclerosing cholangitis
AAV	ANCA-associated vasculitides
HCV	Hepatitis C Virus
HBV	Hepatitis B Virus
EBV	Epstein–Barr virus

Description of study sample set:

776 Clinical samples have been used for the Clinical Evaluation and Method Comparisons studies described herein.

An exhaustive search for US Clinical Samples from 10 BioBanks (BioChain, BioReclamationIVT, Bioserve, ConversantBio, Cureline, DiscoveryLifeSciences, iSpecimen, Precision for Medicine, ProMedDx, and Vitrologic) has been performed and 746 Clinical Samples have been found. The samples have been selected according to their diagnosis, to reflect all important diagnosis for this study (see table). The sample set has been completed with 30 serum samples from a German University Hospital to complement some rare diagnosis (Vasculitis).

Table: 9 Serum samples set for clinical and method comparison studies

Study Sample Set			
Sample Definition		Diagnosis	n
Target Diagnosis	SLE	SLE	277
Differential Diagnosis	APS	APS	19
	Other rheumatic diseases	Mixed connective tissue disease (MCTD)	31
		Undifferentiated connective tissue disease (UCTD)	21
		Systemic sclerosis (SSc)	44
		Sjögren's syndrome	30
		Dermatomyositis (DM) and polymyositis (PM)	37
		Rheumatoid arthritis (RA)	58
	Autoimmune Liver Diseases	Autoimmune Hepatitis	21
		Primary biliary cholangitis (PBC)	20
		Primary sclerosing cholangitis (PSC)	10
	Vasculitis	AAVs	44*
	Fibromyalgia	Fibromyalgia	30
	Infections	HCV	44
		HBV	34
		EBV	25
	Leukemia	Lymphoma, Myeloma	31
Sum			776

*30samples from Germany

The diagnosis criteria of the different samples have been made in agreement with diagnostic standards used in the U.S and Germany. A written statement from different serum suppliers is available on request. Standard criteria are for example ACR criteria.

The US sample set was also selected to contain different ethnic groups (e.g. White, Black/Black African, Asian, Hispanic) to reflect the ethnic composition of the US population as good as possible.

The samples have been checked for purity and volume by visual inspection and for further contaminations by an in house prescreening before inclusion into the study. All 776 sera passed and were determined suitable for the study.

All serum samples which have been used for this studies have been shipped and stored at -20°C. Upon arrival from the Biobanks they were thawed once to aliquot them for the different studies. Subsequently, samples were frozen again. Therefore, samples underwent 2-3 freezing-thawing cycles before they were used in the studies.

To confirm that repeated thawing and freezing and also long term storage at -20°C has no influence on the samples we have performed the following two studies:

Serum Stability AESKUSLIDES nDNA (Crithidia luciliae)

Procedure:
Serum Stability was shown by testing 8 serum samples (7 positive, 1 negative). For each serum, one aliquot has been prepared that serves as control (no freeze-thawing). A second aliquot for each serum has been prepared that underwent 4 freeze-thaw cycles. Then, all samples and controls have been analyzed on AESKUSLIDES nDNA according to the IFU. Slides were read by two independent readers. Each freeze/thaw sample was compared to the respective control sample.

Sample ID	AESKUSLIDES nDNA	
	Result	Grading
1	Pos	low positive
2	Pos	low positive
3	Pos	low positive
4	Pos	medium positive
5	Pos	medium positive
6	Pos	high positive
7	Pos	high positive
8	Neg	Neg

Acceptance criteria:

- Pos/Neg/Overall Agreement: All positive sera have to be found positive and all negative sera have to be found negative.
- Overall Agreement: > 85%.
- FI is allowed to differ maximum + or - 1 from the expected value.
- FI Agreement: > 85%.

Results

- All samples fulfilled the above criteria. No differences were observed between sera thawed a single time and sera that had undergone 4 cycles of freeze/thawing:
- Positive/Negative/Overall Agreement was 100% for AESKUSLIDES nDNA. All positive samples have been found positive, all negative samples have been found negative.
- FI agreement was 100% for AESKUSLIDES nDNA. No deviations of fluorescence intensities greater than +/-1 from the initial value have been observed.

Raw data are in Attachment 10_Section 12_Raw data serum stability

Conclusions
All criteria are fulfilled in each test. The results show that multiple freeze-thaw cycles have no effect on test results.

Long Term Serum Stability AESKUSLIDES nDNA (Crithidia luciliae)

To show that long term storage of serum samples at -20°C has no effect on performance and results of AESKUSLIDES nDNA.

Procedure:
Seven different serum samples (4 positive and 3 negative) have been aliquoted and stored at -20°C for a time period of at least 14 months. Frozen aliquots of the different sera have been thawed and assayed on AESKUSLIDES nDNA at the indicated time points. All tests have been performed manually according to the IFU and subsequently analyzed at the microscope

Sample ID	Expected Result	
	Pos/Neg	FI
Sample 1	Pos	3
Sample 2	Pos	2
Sample 3	Pos	4
Sample 4	Neg	0
Sample 5	Neg	0
Sample 6	Neg	0
Sample 7	Pos	2

Acceptance Criteria:
Positive sera have to be found positive and negative sera have to be found negative throughout the whole testing period. The fluorescence intensity (FI) is allowed to differ maximum +/- 1 level from the expected value at each test time point.

Results:
Positive samples have been found positive, negative samples have been found negative at all test time points. FI did not differ more than +/- 1 level from the expected values.

Table 10: Results long term serum stability

Sample ID	month 0		month 4		month 8		month 10		month 12		month 14	
	Pos/Neg	FI	Pos/Neg	FI	Pos/Neg	FI	Pos/Neg	FI	Pos/Neg	FI	Pos/Neg	FI
Sample 1	Pos	3	Pos	3	Pos	4	Pos	3	Pos	3	Pos	3
Sample 2	Pos	2	Pos	2	Pos	2	Pos	2	Pos	1	Pos	2
Sample 3	Pos	4	Pos	3	Pos	4	Pos	3	Pos	4	Pos	3
Sample 4	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0
Sample 5	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0
Sample 6	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0
Sample 7	Pos	2	Pos	2	Pos	2	Pos	2	Pos	2	Pos	2

Conclusion:
All acceptance criteria have been fulfilled for the tested sera. The results show that long term storage of sera at -20°C over a time period of at least 14 months has no effect on performance and test results of AESKUSLIDES nDNA.

a) Precision/Reproducibilty

Within-Lab Precision AESKUSLIDES nDNA (Crithida luciliae)
To assess Within-Lab Precision of AESKUSLIDES nDNA (Crithidia luciliae) processed on HELIOS (Method A – HELIOS suggestions and Method B – Reader Confirmation of HELIOS images) and processed manually (Method C), based on CLSI Guideline EP12-A2.

Sample Set
11 samples were assayed on AESKUSLIDES nDNA. The sample set includes borderline, low, medium, high positive, and negative samples. The samples are characterized in the following table:

Table 11: Sample table for Within-Lab Precision

Sample ID	AESKUSLIDES nDNA	
	Result	Grading
1	Pos	borderline
2	Pos	low positive
3	Pos	low positive
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

Procedure

11 samples have been tested on five days, two runs per day, three replicates per sample per run, resulting in 30 data points for each sample.

This Within-lab precision study was done for three different Methods:

Method A (HELIOS)	Processing of slides and image recording at HELIOS + HELIOS result suggestion
Method B (Reader Confirmation)	HELIOS images recorded in Method A + result analysis by two independent readers
Method C (Manual)	Manual processing of slides and result analysis at the microscope by two independent readers

All runs have been performed according to the respective IFUs. 1 positive and 1 negative control (kit controls) were included in each run. Samples were tested at 1:10 dilution. Results were analyzed by two independent readers.

Data Analysis

Positive/Negative Classification was recorded for each sample in each run and for each Method. Pos/Neg Classification in Method A (HELIOS) is provided by the nDNA Pattern Plus software tool.

For Method C (Manual performance) fluorescence intensity was also reported.

As an estimate of the imprecision of the three methods A, B and C the percentages of positive and negative results will be calculated for each sample:

- % Positive (number of positive calls divided by the total number of data points for each sample)
- % Negative (number of negative calls divided by the total number of data points per sample)

Results of the two readers (Method B/C) will be calculated and displayed separately as well as combined.

Acceptance Criteria

- Positive sera have to be found positive, and negative sera have to be found negative*.
- Reported FI is allowed to differ max. ± 1 level within the study

* borderline samples are samples with analyte concentration near the cutoff. These samples are very low positive. They can be evaluated also negative in certain cases, depending on the reader, the subjective manner of result analysis and normal test variances.

For Method A we accept lower percentage of positive/negative results for positive and negative samples, respectively, because we state in the IFU that all results generated by the HELIOS (Method A) must be confirmed by trained personnel.

Results

The results are presented in % Positive and % Negative tables of each sample. Percentages were calculated for the two readers separately (30 replicates for each sample) and for both readers combined (60 data points per sample).

Raw Data can be found in 2_Report Within-Lab Precision_re-evaluation_nDNA

Table 12: Results – Within Lab Precision AESKUSLIDES nDNA - Method A, B, and C – number of positive and negative results

Sample ID	N	Method A		Method B				Method C			
		n Neg	n Pos	n Neg		n Pos		n Neg		n Pos	
				Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2
S1	30	30	0	30	6	0	24	4	5	26	25
S2	30	10	20	0	0	30	30	0	0	30	30
S3	30	17	13	0	3	30	27	0	0	30	30
S4	30	14	16	4	1	26	29	0	0	30	30
S5	30	0	30	0	0	30	30	0	0	30	30
S6	30	2	28	0	0	30	30	0	0	30	30
S7	30	0	30	0	0	30	30	0	0	30	30
S8	30	0	30	0	0	30	30	0	0	30	30
S9	30	1	29	0	0	30	30	0	0	30	30
S10	30	25	5	30	30	0	0	29	30	1	0
S11	30	21	9	30	30	0	0	30	30	0	0

Table 13: Results – Within Lab Precision AESKUSLIDES nDNA – Method A, B and C – percentages of positive and negative results

Sample ID	N	Method A		Method B				Method C			
		% Negative	% Positive	% Negative		% Positive		% Negative		% Positive	
				Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2
S1	30	100	0	100	20.0	0	80.0	13.3	16.7	86.7	83.3
S2	30	33.3	66.7	0	0	100	100	0	0	100	100
S3	30	56.7	43.3	0	10	100	90	0	0	100	100
S4	30	46.7	53.3	13.3	3.3	86.7	96.7	0	0	100	100
S5	30	0	100	0	0	100	100	0	0	100	100
S6	30	6.7	93.3	0	0	100	100	0	0	100	100
S7	30	0	100	0	0	100	100	0	0	100	100
S8	30	0	100	0	0	100	100	0	0	100	100
S9	30	3.3	96.7	0	0	100	100	0	0	100	100
S10	30	83.3	16.7	100	100	0	0	96.7	100	3.3	0
S11	30	70.0	30.0	100	100	0	0	100	100	0	0

Table 14: Results – Within Lab Precision AESKUSLIDES nDNA – Method A, B and C – percentages of positive and negative results for both readers combined

Sample ID	N (Method A)	Method A		N (Method B/C)	Method B		Method C	
		% Negative	% Positive		% Negative	% Positive	% Negative	% Positive
S1	30	100	0	60	60.0	40.0	15.0	85.0
S2	30	33.3	66.7	60	0	100	0	100
S3	30	56.7	43.3	60	5.0	95.0	0	100
S4	30	46.7	53.3	60	8.3	91.7	0	100
S5	30	0	100	60	0	100	0	100
S6	30	6.7	93.3	60	0	100	0	100
S7	30	0	100	60	0	100	0	100
S8	30	0	100	60	0	100	0	100
S9	30	3.3	96.7	60	0	100	0	100
S10	30	83.3	16.7	60	100	0	98.3	1.7
S11	30	70.0	30.0	60	100	0	100	0

For manual testing (Method C) fluorescence intensities differed at maximum ±1 level within one run and between the runs.

Conclusion

All pre-determined acceptance criteria were met.

Between-Lab Precision AESKUSLIDES nDNA (Crithida luciliae)
To assess Between-Lab Precision of AESKUSLIDES nDNA (Crithidia luciliae) processed on HELIOS (Method A – HELIOS suggestions and Method B – Reader Confirmation of HELIOS images) and processed manually (Method C).

Sample Set

11 samples were assayed on AESKUSLIDES nDNA. The sample set includes borderline, low, medium, high positive, and negative samples. The samples are characterized in the following table:

Table 15: Sample table for Precision study

Sample ID	AESKUSLIDES nDNA		
	Result	Grading	Fluorescence Intensity (FI)
1	Pos	borderline positive	1
2	Pos	low positive	2
3	Pos	borderline positive	1
4	Pos	medium positive	3
5	Pos	medium positive	3
6	Pos	medium positive	3
7	Pos	high positive	4
8	Pos	high positive	4
9	Pos	high positive	4
10	Neg	negative	0
11	Neg	negative	0

Procedure

11 samples have been tested on five days, two runs per day, three replicates per sample per run, on three different study sites. Two study sites were in the US, one study site was in Germany, with one HELIOS device per study site. Results were analyzed by two different readers per study site, resulting in a total of 90 data points per sample per reader.

This Between-lab precision study was done for three different Methods:

Method A (HELIOS)	Processing of slides and image recording at HELIOS + HELIOS result suggestion
Method B (Reader Confirmation)	HELIOS images recorded in Method A + result analysis by two independent readers
Method C (Manual)	Manual processing of slides and result analysis at the microscope by two independent readers

All runs have been performed according to the respective IFUs. 1 positive and 1 negative control (kit controls) were included in each run. Samples were tested at 1:10 dilution. Results were analyzed by two independent readers.

Data Analysis

Positive/Negative Classification was recorded for each sample in each run and for each Method. Pos/Neg Classification in Method A (HELIOS) is provided by the nDNA Pattern Plus software tool.

For Method C (Manual performance) fluorescence intensity was also reported.

The following % Agreements including 95% CI (confidence intervals) will be calculated for each Method A, B and C:

- Positive % Agreement (across all positive samples: number of correctly found samples divided through number of total positive samples)
- Negative % Agreement (across all negative samples: number of correctly found samples divided through number of total negative samples)
- Overall % Agreement (across all positive and negative samples: number of correctly found samples divided through number of total samples)
- Fluorescence Intensity % Agreement (across all samples, only for Method C: number of correctly found samples divided through number of total samples)

Results of the two readers will be calculated separately as well as combined.

Acceptance Criteria

- Positive sera have to be found positive, and negative sera have to be found negative.
- Reported FI is allowed to differ max. ± 1 level from the expected value.
- Agreements should meet the following criteria:

Table 16: Acceptance criteria for between-lab precision study

Type of Agreement (borderline positive samples excluded*)	Method A	Method B	Method C
Positive Agreement	> 70%	> 90%	> 90%
Negative Agreement	> 70%	> 90%	> 90%
Overall Agreement	> 70%	> 90%	> 90%
Fluorescence Intensity Agreement	---	---	> 90%

* borderline samples are very low positive samples that can be evaluated also negative in certain cases, depending on the reader, the subjective manner of result analysis and normal test variances.

For Method A we accept a lower agreement (>70%), because we state in the IFU that all results generated by the HELIOS (Method A) must be confirmed by trained personnel.

From the results of those study, the following will be calculated:

1. Between-Lab Precisions, 2. Between-Operator Precisions, 3. Single-Operator Precisions, and 4. Instrument Precision

Results

Results are presented in the tables below. Agreements were calculated 1. including the results of the two borderline positive samples, 2. excluding the results of those two samples.
All predetermined acceptance criteria were met.

Between-Site Agreement

Manual (Method C) Between-Site Agreement (Calculation including borderline samples):

Table 17: Between-Site Agreement (Calculation including borderline samples) Number of Correctly/Incorrectly found samples Method C

Number of Samples	Site 1 vs Site 2			Site 2 vs Site 3			Site 1 vs Site 3			All Sites		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	1071	9	1080	1069	11	1080	1060	20	1080	1600	20	1620
Negative Agreement	239	1	240	240	0	240	239	1	240	359	1	360
Overall Agreement	1310	10	1320	1309	11	1320	1299	21	1320	1959	21	1980
FI Agreement	1303	17	1320	1289	31	1320	1286	34	1320	1939	41	1980

Table 18: Between-Site Agreement (Calculation including borderline samples) % Agreements (95% CI) Method C

% Agreement (95% CI)	Site 1 vs Site 2	Site 2 vs Site 3	Site 1 vs Site 3	All Sites
Positive Agreement	99.2 (98.4 - 99.6)	99 (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)	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)

Manual (Method C) Between-Site Agreement (Calculation excluding borderline samples):

Table 19: Between-Site Agreement (Calculation excluding borderline samples) Number of Correctly/Incorrectly found samples Method C

Number of Samples	Site 1 vs Site 2			Site 2 vs Site 3			Site 1 vs Site 3			All Sites		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	840	0	840	834	6	840	834	6	840	1254	6	1260
Negative Agreement	239	1	240	240	0	240	239	1	240	359	1	360
Overall Agreement	1079	1	1080	1074	6	1080	1073	7	1080	1613	7	1620
FI Agreement	1063	17	1080	1049	31	1080	1046	34	1080	1579	41	1620

Table 20: Between-Site Agreement (Calculation excluding borderline samples) % Agreements (95% CI) Method C

% Agreement (95% CI)	Site 1 vs Site 2	Site 2 vs Site 3	Site 1 vs Site 3	All Sites
Positive Agreement	100 (99.5 - 100)	99.3 (98.5 - 99.7)	99.3 (98.5 - 99.7)	99.5 (99 - 99.8)
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	99.9 (99.5 - 100)	99.4 (98.8 - 99.7)	99.4 (98.7 - 99.7)	99.6 (99.1 - 99.8)
FI Agreement	98.4 (97.5 - 99)	97.1 (96 - 98)	96.9 (95.6 - 97.7)	97.5 (96.6 - 98.1)

Reader Confirmation (Method B) Between-Site Agreement (Calculation including borderline samples):

Table 21: Between-Site Agreement (Calculation including borderline samples) Number of Correctly/Incorrectly found samples Method B

Number of Samples	Site 1 vs Site 2			Site 2 vs Site 3			Site 1 vs Site 3			All Sites		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	995	85	1080	912	168	1080	909	171	1080	1408	212	1620
Negative Agreement	238	2	240	238	2	240	240	0	240	358	2	360
Overall Agreement	1233	87	1320	1150	170	1320	1149	171	1320	1766	214	1980
FI Agreement	na	na	na	na	na	na	na	na	na	na	na	na

Table 22: Between-Site Agreement (Calculation including borderline samples) % Agreements (95% CI) 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 - 88.5)
Negative Agreement	99.2 (97 - 99.8)	99.2 (97 - 99.8)	100 (98.4 - 100)	99.4 (98 - 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	na	na	na	na

Reader Confirmation (Method B) Between-Site Agreement (Calculation excluding borderline samples):

Table 23: Between-Site Agreement (Calculation excluding borderline samples) Number of Correctly/Incorrectly found samples Method B

Number of Samples	Site 1 vs Site 2			Site 2 vs Site 3			Site 1 vs Site 3			All Sites		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	833	7	840	818	22	840	815	25	840	1233	27	1260
Negative Agreement	238	2	240	238	2	240	240	0	240	358	2	360
Overall Agreement	1071	9	1080	1056	24	1080	1055	25	1080	1591	29	1620
FI Agreement	na	na	na	na	na	na	na	na	na	na	na	na

Table 24: Between-Site Agreement (Calculation excluding borderline samples) % Agreements (95% CI) Method B

% Agreement (95% CI)	Site 1 vs Site 2	Site 2 vs Site 3	Site 1 vs Site 3	All Sites
Positive Agreement	99.2 (98.3 - 99.6)	97.4 (96.1 - 98.3)	97 (95.6 - 98)	97.9 (96.9 - 98.5)
Negative Agreement	99.2 (97 - 99.8)	99.2 (97 - 99.8)	100 (98.4 - 100)	99.4 (98 - 99.8)
Overall Agreement	99.2 (98.4 - 99.6)	97.8 (96.7 - 98.5)	97.7 (96.6 - 98.4)	98.2 (97.4 - 98.8)
FI Agreement	na	na	na	na

HELIOS (Method A) Between-Site Agreement (Calclation including borderline samples):

Table 25: Between-Site Agreement (Calculation including borderline samples) Number of Correctly/Incorrectly found samples Method A

Number of Samples	Site 1 vs Site 2			Site 2 vs Site 3			Site 1 vs Site 3			All Sites		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	341	199	540	282	258	540	321	219	540	472	338	810
Negative Agreement	105	15	120	114	6	120	105	15	120	162	18	180
Overall Agreement	446	214	660	396	264	660	426	234	660	634	356	990
FI Agreement	na	na	na	na	na	na	na	na	na	na	na	na

Table 26: Between-Site Agreement (Calculation including borderline samples) % Agreements (95% CI) 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 - 67.1)	52.2 (48 - 56.4)	59.4 (55.3 - 63.5)	58.3 (54.8 - 61.6)
Negative Agreement	87.5 (80.4 - 92.3)	95 (89.5 - 97.7)	87.5 (80.4 - 92.3)	90 (84.7 - 93.6)
Overall Agreement	67.6 (63.9 - 71)	60 (56.2 - 63.7)	64.5 (60.8 - 68.1)	64 (61 - 67)
FI Agreement	na	na	na	na

HELIOS (Method A) Between-Site Agreement (Calclation excluding borderline samples):

Table 27: Between-Site Agreement (Calculation excluding borderline samples) Number of Correctly/Incorrectly found samples Method A

Number of Samples	Site 1 vs Site 2			Site 2 vs Site 3			Site 1 vs Site 3			All Sites		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	328	92	420	278	142	420	310	110	420	458	172	630
Negative Agreement	105	15	120	114	6	120	105	15	120	162	18	180
Overall Agreement	433	107	540	392	148	540	415	125	540	620	190	810
FI Agreement	na	na	na	na	na	na	na	na	na	na	na	na

Table 28: Between-Site Agreement (Calculation excluding borderline samples) % Agreements (95% CI) Method A

% Agreement (95% CI)	Site 1 vs Site 2	Site 2 vs Site 3	Site 1 vs Site 3	All Sites
Positive Agreement	78.1 (73.9 - 81.8)	66.2 (61.5 - 70.5)	73.8 (69.4 - 77.8)	72.7 (69.1 - 76)
Negative Agreement	87.5 (80.4 - 92.3)	95 (89.5 - 97.7)	87.5 (80.4 - 92.3)	90 (84.7 - 93.6)
Overall Agreement	80.2 (76.6 - 83.3)	72.6 (68.7 - 76.2)	76.9 (73.1 - 80.2)	76.5 (73.5 - 79.3)
FI Agreement	na	na	na	na

All acceptance critieria were met for Method C (Manual) and Method B (Reader Confirmation). All agreements were >90%. For Method A (HELIOS suggestion) acceptance criteria were met for negative and overall agreement for all study sites (>70%). Positive agreement for Method A was also >70% for site to site precision of site 1 vs site 2 and site 1 vs 3, but not for site 2 vs site 3, where a slightly lower agreement could be observed (66.2%). However, positive agreement met acceptance criteria when calculated across all three sites (72.7%).

Overall-Between-Lab agreements ranged from 72.6% to 80.2% for Method A (HELIOS), from 97.7% to 99.2% for Method B (Reader Confirmation), and from 96.9% to 98.4% for Method C (Manual).

Between-Operator Agreement

Manual (Method C) Between-Operator Agreement (Calculation including borderline samples):

Table 29: Between-Operator Agreement (Calculation including borderline samples) Number of Correctly/Incorrectly found samples Method C

Number of Samples	Site 1			Site 2			Site 3		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	531	9	540	540	0	540	529	11	540
Negative Agreement	119	1	120	120	0	120	120	0	120
Overall Agreement	650	10	660	660	0	660	649	11	660
FI Agreement	650	10	660	653	7	660	636	24	660

Table 30: Between-Operator Agreement (Calculation including borderline samples) % Agreements (95% CI) 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)

Manual (Method C) Between-Operator Agreement (Calculation excluding borderline samples):

Table 31: Between-Operator Agreement (Calculation excluding borderline samples) Number of Correctly/Incorrectly found samples Method C

Number of Samples	Site 1			Site 2			Site 3		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	420	0	420	420	0	420	414	6	420
Negative Agreement	119	1	120	120	0	120	120	0	120
Overall Agreement	539	1	540	540	0	540	534	6	540
FI Agreement	530	10	540	533	7	540	516	24	540

Table 32: Between-Operator Agreement (Calculation excluding borderline samples) % Agreements (95% CI) Method C

% Agreement (95% CI)	Site 1	Site 2	Site 3
Positive Agreement	100 (99.1 - 100)	100 (99.1 - 100)	98.6 (96.9 - 99.3)
Negative Agreement	99.2 (95.4 - 99.9)	100 (96.9 - 100)	100 (96.9 - 100)
Overall Agreement	99.8 (99 - 100)	100 (99.3 - 100)	98.9 (97.6 - 99.5)
FI Agreement	98.1 (96.6 - 99)	98.7 (97.3 - 99.4)	95.6 (93.5 - 97)

Reader Confirmation (Method B) Between-Operator Agreement (Calculation including borderline samples):

Table 33: Between-Operator Agreement (Calculation including borderline samples) Number of Correctly/Incorrectly found samples Method B

Number of Samples	Site 1			Site 2			Site 3		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	496	44	540	499	41	540	413	127	540
Negative Agreement	120	0	120	118	2	120	120	0	120
Overall Agreement	616	44	660	617	43	660	533	127	660
FI Agreement	na	na	na	na	na	na	na	na	na

Table 34: Between-Operator Agreement (Calculation including borderline samples) % Agreements (95% CI) 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)	93.5 (91.3 - 95.1)	80.8 (77.6 - 83.6)
FI Agreement	na	na	na

Reader Confirmation (Method B) Between-Operator Agreement (Calculation excluding borderline samples):

Table 35: Between-Operator Agreement (Calculation excluding borderline samples) Number of Correctly/Incorrectly found samples Method B

Number of Samples	Site 1			Site 2			Site 3		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	415	5	420	418	2	420	400	20	420
Negative Agreement	120	0	120	118	2	120	120	0	120
Overall Agreement	535	5	540	536	4	540	520	20	540
FI Agreement	na	na	na	na	na	na	na	na	na

Table 36: Between-Operator Agreement (Calculation excluding borderline samples) % Agreements (95% CI) Method B

% Agreement (95% CI)	Site 1	Site 2	Site 3
Positive Agreement	98.8 (97.2 - 99.5)	99.5 (98.3 - 99.9)	95.2 (92.8 - 96.9)
Negative Agreement	100 (96.9 - 100)	98.3 (94.1 - 99.5)	100 (96.9 - 100)
Overall Agreement	99.1 (97.9 - 99.6)	99.3 (98.1 - 99.7)	96.3 (94.3 - 97.6)
FI Agreement	na	na	na

Overall-Between-Operator agreements ranged from 96.3% to 99.3% for Method B (Reader Confirmation), and from 98.9% to 100% for Method C (Manual).

Single-Operator Agreement

Manual (Method C) Single-Operator Agreement (Calculation including borderline samples):

Table 37: Single-Operator Agreement (Calculation including borderline samples) Number of Correctly/Incorrectly found samples Method C

Number of Samples	Site 1						Site 2						Site 3					
	Reader 1			Reader 2			Reader 3			Reader 4			Reader 5			Reader 6		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	266	4	270	265	5	270	270	0	270	270	0	270	266	4	270	263	7	270
Negative Agreement	59	1	60	60	0	60	60	0	60	60	0	60	60	0	60	60	0	60
Overall Agreement	325	5	330	325	5	330	330	0	330	330	0	330	326	4	330	323	7	330
FI Agreement	329	1	330	321	9	330	329	1	330	324	6	330	322	8	330	314	16	330

Table 38: Single-Operator Agreement (Calculation including borderline samples) % Agreements (95% CI) 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 - 100)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)	100 (94 - 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)

Manual (Method C) Single-Operator Agreement (Calculation excluding borderline samples):

Table 39: Single-Operator Agreement (Calculation excluding borderline samples) Number of Correctly/Incorrectly found samples Method C

Number of Samples	Site 1						Site 2						Site 3					
	Reader 1			Reader 2			Reader 3			Reader 4			Reader 5			Reader 6		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	210	0	210	210	0	210	210	0	210	210	0	210	209	1	210	205	5	210
Negative Agreement	59	1	60	60	0	60	60	0	60	60	0	60	60	0	60	60	0	60
Overall Agreement	269	1	270	270	0	270	270	0	270	270	0	270	269	1	270	265	5	270
FI Agreement	269	1	270	261	9	270	269	1	270	264	6	270	262	8	270	254	16	270

Table 40: Single-Operator Agreement (Calculation excluding borderline samples) % Agreements (95% CI) 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	100 (98.2 - 100)	100 (98.2 - 100)	100 (98.2 - 100)	100 (98.2 - 100)	99.5 (97.4 - 99.9)	97.6 (94.5 - 99)
Negative Agreement	98.3 (91.1 - 99.7)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)
Overall Agreement	99.6 (97.9 - 99.9)	100 (98.6 - 100)	100 (98.6 - 100)	100 (98.6 - 100)	99.6 (97.9 - 99.9)	98.1 (95.7 - 99.2)
FI Agreement	99.6 (97.9 - 99.9)	96.7 (93.8 - 98.2)	99.6 (97.9 - 99.9)	97.8 (95.2 - 99)	97 (94.3 - 98.5)	94.1 (90.6 - 96.3)

Reader Confirmation (Method B) Single-Operator Agreement (Calculation including borderline samples):

Table 41: Single-Operator Agreement (Calculation including borderline samples) Number of Correctly/Incorrectly found samples Method B

Number of Samples	Site 1						Site 2						Site 3					
	Reader 1			Reader 2			Reader 3			Reader 4			Reader 5			Reader 6		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	236	34	270	260	10	270	257	13	270	242	28	270	209	61	270	204	66	270
Negative Agreement	60	0	60	60	0	60	60	0	60	58	2	60	60	0	60	60	0	60
Overall Agreement	296	34	330	320	10	330	317	13	330	300	30	330	269	61	330	264	66	330
FI Agreement	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na

Table 42: Single-Operator Agreement (Calculation including borderline samples) % Agreements (95% CI) 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)	95.2 (91.9 - 97.2)	89.6 (85.4 - 92.7)	77.4 (72.1 - 82)	75.6 (70.1 - 80.3)
Negative Agreement	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)	96.7 (88.6 - 99.1)	100 (94 - 100)	100 (94 - 100)
Overall Agreement	89.7 (85.9 - 92.5)	97 (94.5 - 98.3)	96.1 (93.4 - 97.7)	90.9 (87.3 - 93.6)	81.5 (77 - 85.3)	80 (75.4 - 84)
FI Agreement	na	na	na	na	na	na

Reader Confirmation (Method B) Single-Operator Agreement (Calculation excluding borderline samples):

Table 43: Single-Operator Agreement (Calculation excluding borderline samples) Number of Correctly/Incorrectly found samples Method B

Number of Samples	Site 1						Site 2						Site 3					
	Reader 1			Reader 2			Reader 3			Reader 4			Reader 5			Reader 6		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	206	4	210	209	1	210	210	0	210	208	2	210	203	7	210	197	13	210
Negative Agreement	60	0	60	60	0	60	60	0	60	58	2	60	60	0	60	60	0	60
Overall Agreement	266	4	270	269	1	270	270	0	270	266	4	270	263	7	270	257	13	270
FI Agreement	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na

Table 44: Single-Operator Agreement (Calculation excluding borderline samples) % Agreements (95% CI) 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	98.1 (95.2 - 99.3)	99.5 (97.4 - 99.9)	100 (98.2 - 100)	99 (96.6 - 99.7)	96.7 (93.3 - 98.4)	93.8 (89.7 - 96.3)
Negative Agreement	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)	96.7 (88.6 - 99.1)	100 (94 - 100)	100 (94 - 100)
Overall Agreement	98.5 (96.3 - 99.4)	99.6 (97.9 - 99.9)	100 (98.6 - 100)	98.5 (96.3 - 99.4)	97.4 (94.7 - 98.7)	95.2 (91.9 - 97.2)
FI Agreement	na	na	na	na	na	na

Overall-Single-Operator agreements ranged from 95.2% to 100% for Method B (Reader Confirmation), and from 98.1% to 100% for Method C (Manual).

Instrument Precision

HELIOS (Method A) Instrument Agreement (Calculation including borderline samples):

Table 45: Instrument Precision (Calculation including borderline samples) Number of Correctly/Incorrectly found samples Method A

Number of Samples	Site 1			Site 2			Site 3		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	190	80	270	151	119	270	131	139	270
Negative Agreement	48	12	60	57	3	60	57	3	60
Overall Agreement	238	92	330	208	122	330	188	142	330
FI Agreement	na	na	na	na	na	na	na	na	na

Table 46: Instrument Precision (Calculation including borderline samples) % Agreements (95% CI) Method A

% Agreement (95% CI)	Site 1	Site 2	Site 3
Positive Agreement	70.4 (64.7 - 75.5)	55.9 (50 - 61.7)	48.5 (42.6 - 54.5)
Negative Agreement	80 (68.2 - 88.2)	95 (86.3 - 98.3)	95 (86.3 - 98.3)
Overall Agreement	72.1 (67 - 76.7)	63 (57.7 - 68.1)	57 (51.6 - 62.2)
FI Agreement	na	na	na

HELIOS (Method A) Instrument Agreement (Calculation excluding borderline samples):

Table 47: Instrument Precision (Calculation excluding borderline samples) Number of Correctly/Incorrectly found samples Method A

Number of Samples	Site 1			Site 2			Site 3		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	180	30	210	148	62	210	130	80	210
Negative Agreement	48	12	60	57	3	60	57	3	60
Overall Agreement	228	42	270	205	65	270	187	83	270
FI Agreement	na	na	na	na	na	na	na	na	na

Table 48: Instrument Precision (Calculation excluding borderline samples) % Agreements (95% CI) Method A

% Agreement (95% CI)	Site 1	Site 2	Site 3
Positive Agreement	85.7 (80.3 - 89.8)	70.5 (64 - 76.2)	61.9 (55.2 - 68.2)
Negative Agreement	80 (68.2 - 88.2)	95 (86.3 - 98.3)	95 (86.3 - 98.3)
Overall Agreement	84.4 (79.6 - 88.3)	75.9 (70.5 - 80.6)	69.3 (63.5 - 74.5)
FI Agreement	na	na	na

Acceptance criteria were met for site 1 and site 2. Positive, Negative and Overall Agreements were >70%, At site 3, positive agreement was slightly below 70% which impacts also on the overall agreement at this site. Upon rechecking the respective HELIOS images we found that some of the images (approx. 11% of all images) that had been suggested false negative by HELIOS were out of focus. Therefore, the DNA Pattern Plus Software was not able to detect the sample as positive, although the readers still could find the sample as positive. This explains the observed lower positive agreement at site 3. This is also a reason why we clearly state that all results suggested by HELIOS have to be confirmed by a trained reader.

Overall, the three different HELIOS devices gave comparable results. Overall-Instrument agreements ranged from 69.3% to 84.4%.

Raw data see Attachment 12_Section 12_ Raw data between lab Precision Study

Conclusions

Overall-Between-Lab agreements ranged from 72.6% to 80.2% for Method A (HELIOS), from 97.7% to 99.2% for Method B (Reader Confirmation), and from 96.9% to 98.4% for Method C (Manual).

Overall-Between-Operator agreements ranged from 96.3% to 99.3% for Method B (Reader Confirmation), and from 98.9% to 100% for Method C (Manual).

Overall-Single-Operator agreements ranged from 95.2% to 100% for Method B (Reader Confirmation), and from 98.1% to 100% for Method C (Manual).

Overall-Instrument agreements ranged from 69.3% to 84.4% (Method A, HELIOS).

The results show that all results suggested by HELIOs have to be confirmed by a trained reader.

Lot to Lot Precision Study AESKUSLIDES nDNA (Crithidia luciliae)
A lot to lot reproducibility study was performed using three reagent lots each for AESKUSLIDES nDNA. 11 sera were tested:

The samples used for this study are the same as for Within Lab and Between Lab Precision Study AESKUSLIDES nDNA (Crithidia luciliae)

Each of the 11 serum samples was assayed 10 times on each reagent lot to give a total of 30 replicates per serum sample.

Slides were processed manually according to the IFU and subsequently analyzed at the microscope by two independent readers.

Agreements were calculated across all 3 reagent lots, for individual as well as for combined readers.

Acceptance Criteria:

- Positive sera have to be found positive, and negative sera have to be found negative.
- Correct pattern has to be found.
- Reported FI is allowed to differ max. ± 1 level from the expected value.
- Agreements should meet the following criteria:

Type of Agreement	% Agreement
Positive Agreement	> 90%
Negative Agreement	> 90%
Overall Agreement	> 90%
Fluorescence Intensity Agreement	> 90%

Results:

Table 49: Lot to lot agreement (across all 3 lots) AESKUSLIDES nDNA – Number of Samples

Number of Samples	Reader 1			Reader 2			Combined Readers		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	270	0	270	270	0	270	540	0	540
Negative Agreement	60	0	60	60	0	60	120	0	120
Overall Agreement	330	0	330	330	0	330	660	0	660
FI Agreement	330	0	330	330	0	330	660	0	660

FI = Fluorescence Intensity

Table 50: Lot to lot agreement (across all 3 lots) AESKUSLIDES nDNA – % Agreement:

% Agreement (95% CI)	Reader 1	Reader 2	Combined Readers
Positive Agreement	100 (98.6 - 100)	100 (98.6 - 100)	100 (99.3 - 100)
Negative Agreement	100 (94 - 100)	100 (94 - 100)	100 (96.9 - 100)
Overall Agreement	100 (98.8 - 100)	100 (98.8 - 100)	100 (99.4 - 100)
FI Agreement	100 (98.8 - 100)	100 (98.8 - 100)	100 (99.4 - 100)

FI = Fluorescence Intensity

All agreements fulfilled the above criteria.

- Positive agreement was 100% for AESKUSLIDES nDNA for both readers.
- Negative agreement for nDNA was 100% for both readers.
- Overall agreement for nDNA was 100% for both readers.
- FI agreement for nDNA was 100% for both readers.
- No deviations of fluorescence intensities greater than +/-1 from the initial value have been observed.

Raw data see Attachment 13_Section 12_ Raw data Lot to Lot Precision Study

Conclusion:
Overall lot to lot agreement (across all 3 lots) for AESKUSLIDES nDNA was 100%.
All acceptance criteria have been fulfilled.

b) Carryover:

To investigate and evaluate the carry over of samples from one reaction well to the following reaction well when slides are processed by HELIOS.

Three high positive samples were run on HELIOS in alternate with a negative serum sample. Results were confirmed by a reader. Tests were performed in accordance with the respective IFUs.

Sample ID	AESKUSLIDES nDNA	
	Pos/Neg	Grading
1	Pos	high positive
2	Pos	high positive
3	Pos	high positive
4	Neg	negative

Acceptance criteria:

- Pos/Neg/Overall Agreement: All positive sera have to be found positive and all negative sera have to be found negative.
- FI is allowed to differ maximum + or - 1 from the expected value.

Results:

Table 51: Results Carry Over

Sample ID	AESKUSLIDES nDNA	
	Result	FI
1	Pos	3
4	Neg	0
4	Neg	0
2	Pos	3
4	Neg	0
4	Neg	0
3	Pos	3
4	Neg	0
4	Neg	0

All samples fulfilled the above criteria. No carry over has been observed from well to well. All positive samples were identified as positive. All negative samples were identified as negative.

Conclusions

No carry over could be observed.

c) Time Extension Study

To investigate the stability of AESKUSLIDES nDNA performance upon shortening and elongation of the recommended incubation times for samples and conjugate.

Procedure:

AESKUSLIDES nDNA was assayed with 10 samples on HELIOS with the following incubation times:

1. 15 min sera incubation + 15min incubation of conjugate
2. 30 min sera incubation + 30min incubation of conjugate
3. 45 min sera incubation + 45min incubation of conjugate

The following samples were used:

Sample ID	Expected Results	
	Result	Grading
Sample 1	Pos	low positive
Sample 2	Pos	medium positive
Sample 3	Pos	low positive
Sample 4	Pos	low positive
Sample 5	Pos	medium positive
Sample 6	Pos	medium positive
Sample 7	Pos	high positive
Sample 8	Pos	high positive
Sample 9	Neg	negative
Sample 10	Neg	Neg

Tests were performed in accordance with the respective IFUs. Results have been confirmed by a trained reader.

Results of the 30 min protocol will be compared to the results of the 15 min and 45 min protocol.

Acceptance criteria:

- Pos/Neg/Overall Agreement: All positive sera have to be found positive and all negative sera have to be found negative.
- FI is allowed to differ maximum + or - 1 from the expected value.

Results

Table 52: Results Time Extention

Incubation Times	2 x 15 Minutes		2 x 30 Minutes		2 x 45 Minutes	
Sample ID	Result	Fluorescence Intensitiy	Result	Fluorescence Intensitiy	Result	Fluorescence Intensitiy
PC	Pos	3	Pos	3	Pos	3
NC	Neg	Neg	Neg	Neg	Neg	Neg
Sample 1	Pos	1	Pos	1	Pos	1
Sample 2	Pos	2	Pos	2	Pos	2
Sample 3	Pos	1	Pos	1	Pos	2
Sample 4	Pos	1	Pos	1	Pos	2
Sample 5	Pos	2	Pos	2	Pos	2
Sample 6	Pos	2	Pos	2	Pos	3
Sample 7	Pos	3	Pos	3	Pos	3
Sample 8	Pos	3	Pos	3	Pos	3
Sample 9	Neg	Neg	Neg	Neg	Neg	Neg
Sample 10	Neg	Neg	Neg	Neg	Neg	Neg

All acceptance criteria were fulfilled in each assay. All positive samples were identified as positive. All negative samples were identified as negative. Fluorescence intensities did not deviate more than +/-1 level.

Conclusions
The results demonstrate stability of the AESKUSLIDES nDNA during the tested incubation times of conjugate and sample.

d) Detection limit
Not applicable

e) Analytical specificity
Interfering substances:
The interference study was performed according to CLSI EP07-A2. Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition. Interference by 8 different substances was assessed by testing 8 serum samples:

Sample ID	AESKUSLIDES nDNA	
	Pos/Neg	Grading
1	Pos	low positive
2	Pos	low positive
3	Pos	medium positive
4	Pos	medium positive
5	Pos	high positive
6	Pos	high positive
7	Neg	negative
8	Neg	negative

The following substances have been tested with the indicated concentrations:

Table 53: Interfering substances with the indicated concentrations

Interfering Substance	Minimum final Concentration tested	Maximum final Concentration tested
Bilirubin conjugated	0.1 mg/mL	0.4 mg/mL
Bilirubin unconjugated	0.1 mg/mL	0.4 mg/mL
Hemoglobin	2.5 mg/mL	5.0 mg/mL
Triglycerides	5 mg/mL	20 mg/mL
RF IgM	200 U/mL	400 U/mL
Rituximab	0.5 mg/mL	2.0 mg/mL
Methylprednisolone	0.2 mg/mL	0.8 mg/mL
Cyclophosphamide	1.0 mg/mL	4.0 mg/mL
Methotrexate	0.025 mg/mL	0.1 mg/mL
Azathioprine	0.0075 mg/mL	0.03 mg/mL
Belimumab	2 mg/mL	8 mg/ml
Hydroxychloroquine	0.006 mg/mL	0.024 mg/mL
Mycophenolat	0.012 mg/mL	0.048 mg/mL
Ibuprofen	0.5 mg/mL	2 mg/mL
Naproxen	0.5 mg/mL	2 mg/mL

Interfering substances were spiked in each of the 8 samples in two different concentrations. Controls were prepared for each serum sample by spiking in only the respective amount of diluent without interfering substances. Spiked serum samples and their controls have been tested in triplicates for each concentration of interferent with AESKUSLIDES nDNA. All tests have been performed manually according to the IFU. Results have been analyzed by two independent readers at the microscope.

The results (positive/negative classification. fluorescence intensity (FI)) of the spiked samples were compared to the results of the respective controls. Agreements have been calculated by dividing the number of correctly found results through the number of total results for spiked samples.

Acceptance criteria:

- Pos/Neg/Overall Agreement: All positive sera have to be found positive and all negative sera have to be found negative.
- Overall Agreement: > 90%.
- FI is allowed to differ maximum + or - 1 from the expected value.
- FI Agreement: > 90%.

Results

All samples fulfilled the above criteria. No significant differences were observed between sera spiked with different concentrations of the interferents and their controls:

- Positive Agreement was 100% for all tested substances and concentrations. except for 20 mg/ml Triglycerides with Reader 1 (97%). There. one out of three repetitions of sample 2 was found negative instead of low positive (FI 1). Reader 2 found the sample correctly. Therefore. we consider this single discrepancy as a result of normal test variances and the subjective nature of the result analysis.

- Negative Agreement was 100% for all tested interferents and concentrations.

- Overall Agreement ranged from 97.9% to 100%. It was 100% for all tested substances and concentrations. except for 20 mg/ml Triglycerides with Reader 1 (97.9%).

- FI Agreement was 100% for all tested interferents and concentrations.

Raw Data see Attachment 14_Section 12_Raw data Interfering Substances

Conclusions

All acceptance criteria are fulfilled in each test. No interference was detected with the tested substances. up to the maximal concentrations indicated above.

f) Assay cut-off:

AESKU recommends a screening dilution of 1:10. 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 1:10 are considered low titers. 1:20 and 1:40 are considered medium titers, and 1:80 and greater are considered high titers.

2. Comparison studies

a. Method comparison with predicate device:

To show comparable performance of AESKUSLIDES nDNA and the predicate assay NOVA Lite dsDNA (Crithidia luciliae) of INOVA.

Procedure:

A clinical study with the same 776 serum samples (comprising 297 serum samples from patients with SLE and 479 samples from patients with other diseases) was performed with AESKUSLIDES nDNA and the predicate assay. A detailed description of the study sample can be found on page 12-50.

For each assay the sample set was processed manually and analyzed (positive/negative classification) at the microscope (Method C).

All assays were performed according to the respective IFUs. Sample were screened at a dilution of 1:10.

From the results of both assays the relative sensitivity and specificity / % positive, negative and overall agreements were calculated.

Furthermore, diagnostic sensitivity and specificity was calculated for both assays, and the cross reactivities in different diagnosis will be shown in a table.

Results:

Table 54: Method comparison with predicate device Diagnostic Sensitivity and Specificity Number of positive and negative results and % agreements:

Diagnostic Sensitivity & Specificity		Diagnosis		
		SLE	Other Diseases	Total
AESKUSLIDES nDNA	Pos	73	25	**98**
	Neg	224	454	**678**
	Total	**297**	**479**	**776**
Predicate Assay	Pos	110	68	**178**
	Neg	187	411	**598**
	Total	**297**	**479**	**776**

% Diagnostic Sensitivity & Specificity (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)	Predictive Value of a Positive Test Result (PPV)	Predictive Value of a Negative Test Result (NPV)
	SLE (n= 297)	OD (n=479)		
AESKUSLIDES nDNA	24.6 (20 - 29.8)	94.8 (92.4 - 96.4)	74.5%	67.0%
Predicate Assay	37 (31.7 - 42.7)	85.8 (82.4 - 88.6)	61.8%	68.7%

Sensitivity of AESKUSLIDES nDNA is a little bit lower (24%.6) than that calculated for the predicate assay (37%) in this study. On the other hand, specificity of AESKU is higher (94.8%) than that of the predicate (85%.8). Those values for sensitivity correlate well with values reported in the literature, where sensitivities of nDNA IFA assays for SLE are described from 13% up to 67%. Anti dsDNA antibodies are associated with disease activity and also have been shown to disappear upon treatment. The SLE cohort tested in this study was not further selected for inactive or active patients, or for treatment naïve patients. Treatment naïve patients are very rare, therefore we expect to have a greater proportion of patients under treatment and also inactive patients in the cohort. This explains why in this study the sensitivities are at the lower end of the reported sensitivities in the literature.

The higher specificity and lower sensitivity of AESKUSLIDES nDNA goes along with a higher PPV (74.5% compared to 61.8% for the predicate). NPVs of both assays are comparable (67% vs 68.7%).

The results for both assays are shown in detail in the crossreactivity table below. With AESKUSLIDES nDNA fewer crossereactivities are seen. Crossreactivities could be detected in APS (8.3%), in Dermato-/Polymyositis (16.1%), RA (17.2%), MCTD (12.5%), AIH (9.5%), PBC (11.1%), and Vasculitis (4.5%). The predicate assay detected positives in almost all diagnosis tested and also higher numbers of positives.

Table 55: Method comparison with predicate device Number and percent positive samples in the different tested diagnosis

Diagnosis			n	AESKUSLIDES nDNA				Predicate			
				n Pos	%Pos	n Neg	%Neg	n Pos	%Pos	n Neg	%Neg
Target disease		Systemic Lupus Erythematosus	297	73	24.6%	224	75.4%	110	37.0%	187	63.0%
Control diseases	Other Rheumatic Diseases	Antiphospholipid Syndrome	12	1	8.3%	11	91.7%	4	33.3%	8	66.7%
		Sjoegren´s Syndrome	30	0	0.0%	30	100.0%	5	16.7%	25	83.3%
		Systemic Sclerosis	44	0	0.0%	44	100.0%	5	11.4%	39	88.6%
		Dermatomyositis/Polymyositis	31	5	16.1%	26	83.9%	3	9.7%	28	90.3%
		Rheumatoid Arthritis	58	10	17.2%	48	82.8%	15	25.9%	43	74.1%
		Mixed Connective Tissue Diseases	24	3	12.5%	21	87.5%	5	20.8%	19	79.2%
		Undifferentiated Connective Tissue Diseases	21	0	0.0%	21	100.0%	0	0.0%	21	100.0%
	Autoimmune Liver Diseases	Autoimmune Hepatitis	21	2	9.5%	19	90.5%	7	33.3%	14	66.7%
		Primary Biliary Cirrhosis	20	2	11.1%	18	88.9%	5	44.4%	15	55.6%
		Primary Sclerosing Cholangitis	10	0	0.0%	10	100.0%	0	0.0%	10	100.0%
	Vasculitis	ANCA associated Vasculitis	44	2	4.5%	42	95.5%	1	2.3%	43	97.7%
	Infectious Diseases	Epstein-Barr Virus Infection	25	0	0.0%	25	100.0%	4	16.0%	21	84.0%
		Hepatitis B Virus Infection	34	0	0.0%	34	100.0%	4	11.8%	30	88.2%
		Hepatitis C Virus Infection	44	0	0.0%	44	100.0%	2	4.5%	42	95.5%
	Leukemia	Lymphoma	31	0	0.0%	31	100.0%	7	22.6%	24	77.4%
	Other	Fibromyalgia	30	0	0.0%	30	100.0%	1	3.3%	29	96.7%
Total			776	98	12.6%	678	87.4%	178	22.9%	598	77.1%

Table 56: Method comparison with predicate device, Relative sensitivity and specificity Number of positive and negative results and % agreements:

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

Method Comparison Predicate vs AESKU	% 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)

Overall Agreement between both assays was good (85.7%), and negative agreement was very good with 97.8%. The rather low positive agreement of 44.9% is due to the fact that the predicate assay detects more positive results.

For the interpretation of the discrepant results we also tested the respective samples by ds DNA ELISA (IgG). The results are shown in the following tables.

Table 57: Method comparison with predicate device, Discrepant samples in the Non-Healthy Control group:

Sample ID	Diagnosis	Predicate		AESKUSLIDES nDNA		AESKULISA dsDNA-G
		Result	FI	Result	FI	U/ml
688	DM/PM	Neg	0	Pos	3	4,9
690	DM/PM	Neg	0	Pos	3	7,4
691	DM/PM	Neg	0	Pos	2	8,7
67	RA	Neg	0	Pos	1	1,8
694	RA	Neg	0	Pos	1	4,3
596	Vasculitis	Neg	0	Pos	1	0,7
632	APS	Pos	1	Neg	0	0,9
758	APS	Pos	1	Neg	0	28,3
759	APS	Pos	1	Neg	0	3,1
243	AIH	Pos	1	Neg	0	4,3

246	AIH	Pos	1	Neg	0	3,1
322	AIH	Pos	1	Neg	0	9,9
361	AIH	Pos	1	Neg	0	3,4
234	PBC	Pos	1	Neg	0	6,5
237	PBC	Pos	1	Neg	0	46,6
240	PBC	Pos	1	Neg	0	4,8
209	EBV	Pos	1	Neg	0	3,2
212	EBV	Pos	1	Neg	0	5,3
220	EBV	Pos	1	Neg	0	12,2
221	EBV	Pos	1	Neg	0	<Min
542	Fibromyalgia	Pos	1	Neg	0	17,2
331	HBV	Pos	1	Neg	0	13,2
338	HBV	Pos	1	Neg	0	2,1
339	HBV	Pos	1	Neg	0	8,4
341	HBV	Pos	1	Neg	0	8,3
78	HCV	Pos	1	Neg	0	5,9
79	HCV	Pos	1	Neg	0	1,1
114	Leukemia	Pos	1	Neg	0	1,5
116	Leukemia	Pos	1	Neg	0	2,2
119	Leukemia	Pos	1	Neg	0	2,8
121	Leukemia	Pos	1	Neg	0	0,7
124	Leukemia	Pos	1	Neg	0	1,3
127	Leukemia	Pos	1	Neg	0	1,6
129	Leukemia	Pos	1	Neg	0	1,5
770	MCTD	Pos	1	Neg	0	2,2
771	MCTD	Pos	1	Neg	0	1,3
767	DM/PM	Pos	1	Neg	0	4,2
178	RA	Pos	1	Neg	0	13,5
180	RA	Pos	1	Neg	0	5,6
185	RA	Pos	1	Neg	0	1,7
188	RA	Pos	1	Neg	0	1,3
191	RA	Pos	3	Neg	0	4,6
193	RA	Pos	1	Neg	0	2,1
698	RA	Pos	1	Neg	0	12,5

271	Sjoegren`s	Pos	1	Neg	0	6,3
281	Sjoegren`s	Pos	1	Neg	0	12,5
397	Sjoegren`s	Pos	1	Neg	0	7,7
422	Sjoegren`s	Pos	1	Neg	0	1,6
423	Sjoegren`s	Pos	1	Neg	0	3,4
393	SSc	Pos	1	Neg	0	3,6
400	SSc	Pos	1	Neg	0	109,7
402	SSc	Pos	1	Neg	0	1,9
579	SSc	Pos	1	Neg	0	71,3
587	SSc	Pos	1	Neg	0	6,2
609	Vasculitis	Pos	1	Neg	0	0,9

55 of 97 discrepant samples have been found in the Non-Healthy Control group. Of those 55 samples, 6 samples are reported positive with the AESKU assay, and negative with the predicate assay. The positive results could not be confirmed by ELISA. Therefore, we think, that for those 6 samples the predicate outcompetes AESKU.

49 of those 55 samples were reported positive with the predicate assay and negative with the AESKU assay. Of these 49 positive samples only 4 could be confirmed by ELISA. Furthermore, all positive results had a low intensity (FI=1) in the predicate assay. Therefore, we think that for this 49 samples, AESKU outcompeted the predicate, by being more specific than the predicate.

Table 58: Method comparison with predicate device, Discrepant samples in the SLE group.

Sample ID	Diagnosis	Predicate		AESKUSLIDES nDNA		AESKULISA dsDNA-G
		Result	FI	Result	FI	U/ml
28	SLE	Neg	0	Pos	2	6,5
56	SLE	Neg	0	Pos	1	3,6
22	SLE	Pos	1	Neg	0	6,1
27	SLE	Pos	1	Neg	0	2,9
32	SLE	Pos	1	Neg	0	15,3
138	SLE	Pos	1	Neg	0	1,4
144	SLE	Pos	1	Neg	0	108,4
148	SLE	Pos	1	Neg	0	6,5
150	SLE	Pos	1	Neg	0	0,6
151	SLE	Pos	1	Neg	0	2,0

153	SLE	Pos	1	Neg	0	4,6
154	SLE	Pos	1	Neg	0	2,1
155	SLE	Pos	2	Neg	0	4,3
156	SLE	Pos	3	Neg	0	296,9
161	SLE	Pos	1	Neg	0	180,4
164	SLE	Pos	1	Neg	0	>Max
200	SLE	Pos	1	Neg	0	1,9
201	SLE	Pos	1	Neg	0	2,4
207	SLE	Pos	1	Neg	0	20,3
317	SLE	Pos	1	Neg	0	28,8
323	SLE	Pos	1	Neg	0	8,5
324	SLE	Pos	1	Neg	0	62,3
328	SLE	Pos	1	Neg	0	3,9
371	SLE	Pos	1	Neg	0	4,8
381	SLE	Pos	1	Neg	0	301,0
383	SLE	Pos	1	Neg	0	2,1
386	SLE	Pos	1	Neg	0	14,0
432	SLE	Pos	2	Neg	0	11,3
433	SLE	Pos	1	Neg	0	13,1
434	SLE	Pos	1	Neg	0	4,6
447	SLE	Pos	1	Neg	0	1,8
451	SLE	Pos	1	Neg	0	52,5
456	SLE	Pos	1	Neg	0	1,8
461	SLE	Pos	1	Neg	0	6,2
466	SLE	Pos	1	Neg	0	57,0
467	SLE	Pos	1	Neg	0	4,3
482	SLE	Pos	1	Neg	0	61,5
499	SLE	Pos	1	Neg	0	7,1
505	SLE	Pos	1	Neg	0	8,3
513	SLE	Pos	1	Neg	0	3,6
514	SLE	Pos	1	Neg	0	14,9
672	SLE	Pos	2	Neg	0	84,7

42 of the 97 of the discrepant samples were found in the SLE cohort. Of these 42 samples, 2 have been reported positive by the AESKU assay and negative by the predicate. However, the positive results could not be confirmed by ELISA.

40 of these 42 samples have been reported positive by the predicate assay and negative by the AESKU assay. Almost all positive samples had a low fluorescence intensity (FI=1). Only 12 samples of those 40 could be confirmed positive by ELISA. Therefore, we think that the predicate outcompetes AESKU in those 12 cases. This is only 4% of the total SLE samples. 28 of the 40 samples could not be confirmed by ELISA which is generally more sensitive than the nDNA immunofluorescence assays.

Overall, the predicate assay detected more positives in the SLE cohort, but most of those results could not be confirmed by ELISA. This matches the observation that INOVA has a lower specificity than AESKU.

Generally, Crithidia luciliae immunofluorescence tests provided by different manufactures on the market are known to deliver different results. This could be explained by differences in the manufacturing process, e.g. cultivation medium, cultivation period, and the fixation method.

Conclusions

AESKUSLIDES nDNA showed a lower diagnostic sensitivity (24.6%) compared to the predicate assay NOVA Lite provided by INOVA (37%). However, the AESKU assay is characterized by a higher specificity (94.8% vs. 85.8%), because of less cross-reactivities with other disease groups.

Raw data see Attachment 15_Section 12_Raw data Method comparison with predicate device

b. Method Comparison of Method A, B, and C and clinical study
To show comparable performance of AESKUSLIDES nDNA when processed manually and on HELIOS.

Procedure:

A clinical study with the same 776 serum samples (comprising 297 serum samples from patients with SLE and 479 samples from patients with other diseases) was performed on 3 different study sites (2 US sites, one German site). A detailed description of the study sample can be found on page 12-50.

At each study site the sample set was processed

1. manually and analyzed (positive/negative classification) at the microscope by two independent readers (Method C)
2. on HELIOS and HELIOS images were analyzed by two independent readers at the PC (Method B)

3. on HELIOS and positive/negative classification was done by the HELIOS DNA Pattern Plus Software (Method A)

All assays were performed according to the IFU.

For each study site, the results of the single and combined readers with the different Methods were compared to each other and % agreements were calculated:

- Method C (Manual) vs Method B (Reader Confirmation)
- Method B (Reader Confirmation) vs Method A (HELIOS)
- Method C (Manual) vs Method A (HELIOS).

Furthermore, the number of positive samples found for each tested diagnosis for all study sites and all Methods will be compared as well as the calculated diagnostic sensitivities and specificities.

Acceptance Criteria:

Positive, Negative, and Overall Agreements between the different Methods should be >85%. The analysis of diagnostic immunofluorescence assays and especially of nDNA assays depends strongly on the readers experience and is very subjective. Therefore, we expect and accept variances up to 15% in this clinical study.

Results:

Table 59: Method Comparison of Method A, B and C – Single Reader Agreements

% Agreement (95% CI)		Method C vs B		Method B vs A		Method C vs A	
		Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2
Site 1	Positive Agreement	85.1 (76.1 - 91.1)	85.5 (75.9 - 91.7)	51.8 (41.3 - 62.1)	50.6 (40 - 61.2)	48.3 (38.1 - 58.6)	51.3 (40.3 - 62.2)
	Negative Agreement	98.4 (97.2 - 99.1)	97.7 (96.3 - 98.6)	84.4 (81.5 - 86.9)	84 (81.1 - 86.6)	84 (81.1 - 86.6)	83.9 (80.9 - 86.4)
	Total Agreement	96.9 (95.4 - 97.9)	96.5 (95 - 97.6)	80.8 (77.9 - 83.4)	80.5 (77.6 - 83.2)	80 (77.1 - 82.7)	80.7 (77.7 - 83.3)
Site 2	Positive Agreement	91.3 (83 - 95.7)	90.7 (82 - 95.4)	54.8 (44.1 - 65)	56.1 (45.3 - 66.3)	53.8 (42.9 - 64.3)	54.7 (43.4 - 65.4)
	Negative Agreement	98.4 (97.2 - 99.1)	98 (96.7 - 98.8)	86.3 (83.5 - 88.6)	86.3 (83.6 - 88.7)	85.9 (83.1 - 88.3)	85.7 (83 - 88.1)
	Total Agreement	97.7 (96.4 - 98.5)	97.3 (95.9 - 98.2)	82.9 (80 - 85.3)	83.1 (80.3 - 85.6)	82.6 (79.8 - 85.1)	82.7 (79.9 - 85.2)
Site 3	Positive Agreement	85.5 (75.9 - 91.7)	87 (77 - 93)	48 (37.1 - 59.1)	47.3 (36.3 - 58.5)	43.4 (32.9 - 54.6)	46.4 (35.1 - 58)
	Negative Agreement	98.6 (97.4 - 99.2)	98 (96.7 - 98.8)	90.6 (88.2 - 92.5)	90.5 (88.1 - 92.4)	90.1 (87.7 - 92.1)	90.1 (87.7 - 92.1)
	Total Agreement	97.3 (95.9 - 98.2)	97 (95.6 - 98)	86.5 (83.9 - 88.7)	86.3 (83.7 - 88.6)	85.6 (82.9 - 87.9)	86.2 (83.6 - 88.5)

Table 60: Method Comparison of Method A, B and C – Combined Reader Agreements

% Agreement (95% CI)		Method C vs B	Method B vs A	Method C vs A
Site 1	Positive Agreement	85.3 (79 - 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)	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 (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)	85.8 (83.9 - 87.6)
	Total Agreement	97.5 (96.6 - 98.2)	83 (81 - 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 - 53)
	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)	85.9 (84.1 - 87.5)

Table 61: Method comparison Number and percent positive samples in the different tested diagnosis

Cross Reactivity		Site 1										Site 2										Site 3									
		Manual Performance				Reader Confirmation at HELIOS				HELIOS suggestion		Manual Performance				Reader Confirmation at HELIOS				HELIOS suggestion		Manual Performance				Reader Confirmation at HELIOS				HELIOS suggestion	
Diagnosis	n	Reader 1		Reader 2		Reader 1		Reader 2				Reader 3		Reader 4		Reader 3		Reader 4				Reader 5		Reader 6		Reader 5		Reader 6			
		n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos
SLE	297	62	20.9	59	19.9	63	21.2	60	20.2	61	20.5	67	22.6	62	20.9	63	21.2	67	22.6	65	21.9	65	21.9	56	18.9	57	19.2	57	19.2	45	15.2
APS	12	0	0.0	0	0.0	1	8.3	0	0.0	5	41.7	0	0.0	0	0.0	1	8.3	0	0.0	5	41.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Sjögren	30	0	0.0	0	0.0	1	3.3	1	3.3	10	33.3	0	0.0	0	0.0	1	3.3	0	0.0	4	13.3	0	0.0	0	0.0	1	3.3	1	3.3	4	13.3
SSc	44	1	2.3	0	0.0	0	0.0	0	0.0	6	13.6	0	0.0	0	0.0	0	0.0	0	0.0	7	15.9	0	0.0	0	0.0	1	2.3	1	2.3	7	15.9
Myositis	31	1	3.2	1	3.2	1	3.2	1	3.2	6	19.4	1	3.2	1	3.2	1	3.2	2	6.5	4	12.9	0	0.0	0	0.0	0	0.0	0	0.0	1	3.2
RA	58	8	13.8	7	12.1	7	12.1	8	13.8	15	25.9	6	10.3	6	10.3	7	12.1	6	10.3	12	20.7	5	8.6	8	13.8	7	12.1	6	10.3	7	12.1
MCTD	24	2	8.3	2	8.3	3	12.5	2	8.3	10	41.7	2	8.3	2	8.3	3	12.5	2	8.3	8	33.3	2	8.3	1	4.2	2	8.3	2	8.3	4	16.7
UCTD	21	0	0.0	0	0.0	0	0.0	0	0.0	2	9.5	0	0.0	0	0.0	0	0.0	0	0.0	3	14.3	0	0.0	0	0.0	0	0.0	0	0.0	2	9.5
AIH	21	2	9.5	2	9.5	2	9.5	2	9.5	5	23.8	2	9.5	2	9.5	2	9.5	1	4.8	3	14.3	1	4.8	0	0.0	2	9.5	2	9.5	3	14.3
AIH/PBC	9	0	0.0	0	0.0	1	11.1	0	0.0	2	22.2	0	0.0	0	0.0	1	11.1	1	11.1	1	11.1	0	0.0	1	11.1	0	0.0	1	11.1	1	11.1
PBC	11	3	27.3	0	0.0	3	27.3	3	27.3	4	36.4	0	0.0	0	0.0	3	27.3	1	9.1	4	36.4	0	0.0	0	0.0	0	0.0	0	0.0	1	9.1
PSC	10	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	30.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Vasculitis	44	5	11.4	5	11.4	2	4.5	2	4.5	11	25.0	1	2.3	1	2.3	2	4.5	1	2.3	4	9.1	2	4.5	1	2.3	2	4.5	2	4.5	6	13.6
EBV	25	0	0.0	0	0.0	0	0.0	0	0.0	3	12.0	0	0.0	0	0.0	0	0.0	0	0.0	1	4.0	0	0.0	0	0.0	0	0.0	0	0.0	3	12.0
HBV	34	2	5.9	0	0.0	1	2.9	1	2.9	1	2.9	1	2.9	1	2.9	1	2.9	1	2.9	4	11.8	1	2.9	0	0.0	2	5.9	1	2.9	5	14.7
HCV	44	0	0.0	0	0.0	0	0.0	0	0.0	3	6.8	0	0.0	0	0.0	0	0.0	0	0.0	5	11.4	0	0.0	0	0.0	0	0.0	0	0.0	2	4.5
Fibromyalgia	30	0	0.0	0	0.0	0	0.0	0	0.0	3	10.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	3.3	0	0.0	0	0.0	4	13.3
Leukemia	31	1	3.2	0	0.0	0	0.0	1	3.2	5	16.1	0	0.0	0	0.0	0	0.0	0	0.0	8	25.8	0	0.0	1	3.2	1	3.2	1	3.2	7	22.6

Table 62: Diagnostic Sensitivity and Specificity

% Diagnostic Sensitivity & Specificity (95% CI)			% Sensitivity (95% CI)	% Specificity (95% CI)		% Sensitivity (95% CI)	% Specificity (95% CI)
			SLE (n= 297)	OD (n=479)		SLE (n= 297)	OD (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 at HELIOS	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 - 96.7)
		Reader 2	20.2 (16 - 25.1)	95.6 (93.4 - 97.1)			
	HELIOS		20.5 (16.3 - 25.5)	81 (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 at HELIOS	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 at HELIOS	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)			

Summary of Results:

Agreements for Method Comparison between Method C (Manual) and Method B (Reader Confirmation of HELIOS images) were > 85% and acceptance criteria were met.

Positive Agreement of Method C vs B ranged from 85.3% to 91%.

Negative Agreement of Method C vs B ranged 98.1% to 98.3%.

Overall Agreement of Method C vs B ranged from 96.7% to 97.5%.

The results show that Methods C (Manual) and B (Reader Confirmation of HELIOS images) give equivalent results. Agreements were > 85%. Furthermore, result of the 3 study site were comparable with each other. We accept an agreement of 85% for AESKUSLIDES nDNA in this study, since the analysis of nDNA slides is very subjective and strongly depends on the experience of the reader.

Agreements for Comparison between Method B (Reader Confirmation of HELIOS images) and Method A (HELIOS positive/negative classification by the DNA Pattern Plus software) were as follows:

Positive Agreement of Method B vs A ranged from 47.7% to 55.4%.

Negative Agreement of Method B vs A ranged from 84.2% to 90.5%.

Overall Agreement of Method B vs A ranged from 80.3% to 85.9%.

According to this acceptance criteria are not met for comparison of Method B vs A in this study. The same holds true for the comparison of Method C (Manual) vs A (HELIOS suggested results).

Positive Agreement of Method C vs A ranged from 44.8% to 54.2%.

Negative Agreement of Method C vs A ranged from 83.9% to 90.1%.

Overall Agreement of Method C vs A ranged from 80.3% to 85.9%.

The reason why acceptance criteria were not met for comparison of Methods B vs A and C vs A is due to the fact that Method A (HELIOS DNA Pattern Plus software) detected only around 50% of the positive samples that had been detected by the readers at the microscope and at the screen.

However, if one takes into account the results of the calculated sensitivities and specificities for the different study sites and methods it can be seen, that the results for sensitivities are very similar between the different methods and study sites:

At site 1, sensitivities ranged from 20.4% to 20.7%.

At site 2, sensitivities ranged from 21.7% to 21.9%.

At site 2, sensitivities ranged from 15.2% to 20.4%.

All sensitivities were around 20% except for site 3, Method A, where only 15.2% were achieved.

Overall these results show, that Method A, B, and C give comparable results for the diagnostic sensitivity. The reason why the positive agreement between Method B vs A and Method C vs A is only around 50% is that in total, the DNA Pattern Plus software detected a similar number of positive samples in the SLE group, but 50% of those were different samples than those detected by the readers in Method B and C.

Furthermore, the calculated specificities for Method C and B were >95% for all three study sites. The specificity for Method A was slightly lower, it ranged from 81% to 88.1%. This is due to the fact that the DNA Pattern Plus Software detected more “false positives” in the non-healthy control group.

The described results are also reflected by the Cross Reactivity Table. Overall, for Method C and B there were only few crossreactivities which is also reflected by the high specificity of this methods (>95%). The most crossreactivities could be seen in RA (up to

13.8%), MCTD (up to 12.5%), in the Autoimmune Liver Disease Group (AIH, PBC, and PCS: up to 11.8%), and in Vasculitis (up to 11.4%). These results reflect values reported in the literature. Method A has a lower specificity as described and therefore, higher crossreactivities can be seen.

Conclusion:

Method C and Method B show substantial equivalence. AESKUSLIDES nDNA performs substantially equivalent when processed manually and on HELIOS with Reader Confirmation of HELIOS images, as shown by the method comparison study, the calculated diagnostic sensitivities and specificities, and the cross reactivity table.

For Method A in summary, when single samples were compared to each other like has been done in the method comparison described, we found a lower agreement of Method A, when compared to Method B and C. But when diagnostic sensitivities are compared, results are comparable between all three Methods. It has been shown that with Method A additional samples could be detected in the SLE group, compared to Method B and C, with a slightly lower specificity.

Our results show that results generated at HELIOS must always be confirmed by trained readers. The DNA Pattern Plus software can support the reader by providing additional information, and might encourage to recheck further samples.

Raw data see Attachment 16_Section 12_Raw data Method comparison of Method A, B, and C and Clinical Study

c. Endpoint Titer Comparison AESKUSLIDES nDNA (Crithidia luciliae)

To show that titration of samples results in the same endpoint titer when assayed manually and on HELIOS.

Procedure:

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:

Sample ID	AESKUSLIDES nDNA	
	Pos/Neg	Expected Endpoint Titer
1	Pos	1280
2	Pos	640
3	Pos	40
4	Pos	320
5	Pos	80

All assays were performed according to the respective IFUs.

Determination of endpoint titer:

The reciprocal of the highest dilution in which both replicates were reported as positive was defined as the endpoint titer of the sample.

Example: Highest dilution in which both replicates are detected as positive = 1:160

=> Endpoint Titer = 160

Acceptance Criteria:

The reported endpoint titer of a sample is allowed to differ maximum 1 titer level between Method B and C and Reader 1 and 2.

Results:

Table 63: Endpoint titer comparison

Sample ID	Method B		Method C	
	Reader 1	Reader 2	Reader 1	Reader 2
Serum 1	1280	1280	1280	1280
Serum 2	640	1280	1280	1280
Serum 3	80	80	40	40
Serum 4	160	160	320	320
Serum 5	80	80	40	80

The reported endpoint titers for the tested sera differ maximally 1 titer level between the different methods and readers.

Raw data see Attachment 17_Section 12_ Raw data Endpoint titer comparison

Conclusion:

Acceptance criteria were met. Sera titrated on HELIOS and manually result in comparable endpoint titers.

3. Clinical cut-off:

See assay cut-off

4. Expected values/Reference range

Normal Range Study AESKUSLIDES nDNA (Crithidia luciliae)

Expected values for nDNA were analyzed with a panel of 164 sera from healthy donors: 114 from Germany (63 females, 51 males, mean age 32 years, range of 18-58 years, Ethnicity: German) and 50 from the US (17 females, 33 males, mean age 34 years, range of 19-62 years, Ethnicity: 30 Black, 10 Hispanic, 10 White).

Slides were processed manually according to the IFU and subsequently analyzed at the microscope by two independent readers.

Results:

With AESKUSLIDES nDNA, there was 1 positive result found by reader 1 and no positive result found by reader 2.

Table 64: Normal Range Study Number and Percent positive and negative samples:

Normal Range Study	Pos (%)	Neg (%)	Total
Reader 1	1 (0.6)	163 (99.4)	164
Reader 2	0 (0)	164 (100)	164

Table 65: Normal Range Study Positive samples

Normal Range Study		Reader 1		Reader 2	
Sample ID	Sample Origin	Result	FI	Result	FI
NC 072	Germany	Pos	1	Neg	0

FI = Fluorescence Intensitiy

Only one sample (NC 072) has been found positive in the AESKUSLIDES nDNA assay (FI = 1) by reader 1.

Raw data see Attachment 18_Section 12_Raw data Normal Range Study

Conclusion:

The low number of positive samples we found with AESKUSLIDES nDNA in the tested healthy population correlate well with numbers reported in the literature.

N. Instrument Name

HELIOS AUTOMATED IFA SYSTEM

O. System Description

The HELIOS AUTOMATED IFA SYSTEM is an automated system including 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 personnel.

1. Modes of Operation:

HELIOS: automated processing with automated imaging and automated reading
HELIOS User Eval: automated processing with automated imaging and manual reading
HELMED Mode: automated processing with manual imaging and manual reading
Manual Mode: manual processing with manual imaging and manual reading

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

Yes

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

No

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 HELIOS camera with an optic (microscope) automatically focuses on the areas of biological material on the slides. Pictures are stored and evaluated by the HELIOS DEVICE SOFTWARE. The software performs a positive/negative of the cells. The software suggests the result as a qualitative result (positive. negative). All suggested

results obtained with the HELIOS AUTOMATED IFA SYSTEM and the HELIOS DEVICE SOFTWARE must be confirmed by trained personnel before the result is finalized.

Level of Concern
The 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 DEVICE SOFTWARE is assigned moderate level of concern.

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

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 (see assay description above).

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

Not applicable

Q. Proposed Labeling:

1. AESKUSLIDES nDNA Package Labels: See Section 13
2. AESKUSLIDES nDNA Package Insert: See Section 13
3. HELIOS Label – "For Use with AESKUSLIDES nDNA (Crithidia luciliae)" See Section 13
4. HELIOS Instruction Manual Draft Section 13
5. All other Labels – see Section 13