510(k) Summary

A. 510(k) Number:
K172461

B. Purpose for Submission:
New Device

C. Measurand:
Anti-neutrophil cytoplasmic autoantibodies (ANCA)

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

E. Applicant:
AESKU.Diagnostics GmbH & Co.KG
Mikro-Forum-Ring 2
55234 Wendelsheim
Germany

Contact Person:
Dr. Sandra Reuter
Regulatory Affairs
registrations@aesku.com
p: +49 6734 9622 0

prepared on March 23rd, 2018

F. Proprietary and Established Names:
AESKUSLIDES® ANCA Ethanol
AESKUSLIDES® ANCA Formalin

G. Regulatory Information:
1. Regulation section:
§CFR 866.5660 – Multiple autoantibodies immunological test system

2. Classification:
Class II

3. Product code:
MOB – Anti-neutrophil cytoplasmic antibodies (ANCA)

4. Clinical use
Immunology (82)

H. Intended Use:

1. Intended use(s):

AESKUSLIDES® ANCA is an indirect immunofluorescence assay utilizing human neutrophil granulocyte coated slides, fixed with Ethanol or Formalin, as a substrate for the qualitative and semi-quantitative determination of anti-neutrophil cytoplasmic autoantibodies (ANCA) in human serum by manual microscopy or with the HELIOS® AUTOMATED IFA SYSTEM.
This in vitro diagnostic assay is used as an aid for the diagnosis of ANCA-associated vasculitides (AAV) in conjunction with other clinical and laboratory findings.
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 ANCA is an indirect immunofluorescence assay utilizing human neutrophil granulocyte coated slides, fixed with Ethanol or Formalin, as a substrate for the qualitative and semi-quantitative determination of anti-neutrophil cytoplasmic autoantibodies (ANCA) in human serum by manual microscopy or with the HELIOS® AUTOMATED IFA SYSTEM.

This in vitro diagnostic assay is used as an aid for the diagnosis of ANCA-associated vasculitides (AAV) in conjunction with other clinical and laboratory findings.
All suggested results obtained with the HELIOS AUTOMATED IFA SYSTEM must be confirmed by trained personnel.

Slides coated with human neutrophil granulocytes for autoantibody detection are fixated by two different methods: ethanol (EtOH) fixation or formalin fixation. Ethanol fixation allows cell components to move through the cells after the fixation process. Formalin fixation causes cellular components to cross-link (a movement of cellular components is abrogated and the patterns are distinct). By processing serum on both Ethanol and Formalin-fixed slides, the user can confirm if the pattern is C-, P-, or A-ANCA, according to the table below.

Ethanol-fixed Result	Formalin-Fixed Result	Pattern
Cytoplasmic (C-ANCA)	Cytoplasmic (C-ANCA)	C-ANCA
Perinuclear (P-ANCA)	Cytoplasmic (C-ANCA)	P-ANCA
Perinuclear (P-ANCA)	Negative / unclear	Confirm with ANA
Mixed (P-ANCA + C-ANCA)	Negative / weak positive	A-ANCA
Very Perinuclear	A-ANCA	A-ANCA

ANCA Formalin test is not intended to be used by itself, but in conjunction with ANCA Ethanol test.

The HELIOS Vasculitis Pattern Plus software is able to detect both C- and P-ANCA. A-ANCA are reported as undefined positive. All suggested results obtained with the HELIOS AUTOMATED IFA SYSTEM must be confirmed by trained personnel.

Each kit of ANCA Ethanol and ANCA Formalin contains (Quantity depends on product variant):

- Slides, each containing 6 or 12 wells coated with human neutrophils (ethanol fixation)/ human neutrophils (formalin fixation) cells
- 2.0 ml/ 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 PBS

Standard Ref.	Description	Tests
54.100.US	ANCA Ethanol (12 wells)	120
54.101.US	ANCA Formalin (12 wells)	120
54.050.US	ANCA Ethanol (6 wells)	60
54.051.US	ANCA Formalin (6 wells)	60
54.100.US.Demo	ANCA Ethanol (12 wells)	24
54.100.US.Bulk5	ANCA Ethanol (12 wells)	600
54.100.US.Bulk10	ANCA Ethanol (12 wells)	1200
54.101.US.Demo	ANCA Formalin (12 wells)	24
54.101.US.Bulk5	ANCA Formalin (12 wells)	600
54.101.US.Bulk10	ANCA Formalin (12 wells)	1200

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 ANCA

1. Predicate 510(k) number(s):
   K961340

2. Comparison with predicate device:

Table 7: comparison table with predicate device

	Item	Predicate NOVA Lite ANCA	AESKUSLIDES® ANCA
Similarities	Intended Use	NOVA Lite® ANCA is an indirect immunofluorescent assay for the screening and semi-quantitative determination of anti-neutrophil cytoplasmic antibodies (ANCA) in human serum. The presence of antineutrophil cytoplasmic antibodies can be used in conjunction with other serological tests and clinical findings aids in the assessment of various systemic vasculitides.	AESKUSLIDES ANCA is an indirect immunofluorescence assay utilizing human neutrophil granulocyte coated slides, fixed with Ethanol or Formalin, as a substrate for the qualitative and semi-quantitative determination of anti-neutrophil cytoplasmic autoantibodies (ANCA) in human serum by manual microscopy or with the HELIOS® AUTOMATED IFA SYSTEM. This in vitro diagnostic assay is used as an aid for the diagnosis of ANCA-associated vasculitides (AAV) in conjunction with other clinical and laboratory findings. All suggested results obtained with the HELIOS AUTOMATED IFA SYSTEM must be confirmed by trained personnel.
	Methology	Immunofluorescence 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	anti-neutrophil cytoplasmic autoantibodies (ANCA)	same as Predicate
	Antigen	ethanol-fixed human neutrophil / formalin-fixed human neutrophil	same as Predicate
	Flurescence Marker	FITC	same as Predicate
	Controls	cANCA Positive, pANCA Positive and one negative control	same as Predicate
	conjugate	Anti-Human IgG Conjugate	same as Predicate
	Screening dilution	1:20	same as Predicate
	Storage	2 - 8 °C	same as Predicate

	Slides	6 or 12 wells coated with antigen	same as Predicate
Differences	Shelf life	18 months	24 months for ANCA Ethanol 18 months for ANCA Formalin
	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 8: 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

AESKUSLIDES® ANCA is an indirect fluorescent antibody assay utilizing human neutrophil granulocyte coated slides, fixed with Ethanol or Formalin, as a substrate for the qualitative and semi-quantitative determination of anti-neutrophil cytoplasmic autoantibodies (ANCA) in human serum by manual microscopy or with HELIOS® AUTOMATED IFA SYSTEM. This in vitro diagnostic assay is used as an aid for the diagnosis of ANCA-associated vascultitides (AAV) in conjunction with other clinical and laboratory findings. All suggested results obtained with the HELIOS® AUTOMATED IFA SYSTEM must be confirmed by trained personnel.

<u>Manual interpretation of AESKUSLIDES® ANCA Ethanol:</u>

The two main patterns seen on an ethanol-fixed substrate are perinuclear (P-ANCA) and cytoplasmic (C-ANCA):

- C-ANCA presents as coarse speckled cytoplasmic fluorescence, often with accentuated staining between the nuclear lobes. This pattern is characteristic for antibodies reacting with PR3.
- P-ANCA presents as perinuclear staining with or without nuclear extension. This pattern is usually characteristic for antibodies reacting with MPO.
  Note that anti-nuclear antibody (ANA) positive samples (containing anti-DNA/histones) may react with the nuclei of ethanol-fixed neutrophils, causing nuclear staining, and may mask or mimic the P-ANCA pattern(s).

A third pattern, less commonly seen, is called atypical ANCA (A-ANCA or X-ANCA):

- A-ANCA presents as a cytoplasmic and perinuclear or very perinuclear staining on ethanol-fixed neutrophil substrate and usually becomes negative on formalin fixed substrate.

Manual interpretation of AESKUSLIDES® ANCA Formalin:

On formalin fixed substrate, both MPO and PR3 antibodies appear as coarse cytoplasmic granular staining with interlobular accentuation.

Results from both AESKUSLIDES® ANCA Ethanol and AESKUSLIDES® ANCA Formalin provide further information on the antibodies present in the serum.

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 fluorescence) 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 fluorescence

AESKU recommends a screening dilution of 1:20, followed by serial dilutions for semi-quantitative 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 ANCA antibodies if the cells exhibit < 1+ fluorescence of the cytoplasm or nucleus. Likewise, a serum dilution is considered positive for ANCA antibodies if the cells exhibit ≥ 1+ fluorescence of the cytoplasm or nucleus. A sample is considered positive for ANCA antibodies if it exhibits ≥ 1+ fluorescence of the cytoplasm or nucleus at a sample dilution of 1:20 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 cytoplasm or nucleus is identifiable.

The titers are classified as:

- 1:20 and 1:40 are considered low titers
- 1:80 and 1:160 are considered medium titers
- 1:320 and greater are considered high titers

Automated instrument interpretation of test results

Software

After slides are processed by the HELIOS®, digital images of representative fields of view in the well are captured and stored on the computer system. The HELIOS Pattern Recognition software recognizes the pattern of the captured image by using SVM (Support Vector Machine) technology. After image pre-processing, feature extraction and classification, the software delivers the results. All suggested results given by the HELIOS® software must be confirmed within the Result Confirmation tool by a trained operator.

The HELIOS PATTERN RECOGNITON software tool identifies the following immunofluorescence patterns, abbreviated below:

Abbreviation	Pattern
P-ANCA	perinuclear
C-ANCA	cytoplasmic
unk	unknown (undefined positive)
neg	negative

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
+	positive
-	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 1:20, followed by serial dilutions for semi-quantitative 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 ANCA
D	Manual	Manual	Manual (read of microscope field)	Manual - Predicate NOVA Lite ANCA

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 9: Nomenclature and acronyms

Nomenclature	Description/ Also known as
AESKUSLIDES® ANCA	ANCA
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 ANCA w/slides read by HELIOS
Method B	HELIOS + AESKUSLIDES ANCA w/slides read by User
Method C	Manual AESKUSLIDES ANCA w/slides read by User
Method D	Manual Nova Lite ANCA w/slides read by User
P-ANCA	perinuclear
C-ANCA	cytoplasmic
unk	unknown (undefined positive)
ANCA	anti-neutrophil cytoplasmic autoantibodies
Negative IFA score/result, negative	neg, negative
Positive IFA score/result	pos, pos~~so~~itive
Diseases Abbreviation	**Diseases Name**
SLE	Systemic lupus erythematosus
MPA	Microscopic polyangiitis
UC	Ulcerative colitis

PMR	Polymyalgia rheumatica
SpA	Spondyloarthropathy
RA	Rheumatoid Arthritis
AAV	ANCA-associated vasculitides
HCV	Hepatitis C Virus
HBV	Hepatitis B Virus

Serum sample:

630 Clinical samples have been used for the Clinical Evaluation and Method Comparison 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 510 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 120 serum samples from a German University Hospital to complement some rare, but important diagnosis (70 sera from patients with Wegener's Granulomatosis, 25 sera from patients with MPA and 25 sera from patients with Churg-Strauss Syndrome).

Table 10: Serum samples set for clinical and method comparison studies

Study Sample Set			
Sample Definition		Diagnosis	n
Target Diagnosis	ANCA associated Vasculitis (AAV)	Wegener's Granulomatosis	80*
		Microscopic polyangiitis (MPA)	29*
		Churg-Strauss Syndrome	26*
Characterized MPO/PR3/ANCA positive sera		PR3 positive	25
		MPO positive	25
		P-ANCA positive	50
		C-ANCA positive	20
Control diagnosis	Autoimmune Liver Diseases	Autoimmune Hepatitis (AIH)	8
		Autoimmune Hepatitis/ Primary biliary cholangitis	6
		Primary biliary cholangitis (PBC)	11
		Primary sclerosing cholangitis (PSC)	10
	Inflammatory bowel diseases	Ulcerative Colitis	71
		Crohn's disease	40
		Inflammatory bowel disease (IBD)	9
	Other rheumatic diseases	Rheumatoid Arthritis (RA)	12
		Systemic Lupus Erythematosus (SLE)	30
		Scleroderma	19
		Myositis	1
	Infections	Hepatitis C Virus (HCV)	16
		Hepatitis B Virus (HBV)	8
		Polymyalgia rheumatica (PMR)	18

	Vasculitides NOT associated with ANCA	Giant cell artertitis (GCA)	4
		Purpura	1
	Leukemia	Lymphoma, Myeloma	15
	Other diagnosis	Celiac disease	10
		Chronic kidney disease	32
		Sinusitis	22
		Asthma	32
Sum			630

*samples from Germany: 70 sera from patients with Wegener's Granulomatosis, 25 sera from patients with MPA and 25 sera from patients with Churg-Strauss Syndrome

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 pre-screening before inclusion into the study. All 630 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: 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® ANCA

Procedure:

Serum Stability was shown by testing 10 serum samples (7 positive, 3 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® ANCA Ethanol and ANCA Formalin according to the IFUs. Slides were read by two independent readers. Each freeze/thaw sample was compared to the respective control sample.

Table 11: Serum sample set for serum stability

Sample ID	AESKUSLIDES ANCA Ethanol			AESKUSLIDES ANCA Formalin		
	Pos/Neg	Pattern ANCA Ethanol	Grading	Pos/Neg	Pattern ANCA Formalin	Grading
1	Pos	P-ANCA	medium positive	Pos	C-ANCA	medium positive
2	Pos	P-ANCA	high positive	Pos	C-ANCA	high positive
3	Pos	P-ANCA	high positive	Pos	C-ANCA	high positive
4	Pos	C-ANCA	low positive	Pos	C-ANCA	medium positive
5	Pos	C-ANCA	high positive	Pos	C-ANCA	high positive
6	Pos	C-ANCA	medium positive	Pos	C-ANCA	medium positive
7	Pos	A-ANCA	medium positive	Neg	Neg	negative
8	Neg	Neg	negative	Neg	Neg	negative
9	Neg	Neg	negative	Neg	Neg	negative
10	Neg	Neg	negative	Neg	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.
  Overall Agreement: > 85%.
- All patterns have to be found correctly.
- Pattern 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 both AESKUSLIDES® ANCA Ethanol and Formalin. All positive samples have been found positive, all negative samples have been found negative.
- Pattern Agreement was 100% for both AESKUSLIDES® ANCA Ethanol and Formalin. All patterns have been found as expected.
- FI agreement was 100% for both AESKUSLIDES® ANCA Ethanol and Formalin. No deviations of fluorescence intensities greater than +/-1 from the initial value have been observed.

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® ANCA

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

Procedure:
Nine different serum samples have been aliquoted and stored at -20°C for a time period of at least 12 months. Frozen aliquots of the different sera have been thawed and assayed on AESKUSLIDES® ANCA at the indicated time points. All tests have been performed manually according to the IFU and subsequently analyzed at the microscope.

Table 12: Serum sample set for long term serum stability

Sample ID	AESKUSLIDES ANCA Ethanol			AESKUSLIDES ANCA Formalin		
	Expected Result			Expected Result		
	Pos/Neg	Pattern	FI	Pos/Neg	Pattern	FI
1	Pos	P	3	Pos	C	3
2	Pos	C	3	Pos	C	3
3	Pos	A	1	Neg	Neg	0
4	Pos	C	1	Pos	C	1
5	Neg	0	0	Neg	0	0
6	Neg	0	0	Neg	0	0
7	Neg	0	0	Neg	0	0
8	Pos	P	3	Pos	P	2
9	Pos	P	3	Pos	C	3

Acceptance Criteria:
Positive sera have to be found positive and negative sera have to be found negative throughout the whole testing period. Correct patterns have to be found. 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, and all patterns have been found correctly at all test time points. FI did not differ more than +/- 1 level from the expected values.

Table 13: Long Term Serum Stability AESKUSLIDES ANCA Ethanol

AESKUSLIDES ANCA Ethanol												
Sample ID	month 0		month 3		month 6		month 8		month 10		month 14	
	Pos/Neg	FI	Pos/Neg	FI	Pos/Neg	FI	Pos/Neg	FI	Pos/Neg	FI	Pos/Neg	FI
1	Pos	3	Pos	3	Pos	3	Pos	3	Pos	3	Pos	3
2	Pos	3	Pos	3	Pos	3	Pos	3	Pos	3	Pos	3
3	Pos	1	Pos	1	Pos	1	Pos	1	Pos	1	Pos	1
4	Pos	1	Pos	1	Pos	1	Pos	1	Pos	1	Pos	1
5	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0
6	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0
7	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0
8	Pos	3	Pos	3	Pos	3	Pos	3	Pos	3	Pos	3
9	Pos	3	Pos	3	Pos	3	Pos	3	Pos	3	Pos	3

Table 14: Long Term Serum Stability AESKUSLIDES® ANCA Formalin

AESKUSLIDES ANCA Formalin												
Sample ID	month 0		month 2		month 5		month 8		month 10		month 12	
	Pos/Neg	FI	Pos/Neg	FI	Pos/Neg	FI	Pos/Neg	FI	Pos/Neg	FI	Pos/Neg	FI
1	Pos	3	Pos	3	Pos	3	Pos	2	Pos	2	Pos	2
2	Pos	3	Pos	3	Pos	3	Pos	3	Pos	3	Pos	3
3	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0
4	Pos	1	Pos	1	Pos	1	Pos	1	Pos	1	Pos	1
5	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0
6	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0
7	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0
8	Pos	1	na	na	Pos	2	na	na	Pos	1	Pos	2
9	Pos	3	Pos	3	Pos	3	Pos	3	Pos	3	Pos	3

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 12 months has no effect on performance and test results of AESKUSLIDES® ANCA.

Precision

a) Within-Lab Precision/Repeatability AESKUSLIDES® ANCA

Within-Lab Precision of AESKUSLIDES® ANCA Ethanol and ANCA Formalin 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
10 samples were assayed on AESKUSLIDES® ANCA Ethanol and Formalin. 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_ANCA Ethanol_Within-Lab

Sample ID	AESKUSLIDES ANCA Ethanol		
	Result	Pattern	Grading
1	Pos	P	high positive
2	Pos	P	high positive
3	Pos	P	medium positive
4	Pos	P	medium positive
5	Pos	C	borderline positive
6	Pos	C	high positive
7	Pos	C	borderline positive
8	Pos	C	medium positive
9	Neg	negative	negative
10	Neg	negative	negative

Table 16: Sample table for Precision study_ANCA Formalin_Within-Lab

Sample ID	AESKUSLIDES ANCA Formalin		
	Result	Pattern	Grading
1	Pos	C	medium positive
2	Pos	C	high positive
3	Pos	C	medium positive
4	Pos	C	high positive
5	Pos	C	low positive
6	Pos	C	high positive
7	Pos	C	medium positive
8	Pos	C	high positive
9	Neg	negative	negative
10	Neg	negative	negative

Procedure

10 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 1:20 dilution. Results were analyzed by two independent readers.

Data Analysis

Positive/Negative Classification and Pattern was recorded for each sample in each run and for each Method. Pattern suggestion in Method A (HELIOS) is provided by the Vasculitis 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
- Calculations should meet the following criteria:

Estimate of imprecision	HELIOS	Reader Confirmation	Manual
% Positives of positive samples (excluding borderline samples)	> 80%	> 90%	> 90%
% Negatives of negative samples	> 80%	> 90%	> 90%

*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

Results are presented as % positive and % negative results 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). All predetermined acceptance criteria were met.

Table 17: Within-Lab Precision AESKUSLIDES ANCA Ethanol number of positive and negative results

Sample ID	N	HELIOS		User Confirmation				Manual			
		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	0	30	0	0	30	30	0	0	30	30
S2	30	4	26	0	0	30	30	0	0	30	30
S3	30	1	29	0	0	30	30	0	0	30	30
S4	30	2	28	0	0	30	30	0	0	30	30
S5	30	23	7	27	19	3	11	27	19	3	11
S6	30	3	27	1	1	29	29	0	0	30	30
S7	30	28	2	19	10	11	20	3	17	27	13
S8	30	2	28	3	3	27	27	0	0	30	30
S9	30	26	4	26	28	4	2	30	30	0	0
S10	30	25	5	30	30	0	0	30	30	0	0

Table 18: Within-Lab Precision AESKUSLIDES ANCA Ethanol percentages of positive and negative results

Sample ID	N	HELIOS		User Confirmation				Manual			
		% Negative	% Positive	% Negative		% Positive		% Negative		% Positive	
				Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2
S1	30	0	100	0	0	100	100	0	0	100	100
S2	30	13.3	86.7	0	0	100	100	0	0	100	100
S3	30	3.3	96.7	0	0	100	100	0	0	100	100
S4	30	6.7	93.3	0	0	100	100	0	0	100	100
S5	30	76.7	23.3	90.0	63.3	10.0	36.7	90.0	63.3	10.0	36.7
S6	30	10.0	90.0	3.3	3.3	96.7	96.7	0	0	100	100
S7	30	93.3	6.7	63.3	33.3	36.7	66.7	10	56.7	90.0	43.3
S8	30	6.7	93.3	10.0	10.0	90.0	90.0	0	0	100	100
S9	30	86.7	13.3	86.7	93.3	13.3	6.7	100	100	0	0
S10	30	83.3	16.7	100	100	0	0	100	100	0	0

Table 19: Within-Lab Precision AESKUSLIDES ANCA Ethanol percentages of positive and negative results for both readers combined

Sample ID	N (Method A)	HELIOS		N (Method B/C)	User Confirmation		Manual	
		% Negative	% Positive		% Negative	% Positive	% Negative	% Positive
S1	30	0	100	60	0	100	0	100
S2	30	13.3	86.7	60	0	100	0	100
S3	30	3.3	96.7	60	0	100	0	100
S4	30	6.7	93.3	60	0	100	0	100
S5	30	77	23	60	76.7	23.3	76.7	23.3
S6	30	10.0	90.0	60	3.3	96.7	0	100
S7	30	93	7	60	48.3	51.7	33.3	66.7
S8	30	7	93	60	10.0	90.0	0	100
S9	30	86.7	13.3	60	90.0	10.0	100	0
S10	30	83.3	16.7	60	100	0	100	0

Table 20: Within-Lab Precision AESKUSLIDES ANCA Formalin number of positive and negative results

Sample ID	N	HELIOS		User Confirmation				Manual			
		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	0	30	0	0	30	30	0	0	30	30
S2	30	0	30	0	0	30	30	0	0	30	30
S3	30	0	30	0	0	30	30	0	0	30	30
S4	30	0	30	0	0	30	30	0	0	30	30
S5	30	0	30	0	0	30	30	0	0	30	30
S6	30	0	30	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	29	1	27	30	3	0	29	26	1	4
S10	30	27	3	30	27	0	3	30	28	0	2

Table 21: Within-Lab Precision AESKUSLIDES ANCA Formalin percentages of positive and negative results

Sample ID	N	HELIOS		User Confirmation				Manual			
		% Negative	% Positive	% Negative		% Positive		% Negative		% Positive	
				Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2
S1	30	0	100	0	0	100	100	0	0	100	100
S2	30	0	100	0	0	100	100	0	0	100	100
S3	30	0	100	0	0	100	100	0	0	100	100
S4	30	0	100	0	0	100	100	0	0	100	100
S5	30	0	100	0	0	100	100	0	0	100	100
S6	30	0	100	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	96.7	3.3	90.0	100	10.0	0	97	86.7	3	13.3
S10	30	90.0	10.0	100	90.0	0	10.0	100	93.3	0	6.7

Table 22: Within-Lab Precision AESKUSLIDES ANCA Formalin percentages of positive and negative results for both readers combined

Sample ID	N (Method A)	HELIOS		N (Method B/C)	User Confirmation		Manual	
		% Negative	% Positive		% Negative	% Positive	% Negative	% Positive
S1	30	0	100	60	0	100	0	100
S2	30	0	100	60	0	100	0	100
S3	30	0	100	60	0	100	0	100
S4	30	0	100	60	0	100	0	100
S5	30	0	100	60	0	100	0	100
S6	30	0	100	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	96.7	3.3	60	95.0	5.0	91.7	8.3
S10	30	90.0	10.0	60	95.0	5.0	96.7	3.3

For manual testing (Method C) of AESKUSLIDES ANCA Ethanol and Formalin fluorescence intensities were at maximum ±1 level within one run (triplicates) and between the runs. For samples found to be positive, pattern determination was consistent for more than 95% of the replicates, using Method B and C, whereas for Method A more than 85% of the determined patterns correspond to each other.

Conclusion

All pre-determined acceptance criteria were met.

b) Between-Lab Precision/Reproducibility AESKUSLIDES ANCA

To assess Between-Lab Precision of AESKUSLIDES ANCA Ethanol and ANCA Formalin processed on HELIOS (Method A – HELIOS suggestions and Method B – Reader Confirmation of HELIOS images) and processed manually (Method C).

Sample Set

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

Table 23: Sample table for Precision study_ANCA Ethanol_Between-Lab

Sample ID	AESKUSLIDES ANCA Ethanol		
	Result	Pattern	Grading
1	Pos	P	high positive
2	Pos	P	high positive
3	Pos	P	medium positive
4	Pos	P	medium positive
5	Pos	C	borderline positive
6	Pos	C	high positive
7	Pos	C	borderline positive
8	Pos	C	medium positive
9	Neg	negative	negative
10	Neg	negative	negative

Table 24: Sample table for Precision study_ANCA Formalin_Between-Lab

Sample ID	AESKUSLIDES ANCA Formalin		
	Result	Pattern	Grading
1	Pos	C	medium positive
2	Pos	C	high positive
3	Pos	C	medium positive
4	Pos	C	high positive
5	Pos	C	low positive
6	Pos	C	high positive
7	Pos	C	medium positive
8	Pos	C	high positive
9	Neg	negative	negative
10	Neg	negative	negative

Procedure

10 samples have been tested on five days, two runs per day, three replicates per sample per run, at 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 1:20 dilution. Results were analyzed by two independent readers.

Data Analysis

Positive/negative classification and pattern was recorded for each sample in each run and for each method. Pattern suggestion in Method A (HELIOS) is provided by the Vasculitis Pattern Plus software tool (ANCA).

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)
- Pattern % agreement (across all across all positive samples: number of correctly found samples divided through number of total positive 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.

Patterns have to be found correctly.

Reported FI is allowed to differ max. ± 1 level from the expected value.

Agreements should meet the following criteria:

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%
Pattern 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. Therefore, they may distort positive and pattern agreement, resulting in lower agreements. Therefore, agreements are calculated once including those borderline samples and once excluding them.

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 for AESKUSLIDES® ANCA Ethanol were calculated 1. including the results of the two borderline positive samples, 2. excluding the results of those two samples. For AESKUSLIDES ANCA Formalin results were only calculated once for all tested sera, since no borderline sera were included.

All predetermined acceptance criteria were met.

Between-Site Agreement ANCA Ethanol

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

Table 25: Between-Site Agreement_ANCA Ethanol_(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	894	66	960	948	12	960	948	12	960	1362	78	1440
Negative Agreement	240	0	240	240	0	240	240	0	240	360	0	360
Overall Agreement	1134	66	1200	1188	12	1200	1188	12	1200	1722	78	1800
Pattern Agreement	894	66	960	945	15	960	945	15	960	1359	81	1440
FI Agreement	1427	13	1440	1416	24	1440	1416	24	1440	2136	24	2160

Table 26: Between-Site Agreement_ANCA Ethanol_(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	93.1 (91.3 - 94.6)	91.9 (90 - 93.4)	98.8 (97.8 - 99.3)	94.6 (93.3 - 95.6)
Negative Agreement	100 (98.4 - 100)	100 (98.4 - 100)	100 (98.4 - 100)	100 (98.9 - 100)
Overall Agreement	94.5 (93.1 - 95.7)	93.5 (92 - 94.8)	99 (98.3 - 99.4)	95.7 (94.6 - 96.5)
Pattern Agreement	93.1 (91.3 - 94.6)	91.6 (89.6 - 93.2)	98.4 (97.4 - 99.1)	94.4 (93.1 - 95.5)
FI Agreement	99.1 (98.5 - 99.5)	99.2 (98.6 - 99.6)	98.3 (97.5 - 98.9)	98.9 (98.4 - 99.3)

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

Table 27: Between-Site Agreement_ANCA Ethanol_(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	720	0	720	720	0	720	720	0	720	1080	0	1080
Negative Agreement	240	0	240	240	0	240	240	0	240	360	0	360
Overall Agreement	960	0	960	960	0	960	960	0	960	1440	0	1440
Pattern Agreement	720	0	720	717	3	720	717	3	720	1077	3	1080
FI Agreement	949	11	960	938	22	960	938	22	960	1418	22	1440

Table 28: Between-Site Agreement_ANCA Ethanol_(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)	100 (99.5 - 100)	100 (99.5 - 100)	100 (99.6 - 100)
Negative Agreement	100 (98.4 - 100)	100 (98.4 - 100)	100 (98.4 - 100)	100 (98.9 - 100)
Overall Agreement	100 (99.6 - 100)	100 (99.6 - 100)	100 (99.6 - 100)	100 (99.7 - 100)
Pattern Agreement	100 (99.5 - 100)	99.6 (98.8 - 99.9)	99.6 (98.8 - 99.9)	99.7 (99.2 - 99.9)
FI Agreement	na	na	na	na

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

Table 29: Between-Site Agreement_ANCA Ethanol_(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
Pos	869	91	960	952	8	960	877	83	960	1349	91	1440
Neg	234	6	240	240	0	240	234	6	240	354	6	360
Overall	1103	97	1200	1192	8	1200	1111	89	1200	1703	97	1800
Total Pattern	837	123	960	897	63	960	850	110	960	1292	148	1440
FI	na	na	na	na	na	na	na	na	na	na	na	na

Table 30: Between-Site Agreement_ANCA Ethanol_(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	90.5 (88.5 - 92.2)	99.2 (98.4 - 99.6)	91.4 (89.4 - 93)	93.7 (92.3 - 94.8)
Negative Agreement	97.5 (94.7 - 98.8)	100 (98.4 - 100)	97.5 (94.7 - 98.8)	98.3 (96.4 - 99.2)
Overall Agreement	91.9 (90.2 - 93.3)	99.3 (98.7 - 99.7)	92.6 (91 - 93.9)	94.6 (93.5 - 95.6)
Total Pattern Agreement	87.2 (84.9 - 89.2)	93.4 (91.7 - 94.8)	88.5 (86.4 - 90.4)	89.7 (88 - 91.2)
FI Agreement	na	na	na	na

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

Table 31: Between-Site Agreement_ANCA Ethanol_(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
Pos	712	8	720	720	0	720	712	8	720	1072	8	1080
Neg	234	6	240	240	0	240	234	6	240	354	6	360
Overall	946	14	960	960	0	960	946	14	960	1426	14	1440
Total Pattern	680	40	720	665	55	720	685	35	720	1015	65	1080
FI	na	na	na	na	na	na	na	na	na	na	na	na

Table 32: Between-Site Agreement_ANCA Ethanol_(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	98.9 (97.8 - 99.4)	100 (99.5 - 100)	98.9 (97.8 - 99.4)	99.3 (98.5 - 99.6)
Negative Agreement	97.5 (94.7 - 98.8)	100 (98.4 - 100)	97.5 (94.7 - 98.8)	98.3 (96.4 - 99.2)
Overall Agreement	98.5 (97.6 - 99.1)	100 (99.6 - 100)	98.5 (97.6 - 99.1)	99 (98.4 - 99.4)
Total Pattern Agreement	94.4 (92.5 - 95.9)	92.4 (90.2 - 94.1)	95.1 (93.3 - 96.5)	94 (92.4 - 95.3)
FI Agreement	na	na	na	na

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

Table 33: Between-Site Agreement_ANCA Ethanol_(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
Pos	411	69	480	472	8	480	415	65	480	649	71	720
Neg	102	18	120	111	9	120	111	9	120	162	18	180
Overall	513	87	600	583	17	600	526	74	600	811	89	900
Total Pattern	298	182	480	345	135	480	309	171	480	476	244	720
FI	na	na	na	na	na	na	na	na	na	na	na	na

Table 34: Between-Site Agreement_ANCA Ethanol_(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	85.6 (82.2 - 88.5)	98.3 (96.7 - 99.2)	86.5 (83.1 - 89.2)	90.1 (87.7 - 92.1)
Negative Agreement	85 (77.5 - 90.3)	92.5 (86.4 - 96)	92.5 (86.4 - 96)	90 (84.7 - 93.6)
Overall Agreement	85.5 (82.5 - 88.1)	97.2 (95.5 - 98.2)	87.7 (84.8 - 90.1)	90.1 (88 - 91.9)
Total Pattern Agreement	62.1 (57.7 - 66.3)	71.9 (67.7 - 75.7)	64.4 (60 - 68.5)	66.1 (62.6 - 69.5)
FI Agreement	na	na	na	na

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

Table 35: Between-Site Agreement_ANCA Ethanol_(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
Pos	342	18	360	352	8	360	346	14	360	520	20	540
Neg	102	18	120	111	9	120	111	9	120	162	18	180
Overall	444	36	480	463	17	480	457	23	480	682	38	720
Total Pattern	278	82	360	308	52	360	292	68	360	439	101	540
FI	na	na	na	na	na	na	na	na	na	na	na	na

Table 36: Between-Site Agreement_ANCA Ethanol_(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	95 (92.2 - 96.8)	97.8 (95.7 - 98.9)	96.1 (93.6 - 97.7)	96.3 (94.3 - 97.6)
Negative Agreement	85 (77.5 - 90.3)	92.5 (86.4 - 96)	92.5 (86.4 - 96)	90 (84.7 - 93.6)
Overall Agreement	92.5 (89.8 - 94.5)	96.5 (94.4 - 97.8)	95.2 (92.9 - 96.8)	94.7 (92.8 - 96.1)
Total Pattern Agreement	77.2 (72.6 - 81.3)	85.6 (81.5 - 88.8)	81.1 (76.7 - 84.8)	81.3 (77.8 - 84.4)
FI Agreement	na	na	na	na

For Between-site Agreement of ANCA Ethanol, all acceptance critieria were met. All agreements were >90% for Method C (Manual) and Method B (Reader Confirmation) for all study sites. All agreements were > 70% for Method A (HELIOS suggestion) for all study sites.

Overall-Between-Lab agreements ranged from 92.5% to 96.5% for Method A (HELIOS), from 98.5% to 100% for Method B (Reader Confirmation), and was 100% for Method C (Manual).

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

Table 37: Between-Operator Agreement_ANCA Ethanol_(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	414	66	480	480	0	480	468	12	480
Negative Agreement	120	0	120	120	0	120	120	0	120
Overall Agreement	534	66	600	600	0	600	588	12	600
Pattern Agreement	414	66	480	480	0	480	465	15	480
FI Agreement	720	0	720	707	13	720	709	11	720

Table 38: Between-Operator Agreement_ANCA Ethanol_(Calculation including borderline samples). % Agreements (95% CI) Method C

% Agreement (95% CI)	Site 1	Site 2	Site 3
Positive Agreement	86.3 (82.9 - 89)	100 (99.2 - 100)	97.5 (95.7 - 98.6)
Negative Agreement	100 (96.9 - 100)	100 (96.9 - 100)	100 (96.9 - 100)
Overall Agreement	89 (86.2 - 91.3)	100 (99.4 - 100)	98 (96.5 - 98.9)
Pattern Agreement	86.3 (82.9 - 89)	100 (99.2 - 100)	96.9 (94.9 - 98.1)
FI Agreement	100 (99.5 - 100)	98.2 (96.9 - 98.9)	98.5 (97.3 - 99.1)

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

Table 39: Between-Operator Agreement_ANCA Ethanol_(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	360	0	360	360	0	360	360	0	360
Negative Agreement	120	0	120	120	0	120	120	0	120
Overall Agreement	480	0	480	480	0	480	480	0	480
Pattern Agreement	360	0	360	360	0	360	357	3	360
FI Agreement	480	0	480	469	11	480	469	11	480

Table 40: Between-Operator Agreement_ANCA Ethanol_(Calculation excluding borderline samples). % Agreements (95% CI) Method C

% Agreement (95% CI)	Site 1	Site 2	Site 3
Positive Agreement	100 (98.9 - 100)	100 (98.9 - 100)	100 (98.9 - 100)
Negative Agreement	100 (96.9 - 100)	100 (96.9 - 100)	100 (96.9 - 100)
Overall Agreement	100 (99.2 - 100)	100 (99.2 - 100)	100 (99.2 - 100)
Pattern Agreement	100 (98.9 - 100)	100 (98.9 - 100)	99.2 (97.6 - 99.7)
FI Agreement	100 (99.2 - 100)	97.7 (95.9 - 98.7)	97.7 (95.9 - 98.7)

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

Table 41: Between-Operator Agreement_ANCA Ethanol_(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
Pos	397	83	480	472	8	480	480	0	480
Neg	114	6	120	120	0	120	120	0	120
Overall	511	89	600	592	8	600	600	0	600
Total Pattern	395	85	480	442	38	480	455	25	480
FI	na	na	na	na	na	na	na	na	na

Table 42: Between-Operator Agreement_ANCA Ethanol_(Calculation including borderline samples). % Agreements (95% CI) Method B

% Agreement (95% CI)	Site 1	Site 2	Site 3
Positive Agreement	82.7 (79.1 - 85.8)	98.3 (96.7 - 99.2)	100 (99.2 - 100)
Negative Agreement	95 (89.5 - 97.7)	100 (96.9 - 100)	100 (96.9 - 100)
Overall Agreement	85.2 (82.1 - 87.8)	98.7 (97.4 - 99.3)	100 (99.4 - 100)
Total Pattern Agreement	82.3 (78.6 - 85.4)	92.1 (89.3 - 94.2)	94.8 (92.4 - 96.4)
FI Agreement	na	na	na

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

Table 43: Between-Operator Agreement_ANCA Ethanol_(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
Pos	352	8	360	360	0	360	360	0	360
Neg	114	6	120	120	0	120	120	0	120
Overall	466	14	480	480	0	480	480	0	480
Total Pattern	350	10	360	330	30	360	335	25	360
FI	na	na	na	na	na	na	na	na	na

Table 44: Between-Operator Agreement_ANCA Ethanol_(Calculation excluding borderline samples). % Agreements (95% CI) Method B

% Agreement (95% CI)	Site 1	Site 2	Site 3
Positive Agreement	97.8 (95.7 - 98.9)	100 (98.9 - 100)	100 (98.9 - 100)
Negative Agreement	95 (89.5 - 97.7)	100 (96.9 - 100)	100 (96.9 - 100)
Overall Agreement	97.1 (95.2 - 98.3)	100 (99.2 - 100)	100 (99.2 - 100)
Total Pattern Agreement	97.2 (95 - 98.5)	91.7 (88.4 - 94.1)	93.1 (89.9 - 95.3)
FI Agreement	na	na	na

For Between-Operator Agreement of ANCA Ethanol, all acceptance criteria were met. All agreements were >90% for Method C (Manual) and Method B (Reader Confirmation) for all study sites.

Overall- Between-Operator agreements ranged from 97.1% to 100% for Method B (Reader Confirmation), and was 100% for Method C (Manual).

<u>Single-Operator Agreement ANCA Ethanol</u>

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

Table 45: Single-Operator Agreement_ANCA Ethanol_(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	210	30	240	204	36	240	240	0	240	240	0	240	228	12	240	240	0	240
Negative Agreement	60	0	60	60	0	60	60	0	60	60	0	60	60	0	60	60	0	60
Overall Agreement	270	30	300	264	36	300	300	0	300	300	0	300	288	12	300	300	0	300
Pattern Agreement	210	30	240	204	36	240	240	0	240	240	0	240	228	12	240	237	3	240
FI Agreement	360	0	360	360	0	360	351	9	360	356	4	360	356	4	360	353	7	360

Table 46: Single-Operator Agreement_ANCA Ethanol_(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	87.5 (82.7 - 91.1)	85 (79.9 - 89)	100 (98.4 - 100)	100 (98.4 - 100)	95 (91.5 - 97.1)	100 (98.4 - 100)
Negative Agreement	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)
Overall Agreement	90 (86.1 - 92.9)	88 (83.8 - 91.2)	100 (98.7 - 100)	100 (98.7 - 100)	96 (93.1 - 97.7)	100 (98.7 - 100)
Pattern Agreement	87.5 (82.7 - 91.1)	85 (79.9 - 89)	100 (98.4 - 100)	100 (98.4 - 100)	95 (91.5 - 97.1)	98.8 (96.4 - 99.6)
FI Agreement	100 (98.9 - 100)	100 (98.9 - 100)	97.5 (95.3 - 98.7)	98.9 (97.2 - 99.6)	98.9 (97.2 - 99.6)	98.1 (96 - 99.1)

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

Table 47: Single-Operator Agreement_ANCA Ethanol_(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	180	0	180	180	0	180	180	0	180	180	0	180	180	0	180	180	0	180
Negative Agreement	60	0	60	60	0	60	60	0	60	60	0	60	60	0	60	60	0	60
Overall Agreement	240	0	240	240	0	240	240	0	240	240	0	240	240	0	240	240	0	240
Pattern Agreement	180	0	180	180	0	180	180	0	180	180	0	180	180	0	180	177	3	180
FI Agreement	240	0	240	240	0	240	231	9	240	238	2	240	236	4	240	233	7	240

Table 48: Single-Operator Agreement_ANCA Ethanol_(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 (97.9 - 100)	100 (97.9 - 100)	100 (97.9 - 100)	100 (97.9 - 100)	100 (97.9 - 100)	100 (97.9 - 100)
Negative Agreement	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)
Overall Agreement	100 (98.4 - 100)	100 (98.4 - 100)	100 (98.4 - 100)	100 (98.4 - 100)	100 (98.4 - 100)	100 (98.4 - 100)
Total Pattern Agreement	100 (97.9 - 100)	100 (97.9 - 100)	100 (97.9 - 100)	100 (97.9 - 100)	100 (97.9 - 100)	98.3 (95.2 - 99.4)
FI Agreement	100 (98.4 - 100)	100 (98.4 - 100)	96.3 (93 - 98)	99.2 (97 - 99.8)	98.3 (95.8 - 99.4)	97.1 (94.1 - 98.6)

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

Table 49: Single-Operator Agreement_ANCA Ethanol_(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	190	50	240	207	33	240	236	4	240	236	4	240	240	0	240	240	0	240
Negative Agreement	56	4	60	58	2	60	60	0	60	60	0	60	60	0	60	60	0	60
Overall Agreement	246	54	300	265	35	300	296	4	300	296	4	300	300	0	300	300	0	300
Pattern Agreement	190	50	240	205	35	240	221	19	240	221	19	240	228	12	240	227	13	240
FI Agreement	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na

Table 50: Single-Operator Agreement_ANCA Ethanol_(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	79.2 (73.6 - 83.8)	86.3 (81.3 - 90)	98.3 (95.8 - 99.4)	98.3 (95.8 - 99.4)	100 (98.4 - 100)	100 (98.4 - 100)
Negative Agreement	93.3 (84.1 - 97.4)	96.7 (88.6 - 99.1)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)
Overall Agreement	82 (77.3 - 85.9)	88.3 (84.2 - 91.5)	98.7 (96.6 - 99.5)	98.7 (96.6 - 99.5)	100 (98.7 - 100)	100 (98.7 - 100)
Total Pattern Agreement	79.2 (73.6 - 83.8)	85.4 (80.4 - 89.3)	92.1 (88 - 94.9)	92.1 (88 - 94.9)	95 (91.5 - 97.1)	94.6 (91 - 96.8)
FI Agreement	na	na	na	na	na	na

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

Table 51: Single-Operator Agreement_ANCA Ethanol_(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 Incorrectl	Expected Numbers	Found Correctly	Found Incorrectl	Expected Numbers	Found Correctly	Found Incorrectl	Expected Numbers	Found Correctly	Found Incorrectl	Expected Numbers	Found Correctly	Found Incorrectl	Expected Numbers	Found Correctly	Found Incorrectl	Expected Numbers
Positive Agreement	176	4	180	176	4	180	180	0	180	180	0	180	180	0	180	180	0	180
Negative Agreement	56	4	60	58	2	60	60	0	60	60	0	60	60	0	60	60	0	60
Overall Agreement	232	8	240	234	6	240	240	0	240	240	0	240	240	0	240	240	0	240
Pattern Agreement	176	4	180	174	6	180	165	15	180	165	15	180	168	12	180	167	13	180
FI Agreement	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na

Table 52: Single-Operator Agreement_ANCA Ethanol_(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	97.8 (94.4 - 99.1)	97.8 (94.4 - 99.1)	100 (97.9 - 100)	100 (97.9 - 100)	100 (97.9 - 100)	100 (97.9 - 100)
Negative Agreement	93.3 (84.1 - 97.4)	96.7 (88.6 - 99.1)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)
Overall Agreement	96.7 (93.6 - 98.3)	97.5 (94.7 - 98.8)	100 (98.4 - 100)	100 (98.4 - 100)	100 (98.4 - 100)	100 (98.4 - 100)
Total Pattern Agreement	97.8 (94.4 - 99.1)	96.7 (92.9 - 98.5)	91.7 (86.7 - 94.9)	91.7 (86.7 - 94.9)	93.3 (88.7 - 96.1)	92.8 (88 - 95.7)
FI Agreement	na	na	na	na	na	na

For Single-Operator Agreement of ANCA Ethanol, all acceptance criteria were met. All agreements were >90% for Method C (Manual) and Method B (Reader Confirmation) for all study sites.

Overall- Single-Operator agreements ranged from 96.7% to 100% for Method B (Reader Confirmation), and was 100% for Method C (Manual).

Instrument Precision ANCA Ethanol

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

Table 53: Instrument Agreement_ANCA Ethanol_(Calculation including borderline samples). Number of correctly/incorrectly found samples Method A

Number of Samples	Site 1			Site 2			Site 3		
	HELIOS 1			HELIOS 2			HELIOS 3		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	177	63	240	234	6	240	238	2	240
Negative Agreement	51	9	60	51	9	60	60	0	60
Overall Agreement	228	72	300	285	15	300	298	2	300
Pattern Agreement	131	109	240	167	73	240	178	62	240
FI Agreement	na	na	na	na	na	na	na	na	na

Table 54: Instrument Agreement_ANCA Ethanol_(Calculation including borderline samples). % Agreements (95% CI) Method A

% Agreement (95% CI)	Site 1	Site 2	Site 3
	HELIOS 1	HELIOS 2	HELIOS 3
Positive Agreement	73.8 (67.8 - 78.9)	97.5 (94.7 - 98.8)	99.2 (97 - 99.8)
Negative Agreement	85 (73.9 - 91.9)	85 (73.9 - 91.9)	100 (94 - 100)
Overall Agreement	76 (70.9 - 80.5)	95 (91.9 - 96.9)	99.3 (97.6 - 99.8)
Pattern Agreement	54.6 (48.3 - 60.8)	69.6 (63.5 - 75.1)	74.2 (68.3 - 79.3)
FI Agreement	na	na	na

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

Table 55: Instrument Agreement_ANCA Ethanol_(Calculation excluding borderline samples). Number of correctly/incorrectly found samples Method A

Number of Samples	Site 1			Site 2			Site 3		
	HELIOS 1			HELIOS 2			HELIOS 3		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	168	12	180	174	6	180	178	2	180
Negative Agreement	51	9	60	51	9	60	60	0	60
Overall Agreement	219	21	240	225	15	240	238	2	240
Pattern Agreement	131	49	180	147	33	180	161	19	180
FI Agreement	na	na	na	na	na	na	na	na	na

Table 56: Instrument Agreement_ANCA Ethanol_(Calculation excluding borderline samples). % Agreements (95% CI) Method A

% Agreement (95% CI)	Site 1	Site 2	Site 3
	HELIOS 1	HELIOS 2	HELIOS 3
Positive Agreement	93.3 (88.7 - 96.1)	96.7 (92.9 - 98.5)	98.9 (96 - 99.7)
Negative Agreement	85 (73.9 - 91.9)	85 (73.9 - 91.9)	100 (94 - 100)
Overall Agreement	91.3 (87 - 94.2)	93.8 (89.9 - 96.2)	99.2 (97 - 99.8)
Pattern Agreement	72.8 (65.9 - 78.8)	81.7 (75.4 - 86.6)	89.4 (84.1 - 93.1)
FI Agreement	na	na	na

For Instrument to Instrument Agreement with ANCA Ethanol, all acceptance criteria were met. All agreements were >70% for all study sites.

Overall- Instrument to Instrument agreements ranged from 91.3% to 99.2%.

We clearly state that all results suggested by HELIOS have to be confirmed by a trained reader.

Between-Site Agreement ANCA Formalin

Manual (Method C) Between-Site Agreement:

Table 57: Between-Site Agreement_ANCA Formalin_Number of correctly/incorrectly found samples Method C

AESKUSLIDES ANCA Formalin Manual Performance (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	960	0	960	945	15	960	945	15	960	1425	15	1440
Negative Agreement	233	7	240	240	0	240	233	7	240	353	7	360
Overall Agreement	1193	7	1200	1185	15	1200	1178	22	1200	1778	22	1800
Pattern Agreement	960	0	960	945	15	960	945	15	960	1425	15	1440
FI Agreement	897	3	900	1166	34	1200	869	31	900	1466	34	1500

Table 58: Between-Site Agreement_ANCA Formalin % 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 (99.6 - 100)	98.4 (97.4 - 99.1)	98.4 (97.4 - 99.1)	99 (98.3 - 99.4)
Negative Agreement	97.1 (94.1 - 98.6)	100 (98.4 - 100)	97.1 (94.1 - 98.6)	98.1 (96 - 99.1)
Overall Agreement	99.4 (98.8 - 99.7)	98.8 (97.9 - 99.2)	98.2 (97.2 - 98.8)	98.8 (98.2 - 99.2)
Pattern Agreement	100 (99.6 - 100)	98.4 (97.4 - 99.1)	98.4 (97.4 - 99.1)	99 (98.3 - 99.4)
FI Agreement	99.7 (99 - 99.9)	97.2 (96.1 - 98)	96.6 (95.2 - 97.6)	97.7 (96.8 - 98.4)

Reader Confirmation (Method B) Between-Site Agreement:

Table 59: Between-Site Agreement_ANCA Formalin_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	931	29	960	924	36	960	953	7	960	1404	36	1440
Negative Agreement	224	16	240	226	14	240	230	10	240	340	20	360
Overall Agreement	1155	45	1200	1150	50	1200	1183	17	1200	1744	56	1800
Pattern Agreement	914	46	960	898	62	960	944	16	960	1378	62	1440
FI Agreement	na	na	na	na	na	na	na	na	na	na	na	na

Table 60: Between-Site Agreement_ANCA Formalin % 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	97 (95.7 - 97.9)	96.3 (94.9 - 97.3)	99.3 (98.5 - 99.6)	97.5 (96.6 - 98.2)
Negative Agreement	93.3 (89.4 - 95.9)	94.2 (90.4 - 96.5)	95.8 (92.5 - 97.7)	94.4 (91.6 - 96.4)
Overall Agreement	96.3 (95 - 97.2)	95.8 (94.5 - 96.8)	98.6 (97.7 - 99.1)	96.9 (96 - 97.6)
Pattern Agreement	95.2 (93.7 - 96.4)	93.5 (91.8 - 94.9)	98.3 (97.3 - 99)	95.7 (94.5 - 96.6)
FI Agreement	na	na	na	na

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

Table 61: Between-Site Agreement_ANCA Formalin_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	461	19	480	456	24	480	475	5	480	696	24	720
Negative Agreement	102	18	120	83	37	120	93	27	120	139	41	180
Overall Agreement	563	37	600	539	61	600	568	32	600	835	65	900
Pattern Agreement	422	58	480	404	76	480	440	40	480	633	87	720
FI Agreement	na	na	na	na	na	na	na	na	na	na	na	na

Table 62: Between-Site Agreement_ANCA Formalin. % 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	96 (93.9 - 97.5)	95 (92.7 - 96.6)	99 (97.6 - 99.6)	96.7 (95.1 - 97.7)
Negative Agreement	85 (77.5 - 90.3)	69.2 (60.4 - 76.7)	77.5 (69.2 - 84.1)	77.2 (70.6 - 82.7)
Overall Agreement	93.8 (91.6 - 95.5)	89.8 (87.2 - 92)	94.7 (92.6 - 96.2)	92.8 (90.9 - 94.3)
Pattern Agreement	87.9 (84.7 - 90.5)	84.2 (80.6 - 87.2)	91.7 (88.9 - 93.8)	87.9 (85.3 - 90.1)
FI Agreement	na	na	na	na

For Between-site Agreement of ANCA Formalin, all acceptance criteria were met. All agreements were >90% for Method C (Manual) and Method B (Reader Confirmation) for all study sites. All agreements were > 70% for Method A (HELIOS suggestion) for all study sites.

Overall-Between-Lab agreements ranged from 89.8% to 94.7% for Method A (HELIOS), from 95.8% to 98.6% for Method B (Reader Confirmation), and from 98.2% to 99.4% for Method C (Manual).

Between-Operator Agreement ANCA Formalin

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

Table 63: Between-Operator Agreement_ANCA Formalin_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
Pos	480	0	480	480	0	480	465	15	480
Neg	113	7	120	120	0	120	120	0	120
Overall	593	7	600	600	0	600	585	15	600
Single Pattern C-ANCA	480	0	480	480	0	480	465	15	480
FI	300	0	300	597	3	600	569	31	600

Table 64: Between-Operator Agreement_ANCA Formalin. % Agreements (95% CI) Method C

% Agreement (95% CI)	Site 1	Site 2	Site 3
Positive Agreement	100 (99.2 - 100)	100 (99.2 - 100)	96.9 (94.9 - 98.1)
Negative Agreement	94.2 (88.4 - 97.1)	100 (96.9 - 100)	100 (96.9 - 100)
Overall Agreement	98.8 (97.6 - 99.4)	100 (99.4 - 100)	97.5 (95.9 - 98.5)
Single Pattern C-ANCA Agreement	100 (99.2 - 100)	100 (99.2 - 100)	96.9 (94.9 - 98.1)
FI Agreement	100 (98.7 - 100)	99.5 (98.5 - 99.8)	94.8 (92.8 - 96.3)

Reader Confirmation (Method B) Between-Operator Agreement:

Table 65: Between-Operator Agreement_ANCA Formalin_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	480	0	480	451	29	480	473	7	480
Negative Agreement	114	6	120	110	10	120	116	4	120
Overall Agreement	594	6	600	561	39	600	589	11	600
Pattern Agreement	480	0	480	434	46	480	464	16	480
FI Agreement	na	na	na	na	na	na	na	na	na

Table 66: Between-Operator Agreement_ANCA Formalin. % Agreements (95% CI) Method B

% Agreement (95% CI)	Site 1	Site 2	Site 3
Positive Agreement	100 (99.2 - 100)	94 (91.5 - 95.8)	98.5 (97 - 99.3)
Negative Agreement	95 (89.5 - 97.7)	91.7 (85.3 - 95.4)	96.7 (91.7 - 98.7)
Overall Agreement	99 (97.8 - 99.5)	93.5 (91.2 - 95.2)	98.2 (96.7 - 99)
Pattern Agreement	100 (99.2 - 100)	90.4 (87.5 - 92.7)	96.7 (94.7 - 97.9)
FI Agreement	na	na	na

For Between-Operator Agreement of ANCA Formalin, all acceptance critieria were met. All agreements were >90% for Method C (Manual) and Method B (Reader Confirmation) for all study sites.

Overall- Between-Operator agreements ranged from 93.5% to 99% for Method B (Reader Confirmation), and from 97.5% to 100% for Method C (Manual).

Single-Operator Agreement ANCA Formalin

Manual (Method C) Single-Operator Agreement:

Table 67: Single-Operator Agreement_ANCA Formalin_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	240	0	240	240	0	240	240	0	240	240	0	240	234	6	240	231	9	240
Negative Agreement	59	1	60	54	6	60	60	0	60	60	0	60	60	0	60	60	0	60
Overall Agreement	299	1	300	294	6	300	300	0	300	300	0	300	294	6	300	291	9	300
Pattern Agreement	240	0	240	240	0	240	240	0	240	240	0	240	234	6	240	231	9	240
FI Agreement	300	0	300	300	0	300	300	0	300	297	3	300	293	7	300	276	24	300

Table 68: Single-Operator Agreement_ANCA Formalin. % 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 (98.4 - 100)	100 (98.4 - 100)	100 (98.4 - 100)	100 (98.4 - 100)	97.5 (94.7 - 98.8)	96.3 (93 - 98)
Negative Agreement	98.3 (91.1 - 99.7)	90 (79.9 - 95.3)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)
Overall Agreement	99.7 (98.1 - 99.9)	98 (95.7 - 99.1)	100 (98.7 - 100)	100 (98.7 - 100)	98 (95.7 - 99.1)	97 (94.4 - 98.4)
Single Pattern C-ANCA Agreement	100 (98.4 - 100)	100 (98.4 - 100)	100 (98.4 - 100)	100 (98.4 - 100)	97.5 (94.7 - 98.8)	96.3 (93 - 98)
FI Agreement	100 (98.7 - 100)	100 (98.7 - 100)	100 (98.7 - 100)	99 (97.1 - 99.7)	97.7 (95.3 - 98.9)	92 (88.4 - 94.6)

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

Table 69: Single-Operator Agreement_ANCA Formalin_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	240	0	240	240	0	240	223	17	240	228	12	240	235	5	240	238	2	240
Negative Agreement	57	3	60	57	3	60	55	5	60	55	5	60	59	1	60	57	3	60
Overall Agreement	297	3	300	297	3	300	278	22	300	283	17	300	294	6	300	295	5	300
Pattern Agreement	240	0	240	240	0	240	218	22	240	216	24	240	232	8	240	232	8	240
FI Agreement	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na

Table 70: Single-Operator Agreement_ANCA Formalin. % 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	100 (98.4 - 100)	100 (98.4 - 100)	92.9 (89 - 95.5)	95 (91.5 - 97.1)	97.9 (95.2 - 99.1)	99.2 (97 - 99.8)
Negative Agreement	95 (86.3 - 98.3)	95 (86.3 - 98.3)	91.7 (81.9 - 96.4)	91.7 (81.9 - 96.4)	98.3 (91.1 - 99.7)	95 (86.3 - 98.3)
Overall Agreement	99 (97.1 - 99.7)	99 (97.1 - 99.7)	92.7 (89.1 - 95.1)	94.3 (91.1 - 96.4)	98 (95.7 - 99.1)	98.3 (96.2 - 99.3)
Pattern Agreement	100 (98.4 - 100)	100 (98.4 - 100)	90.8 (86.5 - 93.9)	90 (85.6 - 93.2)	96.7 (93.6 - 98.3)	96.7 (93.6 - 98.3)
FI Agreement	na	na	na	na	na	na

For Single-Operator Agreement of ANCA Formalin, all acceptance critieria were met. All agreements were >90% for Method C (Manual) and Method B (Reader Confirmation) for all study sites.

Overall- Single-Operator agreements ranged from 92.7% to 99% for Method B (Reader Confirmation), and from 97% to 100% for Method C (Manual).

Instrument Precision ANCA Formalin

HELIOS (Method A) Instrument Agreement:

Table 71: Instrument Agreement_ANCA Formalin_Number of correctly/incorrectly found samples Method A

Number of Samples	Site 1			Site 2			Site 3		
	HELIOS 1			HELIOS 2			HELIOS 3		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	240	0	240	221	19	240	235	5	240
Negative Agreement	56	4	60	46	14	60	37	23	60
Overall Agreement	296	4	300	267	33	300	272	28	300
Pattern Agreement	229	11	240	193	47	240	211	29	240
FI Agreement	na	na	na	0	0	0	0	0	0

Table 72: Instrument Agreement_ANCA Formalin. % Agreements (95% CI) Method A

% Agreement (95% CI)	Site 1	Site 2	Site 3
	HELIOS 1	HELIOS 2	HELIOS 3
Positive Agreement	100 (98.4 - 100)	92.1 (88 - 94.9)	97.9 (95.2 - 99.1)
Negative Agreement	93.3 (84.1 - 97.4)	76.7 (64.6 - 85.6)	61.7 (49 - 72.9)
Overall Agreement	98.7 (96.6 - 99.5)	89 (85 - 92.1)	90.7 (86.8 - 93.5)
Pattern Agreement	95.4 (92 - 97.4)	80.4 (74.9 - 84.9)	87.9 (83.2 - 91.5)
FI Agreement	na	0	0

For Instrument to Instrument Agreement with ANCA Formalin, all acceptance criteria were met, except for negative agreement at site 3. There, negative agreement was 61.7% whereas negative agreement at site 1 and was 93.3% and 76.7%. Sometimes it may occur, that HELIOS focusses falsly on unspecific, fluorescing particles, thereby reporting the sample as positive, while the reader can still see from the images that the cells are negative. Therefore, we clearly state that all results suggested by HELIOS have to be confirmed by a trained reader.

Overall- Instrument to Instrument agreements ranged from 89% to 90.7%.

Conclusions

ANCA Ethanol
Overall-Between-Lab agreements ranged from 92.5% to 96.5% for Method A (HELIOS), from 98.5% to 100% for Method B (Reader Confirmation), and was 100% for Method C (Manual).

Overall- Between-Operator agreements ranged from 97.1% to 100% for Method B (Reader Confirmation), and was 100% for Method C (Manual).

Overall- Single-Operator agreements ranged from 96.7% to 100% for Method B (Reader Confirmation), and was 100% for Method C (Manual).

Overall- Instrument to Instrument agreements ranged from 91.3% to 99.2%.

ANCA Formalin
Overall-Between-Lab agreements ranged from 89.8% to 94.7% for Method A (HELIOS), from 95.8% to 98.6% for Method B (Reader Confirmation), and from 98.2% to 99.4% for Method C (Manual).

Overall- Between-Operator agreements ranged from 93.5% to 99% for Method B (Reader Confirmation), and from 97.5% to 100% for Method C (Manual).

Overall- Single-Operator agreements ranged from 92.7% to 99% for Method B (Reader Confirmation), and from 97% to 100% for Method C (Manual).

Overall- Instrument to Instrument agreements ranged from 89% to 90.7%.

c) Lot to Lot Precision/Reproducibility Study AESKUSLIDES® ANCA
A lot to lot reproducibility study was performed using three reagent lots each for AESKUSLIDES® ANCA Ethanol and ANCA Formalin. 12 sera were tested:

Table 73: Serum sample set for Lot to Lot Precision Study

Sample ID	AESKUSLIDES ANCA Ethanol			AESKUSLIDES ANCA Formalin		
	Result	Pattern ANCA Ethanol	Grading	Result	Pattern ANCA Formalin	Grading
1	Pos	P-ANCA	high positive	Pos	C-ANCA	high positive
2	Pos	P-ANCA	medium positive	Pos	C-ANCA	medium positive
3	Pos	P-ANCA	high positive	Pos	C-ANCA	high positive
4	Pos	P-ANCA	medium positive	Pos	C-ANCA	medium positive
5	Pos	C-ANCA	high positive	Pos	C-ANCA	high positive
6	Pos	C-ANCA	medium positive	Pos	C-ANCA	medium positive
7	Pos	C-ANCA	high positive	Pos	C-ANCA	high positive
8	Pos	C-ANCA	low positive	Pos	C-ANCA	low positive
9	Pos	A-ANCA	high positive	Neg	Neg	negative
10	Pos	A-ANCA	medium positive	Neg	Neg	negative
11	Neg	Neg	negative	Neg	Neg	negative
12	Neg	Neg	negative	Neg	Neg	negative

Each of the 12 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 between the three reagent lots (lot 1 vs lo2 , lot 2 vs lot 3, lot 1 vs lot 3), for individual as well as for both readers combined.

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	> 85%
Negative Agreement	> 85%
Overall Agreement	> 85%
Total Pattern Agreement	> 85%
Single Pattern Agreement P-ANCA	> 85%
Single Pattern Agreement C-ANCA	> 85%
Single Pattern % Agreement A-ANCA	> 85%
Fluorescence Intensity Agreement	> 85%

Results:

Table 74a: Lot to Lot Precision AESKUSLIDES ANCA Ethanol, Lot to lot agreements – Lot 1 vs Lot 2:

% Agreement (95% CI) Lot 1 vs Lot 2	Reader 1	Reader 2	Combined Readers
Positive agreement	100 (97.1 - 100)	92.9 (87.4 - 96.1)	96.3 (93.3 - 98)
Negative agreement	100 (88.6 - 100)	100 (83.9 - 100)	100 (92.9 - 100)
Overall agreement	100 (97.7 - 100)	93.8 (88.9 - 96.6)	96.9 (94.3 - 98.3)
Single Pattern C-ANCA agreement	100 (92.9 - 100)	100 (92.9 - 100)	100 (96.3 - 100)
Single Pattern P-ANCA agreement	100 (94 - 100)	100 (94 - 100)	100 (96.9 - 100)
Single Pattern A-ANCA agreement	100 (83.9 - 100)	100 (83.9 - 100)	100 (91.2 - 100)
Total Pattern agreement	100 (97.1 - 100)	100 (97.1 - 100)	100 (98.5 - 100)
FI agreement	99.4 (96.5 - 99.9)	98.8 (95.6 - 99.7)	99.1 (97.3 - 99.7)

FI = Fluorescence Intensity

Table 74b: Lot to Lot Precision AESKUSLIDES ANCA Ethanol, Lot to lot agreements – Lot 2 vs Lot 3:

% Agreement (95% CI) Lot 2 vs Lot 3	Reader 1	Reader 2	Combined Readers
Positive agreement	100 (97.1 - 100)	100 (97.1 - 100)	100 (98.5 - 100)
Negative agreement	100 (88.6 - 100)	100 (88.6 - 100)	100 (94 - 100)
Overall agreement	100 (97.7 - 100)	100 (97.7 - 100)	100 (98.8 - 100)
Single Pattern C-ANCA agreement	100 (92.9 - 100)	100 (92.9 - 100)	100 (96.3 - 100)
Single Pattern P-ANCA agreement	100 (94 - 100)	100 (94 - 100)	100 (96.9 - 100)
Single Pattern A-ANCA agreement	100 (83.9 - 100)	100 (83.9 - 100)	100 (91.2 - 100)
Total Pattern agreement	100 (97.1 - 100)	100 (97.1 - 100)	100 (98.5 - 100)
FI agreement	98.1 (94.6 - 99.4)	97.5 (93.7 - 99)	97.8 (95.6 - 98.9)

FI = Fluorescence Intensity

Table 74c: Lot to Lot Precision AESKUSLIDES ANCA Ethanol, Lot to lot agreements – Lot 1 vs Lot 3:

% Agreement (95% CI) Lot 1 vs Lot 3	Reader 1	Reader 2	Combined Readers
Positive agreement	100 (97.1 - 100)	92.9 (87.4 - 96.1)	96.3 (93.3 - 98)
Negative agreement	100 (88.6 - 100)	100 (83.9 - 100)	100 (92.9 - 100)
Overall agreement	100 (97.7 - 100)	93.8 (88.9 - 96.6)	96.9 (94.3 - 98.3)
Single Pattern C-ANCA agreement	100 (92.9 - 100)	100 (92.9 - 100)	100 (96.3 - 100)
Single Pattern P-ANCA agreement	100 (94 - 100)	100 (94 - 100)	100 (96.9 - 100)
Single Pattern A-ANCA agreement	100 (83.9 - 100)	100 (83.9 - 100)	100 (91.2 - 100)
Total Pattern agreement	100 (97.1 - 100)	100 (97.1 - 100)	100 (98.5 - 100)
FI agreement	100 (97.7 - 100)	100 (97.7 - 100)	100 (98.8 - 100)

FI = Fluorescence Intensity

Table 75: Overview Lot to lot agreements (combined readers):

% Agreement (95% CI)	Lot 1 vs Lot 2	Lot 2 vs Lot 3	Lot 1 vs Lot 3
Positive agreement	96.3 (93.3 - 98)	100 (98.5 - 100)	96.3 (93.3 - 98)
Negative agreement	100 (92.9 - 100)	100 (94 - 100)	100 (92.9 - 100)
Overall agreement	96.9 (94.3 - 98.3)	100 (98.8 - 100)	96.9 (94.3 - 98.3)
Single Pattern C-ANCA agreement	100 (96.3 - 100)	100 (96.3 - 100)	100 (96.3 - 100)
Single Pattern P-ANCA agreement	100 (96.9 - 100)	100 (96.9 - 100)	100 (96.9 - 100)
Single Pattern A-ANCA agreement	100 (91.2 - 100)	100 (91.2 - 100)	100 (91.2 - 100)
Total Pattern agreement	100 (98.5 - 100)	100 (98.5 - 100)	100 (98.5 - 100)
FI agreement	99.1 (97.3 - 99.7)	97.8 (95.6 - 98.9)	100 (98.8 - 100)

FI = Fluorescence Intensity

All agreements fulfilled the above criteria.

Positive agreement ranged from 96.3% to 100% for AESKUSLIDES ANCA Ethanol for both readers.

Negative agreement for ANCA Ethanol was 100% for both readers.

Overall agreement for ANCA Ethanol ranged from 96.9% to 100% for combined readers.

Pattern agreement for ANCA Ethanol was 100% for both readers, for all single pattern and total pattern agreement.

FI agreement for ANCA Ethanol ranged from 97.8% to 100% for combined readers.

Table 76a: Lot to Lot Precision AESKUSLIDES ANCA Formalin, Lot to lot agreements – Lot 1 vs Lot 2

% Agreement (95% CI) Lot 1 vs Lot 2	Reader 1	Reader 2	Combined Readers
Positive agreement	100 (96.6 - 100)	99.1 (94.9 - 99.8)	99.5 (97.4 - 99.9)
Negative agreement	98 (89.7 - 99.7)	94.3 (84.6 - 98.1)	96.2 (90.5 - 98.5)
Overall agreement	99.4 (96.5 - 99.9)	97.5 (93.7 - 99)	98.4 (96.4 - 99.3)
Single Pattern C-ANCA agreement	100 (96.3 - 100)	100 (96.2 - 100)	100 (98.1 - 100)
Total Pattern agreement	100 (96.6 - 100)	100 (96.5 - 100)	100 (98.2 - 100)
FI agreement	98.8 (95.6 - 99.7)	96.9 (92.9 - 98.7)	97.8 (95.6 - 98.9)

FI = Fluorescence Intensity

Table 76b: Lot to Lot Precision AESKUSLIDES ANCA Formalin, Lot to lot agreements – Lot 2 vs Lot 3:

% Agreement (95% CI) Lot 2 vs Lot 3	Reader 1	Reader 2	Combined Readers
Positive agreement	100 (96.6 - 100)	100 (96.6 - 100)	100 (98.3 - 100)
Negative agreement	100 (92.9 - 100)	98 (89.7 - 99.7)	99 (94.6 - 99.8)
Overall agreement	100 (97.7 - 100)	99.4 (96.5 - 99.9)	99.7 (98.3 - 99.9)
Single Pattern C-ANCA agreement	100 (96.3 - 100)	100 (96.3 - 100)	100 (98.1 - 100)
Total Pattern agreement	100 (96.6 - 100)	100 (96.6 - 100)	100 (98.3 - 100)
FI agreement	99.4 (96.5 - 99.9)	98.8 (95.6 - 99.7)	99.1 (97.3 - 99.7)

FI = Fluorescence Intensity

Table 76a: Lot to Lot Precision AESKUSLIDES ANCA Formalin, Lot to lot agreements – Lot 1 vs Lot 3:

% Agreement (95% CI) Lot 1 vs Lot 3	Reader 1	Reader 2	Combined Readers
Positive agreement	100 (96.6 - 100)	100 (96.5 - 100)	100 (98.3 - 100)
Negative agreement	98 (89.7 - 99.7)	94.3 (84.6 - 98.1)	96.2 (90.5 - 98.5)
Overall agreement	99.4 (96.5 - 99.9)	98.1 (94.6 - 99.4)	98.8 (96.8 - 99.5)
Single Pattern C-ANCA agreement	100 (96.3 - 100)	100 (96.2 - 100)	100 (98.1 - 100)
Total Pattern agreement	100 (96.6 - 100)	100 (96.5 - 100)	100 (98.3 - 100)
FI agreement	98.1 (94.6 - 99.4)	96.9 (92.9 - 98.7)	97.5 (95.1 - 98.7)

FI = Fluorescence Intensity

Table 77: Overview Lot to Lot agreements (combined readers):

% Agreement (95% CI)	Lot 1 vs Lot 2	Lot 2 vs Lot 3	Lot 1 vs Lot 3
Positive agreement	99.5 (97.4 - 99.9)	100 (98.3 - 100)	100 (98.3 - 100)
Negative agreement	96.2 (90.5 - 98.5)	99 (94.6 - 99.8)	96.2 (90.5 - 98.5)
Overall agreement	98.4 (96.4 - 99.3)	99.7 (98.3 - 99.9)	98.8 (96.8 - 99.5)
Single Pattern C-ANCA agreement	100 (98.1 - 100)	100 (98.1 - 100)	100 (98.1 - 100)
Total Pattern agreement	100 (98.2 - 100)	100 (98.3 - 100)	100 (98.3 - 100)
FI agreement	97.8 (95.6 - 98.9)	99.1 (97.3 - 99.7)	97.5 (95.1 - 98.7)

FI = Fluorescence Intensity

All agreements fulfilled the above criteria.

Positive agreement for AESKUSLIDES ANCA Formalin ranged from 99.5% to 100% for combined readers.

Negative agreement for ANCA Formalin ranged from 96.2% to 99.0% for both readers.

Overall agreement for ANCA Formalin ranged from 98.4% to 99.7% for combined readers.

Pattern agreement for ANCA Formalin was 100% for both readers, for all single pattern and total pattern agreement.

FI agreement for ANCA Formalin ranged from 97.8% to 99.1%% for combined readers.

Conclusion:
Overall agreement for AESKUSLIDES ANCA Ethanol ranged from 96.9% to 100% and was acceptable. Overall agreement for AESKUSLIDES ANCA Formalin ranged from 98.4% to 99.7% and was acceptable.

Carry over:

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.

Table 78: Serum sample set for Carry Over study

Sample ID	AESKUSLIDES ANCA Ethanol			AESKUSLIDES ANCA Formalin		
	Pos/Neg	Pattern ANCA Ethanol	Grading	Pos/Neg	Pattern ANCA Formalin	Grading
1	Pos	P-ANCA	high positive	Pos	C-ANCA	high positive
2	Pos	C-ANCA	high positive	Pos	C-ANCA	high positive
3	Pos	P-ANCA	high positive	Pos	C-ANCA	high positive
4	Neg	Neg	negative	Neg	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.
- All patterns have to be found correctly.
- FI is allowed to differ maximum + or - 1 from the expected value.

Results:

Table 79: Results Carry over for AESKUSLIDES ANCA

Sample ID	AESKUSLIDES ANCA Ethanol			AESKUSLIDES ANCA Formalin		
	Result	Pattern	FI	Result	Pattern	FI
1	Pos	P	3	Pos	C	3
4	Neg	Neg	0	Neg	Neg	0
4	Neg	Neg	0	Neg	Neg	0
2	Pos	C	3	Pos	C	3
4	Neg	Neg	0	Neg	Neg	0
4	Neg	Neg	0	Neg	Neg	0
3	Pos	P	3	Pos	C	3
4	Neg	Neg	0	Neg	Neg	0
4	Neg	Neg	0	Neg	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. All patterns have been identified correctly.

Conclusions
No carry over could be observed.

Time Extension Study

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

Procedure:

AESKUSLIDES ANCA Ethanol and ANCA Formalin 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

Tabel 80: Samples used for Time Extension

Sample ID	AESKUSLIDES ANCA Ethanol			AESKUSLIDES ANCA Formalin		
	Pos/Neg	Pattern ANCA Ethanol	Grading	Pos/Neg	Pattern ANCA Formalin	Grading
1	Pos	P-ANCA	high positive	Pos	C-ANCA	high positive
2	Pos	P-ANCA	medium positive	Pos	C-ANCA	medium positive
3	Pos	P-ANCA	high positive	Pos	C-ANCA	high positive
4	Pos	P-ANCA	medium positive	Pos	C-ANCA	medium positive
5	Pos	P-ANCA	medium positive	Pos	C-ANCA	medium positive
6	Pos	C-ANCA	high positive	Pos	C-ANCA	high positive
7	Pos	C-ANCA	medium positive	Pos	C-ANCA	medium positive
8	Pos	C-ANCA	medium positive	Pos	C-ANCA	medium positive
9	Neg	Neg	negative	Neg	Neg	negative
10	Neg	Neg	negative	Neg	Neg	negative

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 81: Results Time extension study AESKUSLIDES ANCA Ethanol

Incubation Times	2 x 15 Minutes			2 x 30 Minutes			2 x 45 Minutes		
Sample ID	Result	Pattern	FI	Result	Pattern	FI	Result	Pattern	FI
PC-C	Pos	C	3	Pos	C	3	Pos	C	3
PC-P	Pos	P	3	Pos	P	3	Pos	P	3
NC	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Sample 1	Pos	P	3	Pos	P	3	Pos	P	3
Sample 2	Pos	P	2	Pos	P	3	Pos	P	2
Sample 3	Pos	P	3	Pos	P	3	Pos	P	3
Sample 4	Pos	P	3	Pos	P	3	Pos	P	3
Sample 5	Pos	P	2	Pos	P	3	Pos	P	3
Sample 6	Pos	C	3	Pos	C	3	Pos	C	3
Sample 7	Pos	C	2	Pos	C	2	Pos	C	2
Sample 8	Pos	C	3	Pos	C	3	Pos	C	3
Sample 9	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Sample 10	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

FI = Fluorescence Intensity

Table 82: Results Time extension study AESKUSLIDES ANCA Formalin

Incubation Times	2 x 15 Minutes			2 x 30 Minutes			2 x 45 Minutes		
Sample ID	Result	Pattern	FI	Result	Pattern	FI	Result	Pattern	FI
PC-C	Pos	C	3	Pos	C	3	Pos	C	3
PC-P	Pos	C	3	Pos	C	3	Pos	C	3
NC	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Sample 1	Pos	C	3	Pos	C	3	Pos	C	3
Sample 2	Pos	C	2	Pos	C	3	Pos	C	2
Sample 3	Pos	C	3	Pos	C	2	Pos	C	3
Sample 4	Pos	C	3	Pos	C	3	Pos	C	3
Sample 5	Pos	C	2	Pos	C	3	Pos	C	2
Sample 6	Pos	C	3	Pos	C	3	Pos	C	3
Sample 7	Pos	C	3	Pos	C	3	Pos	C	3
Sample 8	Pos	C	3	Pos	C	3	Pos	C	3
Sample 9	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Sample 10	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

FI = Fluorescence Intensity

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

Conclusions
The results demonstrate stability of the AESKUSLIDES ANCA Ethanol, as well as AESKUSLIDES ANCA Formalin during the tested incubation times of sample and conjugate.

Detection limit

Not applicable

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 10 different substances was assessed by testing 14 serum samples.

Table 83: Samples used for Interfering substances study

Sample ID	AESKUSLIDES ANCA Ethanol			AESKUSLIDES ANCA Formalin		
	Pos/Neg	Pattern ANCA Ethanol	Grading	Pos/Neg	Pattern ANCA Formalin	Grading
1	Pos	P-ANCA	medium positive	Pos	C-ANCA	medium positive
2	Pos	P-ANCA	high positive	Pos	C-ANCA	high positive
3	Pos	C-ANCA	medium positive	Pos	C-ANCA	medium positive
4	Pos	C-ANCA	low positive	Pos	C-ANCA	high positive
5	Pos	A-ANCA	medium positive	Neg	Neg	negative
6	Pos	A-ANCA	low positive	Neg	Neg	negative
7	Neg	Neg	negative	Neg	Neg	negative
8	Neg	Neg	negative	Neg	Neg	negative
9	Pos	P-ANCA	low positive	Pos	C-ANCA	low positive
10	Pos	P-ANCA	low positive	Pos	C-ANCA	low positive
11	Pos	P-ANCA	low positive	Pos	C-ANCA	low positive
12	Pos	C-ANCA	high positive	Pos	C-ANCA	high positive
13	Pos	C-ANCA	low positive	Pos	C-ANCA	low positive
14	Pos	C-ANCA	low positive	Pos	C-ANCA	low positive

The following substances have been tested with the indicated concentrations:

Table 84: Concentrations of Interfering substances

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 IU/mL	400 IU/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

Interfering substances were spiked in each of the 14 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 ANCA Ethanol and ANCA Formalin. All tests have been performed manually according to the IFUs. Results have been analyzed by two independent readers at the microscope.

The results (positive/negative classification, pattern, 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.

<u>Acceptance criteria:</u>

- Pos/Neg/Overall Agreement: All positive sera have to be found positive and all negative sera have to be found negative.
- Overall Agreement: > 90%.
- All patterns have to be found correctly.
  Pattern 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:

ANCA Ethanol:

- Positive Agreement ranged from 97% to 100% for all tested interferents and concentrations.
  It was 100% for all tested substances and concentrations, except for 5 mg/ml Triglycerides with Reader 2 (97%), 200 IU/ml RF with both readers (99%), and 1 mg/ml Cyclophosphamide with both readers (99%).

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

- Overall Agreement ranged from 98% to 100% for all tested interferents and concentrations.
  It was 100% for all tested substances and concentrations, except for 5 mg/ml Triglycerides with Reader 2 (98%), 200 IU/ml RF with both readers (99%), and 1 mg/ml Cyclophosphamide with both readers (99%).

- Pattern Agreement ranged from 97% to 100% for all tested interferents and concentrations.
  It was 100% for all tested substances and concentrations, except for 5 mg/ml Triglycerides with Reader 2 (97%), 200 IU/ml RF with both readers (99%), 1 mg/ml Cyclophosphamide with both readers (99%), and 0.0075 mg/ml Azathioprine with both readers (99%).

- FI agreement ranged from 98% to 100% for all tested interferents and concentrations.
  It was 100% for all tested substances and concentrations, except for 5 mg/ml Triglycerides with Reader 2 (98%), and 1 mg/ml Cyclophosphamide with both readers (99%).

ANCA Formalin:

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

- Negative Agreement ranged from 92% to 100% for all tested interferents and concentrations.
  It was 100% for all tested substances and concentrations, except for 0.025 mg/ml Methotrexate with Reader 1 (92%) and with Reader 2 (96%).

- Overall Agreement ranged from 98% to 100% for all tested interferents and concentrations.
  It was 100% for all tested substances and concentrations, except for 0.025 mg/ml Methotrexate with Reader 1 (98%) and with Reader 2 (99%).

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

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

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

Assay cut-off:

AESKU recommends a screening dilution of 1:20. 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 and 1:20 are considered low titers. 1:40 and 1:80 are considered medium titers. and 1:160 and greater are considered high titers.

Stability studies

Accelerated Stability Report

3 lots of complete kits (including slides, controls, conjugate, & sample buffer) of AESKUSLIDES ANCA Ethanol and Formalin were stored at 37°C for 6 weeks. At t=0, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, and 6 weeks, a kit was manually tested on the same set of 8 samples:

Sample ID	AESKUSLIDES ANCA Ethanol			AESKUSLIDES ANCA Formalin		
	Pos/Neg	Pattern ANCA Ethanol	Grading	Pos/Neg	Pattern ANCA Formalin	Grading
1	Pos	C-ANCA	high positive	Pos	C-ANCA	high positive
2	Pos	C-ANCA	medium positive	Pos	C-ANCA	medium positive
3	Pos	C-ANCA	low positive	Pos	C-ANCA	low positive
4	Pos	P-ANCA	high positive	Pos	C-ANCA	medium positive
5	Pos	P-ANCA	medium positive	Pos	C-ANCA	borderline positive
6	Pos	P-ANCA	low positive	Pos	Neg	Neg
7	Pos	A-ANCA	medium positive	Neg	Neg	Neg
8	Neg	Neg	Neg	Neg	Neg	Neg

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

The stability data is covering the stability of all components of the kit. Agreements were calculated for individual and combined readers. Results of the kit controls were not included in the agreement calculations.

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	> 85%
Negative Agreement	> 85%
Overall Agreement	> 85%
Total Pattern Agreement	> 85%
Fluorescence Intensity Agreement	> 85%

Results:

Table 85: Accelerated Stability AESKUSLIDES ANCA Ethanol – Number of Samples:

No of samples (Incorrect/total)	Lot 1			Lot 2			Lot 3		
	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers
Positive Agreement	3 / 98	5 / 98	8 / 196	9 / 98	12 / 98	21 / 196	2 / 98	5 / 98	7 / 196
Negative Agreement	1 / 14	1 / 14	2 / 28	0 / 14	0 / 14	0 / 28	0 / 14	0 / 14	0 / 28
Overall Agreement	4 / 112	6 / 112	10 / 224	9 / 112	12 / 112	21 / 224	2 / 112	5 / 112	7 / 224
Pattern Agreement	3 / 98	5 / 98	8 / 196	11 / 98	15 / 98	26 / 196	2 / 98	8 / 98	10 / 196
FI Agreement	3 / 112	4 / 112	7 / 224	4 / 112	6 / 112	10 / 224	2 / 112	2 / 112	4 / 224

FI = Fluorescence Intensity

Table 86: Accelerated Stability AESKUSLIDES ANCA Ethanol – % Agreement:

% Agreement (95% CI)	Lot 1			Lot 2			Lot 3		
	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers
Positive Agreement	96.9 (91.4 - 99)	94.9 (88.6 - 97.8)	95.9 (92.2 - 97.9)	90.8 (83.5 - 95.1)	87.8 (79.8 - 92.9)	89.3 (84.2 - 92.9)	98 (92.9 - 99.4)	94.9 (88.6 - 97.8)	96.4 (92.8 - 98.3)
Negative Agreement	92.9 (68.5 - 98.7)	92.9 (68.5 - 98.7)	92.9 (77.4 - 98)	100 (78.5 - 100)	100 (78.5 - 100)	100 (87.9 - 100)	100 (78.5 - 100)	100 (78.5 - 100)	100 (87.9 - 100)
Overall Agreement	96.4 (91.2 - 98.6)	94.6 (88.8 - 97.5)	95.5 (92 - 97.6)	92 (85.4 - 95.7)	89.3 (82.2 - 93.8)	90.6 (86.1 - 93.8)	98.2 (93.7 - 99.5)	95.5 (90 - 98.1)	96.9 (93.7 - 98.5)
Pattern Agreement	96.9 (91.4 - 99)	94.9 (88.6 - 97.8)	95.9 (92.2 - 97.9)	88.8 (81 - 93.6)	84.7 (76.3 - 90.5)	86.7 (81.3 - 90.8)	98 (92.9 - 99.4)	91.8 (84.7 - 95.8)	94.9 (90.9 - 97.2)
FI Agreement	97.3 (92.4 - 99.1)	96.4 (91.2 - 98.6)	96.9 (93.7 - 98.5)	96.4 (91.2 - 98.6)	94.6 (88.8 - 97.5)	95.5 (92 - 97.6)	98.2 (93.7 - 99.5)	98.2 (93.7 - 99.5)	98.2 (95.5 - 99.3)

FI = Fluorescence Intens

Agreement calculations for AESKUSLIDES ANCA Ethanol included all data points of the whole test time span of 6 weeks at 37°C:

All agreements fulfilled the above criteria.

Positive agreement for AESKUSLIDES ANCA Ethanol for both readers ranged from 89.3% to 96.4% for all 3 reagent lots.

Negative agreement for ANCA Ethanol for both readers ranged from 92.9% to 100% for all 3 reagent lots.

Overall agreement for ANCA Ethanol for both readers ranged from 90.6% to 96.9% for all 3 reagent lots.

Pattern agreement for ANCA Ethanol for both readers ranged from 86.7% to 95.9% for all 3 reagent lots.

FI agreement for ANCA Ethanol for both readers ranged from 95.5% to 98.2% for all 3 reagent lots.

No deviations of fluorescence intensities greater than +/-1 from the initial value have been observed.

Table 87: Accelerated Stability AESKUSLIDES ANCA Formalin – Number of Samples:

No of samples (Incorrect/total)	Lot 1			Lot 2			Lot 3		
	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers
Positive Agreement	4 / 50	14 / 50	18 / 100	4 / 50	12 / 50	16 / 100	5 / 50	16 / 50	21 / 100
Negative Agreement	0 / 30	0 / 30	0 / 60	0 / 30	0 / 30	0 / 60	0 / 30	0 / 30	0 / 60
Overall Agreement	4 / 80	14 / 80	18 / 160	4 / 80	12 / 80	16 / 160	5 / 80	16 / 80	21 / 160
Pattern Agreement	4 / 50	14 / 50	18 / 100	4 / 50	12 / 50	16 / 100	5 / 50	16 / 50	21 / 100
FI Agreement	0 / 80	3 / 80	3 / 160	0 / 80	2 / 80	2 / 160	1 / 80	4 / 80	5 / 160

FI = Fluorescence Intensity

Table 88: Accelerated Stability AESKUSLIDES ANCA Formalin – % Agreement:

% Agreement (95% CI)	Lot 1			Lot 2			Lot 3		
	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers
Positive Agreement	92 (81.2 - 96.8)	72 (58.3 - 82.5)	82 (73.3 - 88.3)	92 (81.2 - 96.8)	76 (62.6 - 85.7)	84 (75.6 - 89.9)	90 (78.6 - 95.7)	68 (54.2 - 79.2)	79 (70 - 85.8)
Negative Agreement	100 (88.6 - 100)	100 (88.6 - 100)	100 (94 - 100)	100 (88.6 - 100)	100 (88.6 - 100)	100 (94 - 100)	100 (88.6 - 100)	100 (88.6 - 100)	100 (94 - 100)
Overall Agreement	95 (87.8 - 98)	82.5 (72.7 - 89.3)	88.8 (82.9 - 92.8)	95 (87.8 - 98)	85 (75.6 - 91.2)	90 (84.4 - 93.8)	93.8 (86.2 - 97.3)	80 (70 - 87.3)	86.9 (80.8 - 91.3)
Pattern Agreement	92 (81.2 - 96.8)	72 (58.3 - 82.5)	82 (73.3 - 88.3)	92 (81.2 - 96.8)	76 (62.6 - 85.7)	84 (75.6 - 89.9)	90 (78.6 - 95.7)	68 (54.2 - 79.2)	79 (70 - 85.8)
FI Agreement	100 (95.4 - 100)	96.3 (89.5 - 98.7)	98.1 (94.6 - 99.4)	100 (95.4 - 100)	97.5 (91.3 - 99.3)	98.8 (95.6 - 99.7)	98.8 (93.3 - 99.8)	95 (87.8 - 98)	96.9 (92.9 - 98.7)

FI = Fluorescence Intensity

Table 89: Accelerated Stability AESKUSLIDES ANCA Formalin – Number of Samples, borderline positive samples excluded:

No of samples (Incorrect/total)	Lot 1			Lot 2			Lot 3		
	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers
Positive Agreement	0 / 40	5 / 40	5 / 80	0 / 40	2 / 40	2 / 80	1 / 40	6 / 40	7 / 80
Negative Agreement	0 / 30	0 / 30	0 / 60	0 / 30	0 / 30	0 / 60	0 / 30	0 / 30	0 / 60
Overall Agreement	0 / 70	5 / 70	5 / 140	0 / 70	2 / 70	2 / 140	1 / 70	6 / 70	7 / 140
Pattern Agreement	0 / 40	5 / 40	5 / 80	0 / 40	2 / 40	2 / 80	1 / 40	6 / 40	7 / 80
FI Agreement	0 / 70	3 / 70	3 / 140	0 / 70	2 / 70	2 / 140	1 / 70	4 / 70	5 / 140

FI = Fluorescence Intensity

Table 90: Accelerated Stability AESKUSLIDES ANCA Formalin – % Agreement, borderline positive samples excluded:

% Agreement (95% CI)	Lot 1			Lot 2			Lot 3		
	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers
Positive Agreement	100 (91.2 - 100)	87.5 (73.9 - 94.5)	93.8 (86.2 - 97.3)	100 (91.2 - 100)	95 (83.5 - 98.6)	97.5 (91.3 - 99.3)	97.5 (87.1 - 99.6)	85 (70.9 - 92.9)	91.3 (83 - 95.7)
Negative Agreement	100 (88.6 - 100)	100 (88.6 - 100)	100 (94 - 100)	100 (88.6 - 100)	100 (88.6 - 100)	100 (94 - 100)	100 (88.6 - 100)	100 (88.6 - 100)	100 (94 - 100)
Overall Agreement	100 (94.8 - 100)	92.9 (84.3 - 96.9)	96.4 (91.9 - 98.5)	100 (94.8 - 100)	97.1 (90.2 - 99.2)	98.6 (94.9 - 99.6)	98.6 (92.3 - 99.7)	91.4 (82.5 - 96)	95 (90 - 97.6)
Pattern Agreement	100 (91.2 - 100)	87.5 (73.9 - 94.5)	93.8 (86.2 - 97.3)	100 (91.2 - 100)	95 (83.5 - 98.6)	97.5 (91.3 - 99.3)	97.5 (87.1 - 99.6)	85 (70.9 - 92.9)	91.3 (83 - 95.7)
FI Agreement	100 (95.4 - 100)	96.3 (89.5 - 98.7)	97.9 (93.9 - 99.3)	100 (95.4 - 100)	97.5 (91.3 - 99.3)	98.6 (94.9 - 99.6)	98.8 (93.3 - 99.8)	95 (87.8 - 98)	96.4 (91.9 - 98.5)

FI = Fluorescence Intensity

Agreement calculations for AESKUSLIDES ANCA Formalin included all data points of a test time span of 4 weeks at 37°C:

The sample set for AESKUSLIDES ANCA Formalin included one sample (sample 5) that was borderline positive. For this sample we expect either a low positive result (FI=1) or a negative result (FI=0). Therefore, this sample leads to many false negatives and therefore has a big impact on positive agreement and on pattern agreement which is not due to stability issues of the assay.

Therefore, we did an alternative agreement calculation excluding this borderline positive sample. In this calculation all acceptance criteria have been met:

Positive agreement for AESKUSLIDES ANCA Formalin for both readers ranged from 91.3% to 97.5% for all 3 reagent lots.

Negative agreement for ANCA Formalin for both readers was 100% for all 3 reagent lots.

Overall agreement for ANCA Formalin for both readers ranged from 95% to 98.6% for all 3 reagent lots.

Pattern agreement for ANCA Formalin for both readers ranged from 91.3% to 97.5% for all 3 reagent lots.

FI agreement for ANCA Formalin for both readers ranged from 96.4% to 98.6% for all 3 reagent lots.

No deviations of fluorescence intensities greater than +/-1 from the initial value have been observed.

Conclusion:

For AESKUSLIDES ANCA Ethanol all acceptance criteria were met for a time span of 6 weeks at 37°C. Therefore, according to Arrhenius equation, we claim a shelf life of at least 24 months (24+3 months) at 2-8°C.

For AESKUSLIDES ANCA Formalin all acceptance criteria were met for a time span of 4 weeks at 37°C. Therefore, according to Arrhenius equation, we claim a shelf life of at least 18 months at 2-8°C.

Real Time Stability Report

Shelf life of AESKUSLIDES ANCA Ethanol has been claimed to be at least 24 months at 2-8°C storage temperature based on the results of the Accelerated Stability Study of AESKUSLIDES ANCA Ethanol. Shelf life of AESKUSLIDES ANCA Formalin has been claimed to be at least 18 months at 2-8°C storage temperature based on the results of the Accelerated Stability Study of AESKUSLIDES ANCA Formalin. To confirm this, a real time / long term stability study over a time period of 27 months is conducted.

Procedure:

Three different reagent lots of AESKUSLIDES ANCA Ethanol and ANCA Formalin will be stored at 2-8°C over a time period of 27 months. The same eight sera will be assayed at each of the following time points:

t_0 Starting point of the stability study

t_1 3 months after t_0

t_2 6 months after t_0

t_3 12 months after t_0

t_4 18 months after t_0

t_5 24 months after t_0

t_6 27 months after t_0

Sample description:

Sample ID	AESKUSLIDES ANCA Ethanol			AESKUSLIDES ANCA Formalin		
	Expected Result	Expected Pattern	Grading	Expected Result	Expected Pattern	Grading
Sample 1	Pos	C	high positive	Pos	C	high positive
Sample 2	Pos	C	medium positive	Pos	C	medium positive
Sample 3	Pos	C	low positve	Pos	C	low posiitve
Sample 4	Pos	P	high positive	Pos	C	medium positive
Sample 5	Pos	P	medium positive	Pos	C	low posiitve
Sample 6	Pos	P	low positve	Neg	Neg	negative
Sample 7	Pos	A	medium positive	Neg	Neg	negative
Sample 8	Neg	Neg	negative	Neg	Neg	negative

Each serum is tested in triplicates in each run.

At time point t_0 three runs will be performed for each of the three different kit lots to give a total of 9 repetitions per serum per lot.

At the following time points (t_1 to t_6) only two runs will be performed for each of the three different kit lots to give a total of 6 repetitions per serum per lot.

All tests are performed manually according to the IFU. Results are analyzed by two independent readers at the microscope.

The results (pos/neg, pattern, FI) at each time point and for each sample are compared to the respective results, determined at start of the stability study ($t_{0)}$ and will be displayed as % agreements (pos/neg/overall agreement, pattern agreement, FI agreement).

The results of both readers are combined for agreement calculations. Results of the kit controls are not included in the agreement calculations.

The stability data is covering the stability of all (undiluted) components of the kit. For each test time point only the minimal needed amount of concentrated wash buffer will be removed from the original bottle and diluted to give 1x wash buffer.

Acceptance criteria:
Positive/Negative /Overall Agreement: All positive sera have to be found positive and all negative sera have to be found negative. Agreement is calculated from the number of correctly and incorrectly found samples, for positive samples (positive agreement), negative samples (negative agreement) and total samples (overall agreement).

Positive/Negative/Overall Agreement > 85%.

Patterns have to be found correctly.

Pattern Agreement > 85%.

FI is allowed to differ maximum + or - 1 from the expected value. Agreement is calculated from the number of correctly and incorrectly found FI values (of all tested samples).

FI Agreement > 85%.

Interpretation for Real time stability:

Acceptance criteria have to be fulfilled over the whole time period.

Results:
At the time of preparation of this report, only the results for time points t_0 and t_1 have been acquired so far, covering a time period of 3 months at 2-8°C.

Real time stability study is still ongoing. Further time points to be tested are 6, 12, 18, 24, and 27 months.

The results of the In Use Stability Study of AESKUSLIDES ANCA Ethanol and ANCA Formalin described elsewhere, can also serve as a real time stability study, covering a time period of 6 weeks at 2-8°C. In this In Use study the same sera have been used as for the herein described real time / long term stability study.

Table 91: Real time Stability AESKUSLIDES ANCA Ethanol (Reader 1) – Number of Samples:

Number of Samples	Reader 1								
	Lot 1			Lot 2			Lot 3		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	101	4	105	105	0	105	105	0	105
Negative Agreement	15	0	15	15	0	15	15	0	15
Overall Agreement	116	4	120	120	0	120	120	0	120
Pattern Agreement	101	4	105	105	0	105	104	1	105
FI Agreement	119	1	120	120	0	120	120	0	120

FI = Fluorescence Intensity

Table 92: Real time Stability AESKUSLIDES ANCA Ethanol (Reader 2) – Number of Samples:

Number of Samples	Reader 2								
	Lot 1			Lot 2			Lot 3		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	98	7	105	105	0	105	101	4	105
Negative Agreement	14	1	15	15	0	15	15	0	15
Overall Agreement	112	8	120	120	0	120	116	4	120
Pattern Agreement	98	7	105	105	0	105	100	5	105
FI Agreement	119	1	120	120	0	120	120	0	120

FI = Fluorescence Intensity

Table 93: Real time Stability AESKUSLIDES ANCA Ethanol (Combined Readers) – Number of Samples:

Number of Samples	Combined Readers								
	Lot 1			Lot 2			Lot 3		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	199	11	210	210	0	210	206	4	210
Negative Agreement	29	1	30	30	0	30	30	0	30
Overall Agreement	228	12	240	240	0	240	236	4	240
Pattern Agreement	199	11	210	210	0	210	204	6	210
FI Agreement	238	2	240	240	0	240	240	0	240

FI = Fluorescence Intensity

Table 94: Real time Stability AESKUSLIDES ANCA Ethanol (Reader 1) – %Agreement:

% Agreement (95% CI)	Reader 1		
	Lot 1	Lot 2	Lot 3
Positive Agreement	96.2 (90.6 - 98.5)	100 (96.5 - 100)	100 (96.5 - 100)
Negative Agreement	100 (79.6 - 100)	100 (79.6 - 100)	100 (79.6 - 100)
Overall Agreement	96.7 (91.7 - 98.7)	100 (96.9 - 100)	100 (96.9 - 100)
Pattern Agreement	96.2 (90.6 - 98.5)	100 (96.5 - 100)	99 (94.8 - 99.8)
FI Agreement	99.2 (95.4 - 99.9)	100 (96.9 - 100)	100 (96.9 - 100)

FI = Fluorescence Intensity

Table 95: Real time Stability AESKUSLIDES ANCA Ethanol (Reader 2) – %Agreement:

% Agreement (95% CI)	Reader 2		
	Lot 1	Lot 2	Lot 3
Positive Agreement	93.3 (86.9 - 96.7)	100 (96.5 - 100)	96.2 (90.6 - 98.5)
Negative Agreement	93.3 (70.2 - 98.8)	100 (79.6 - 100)	100 (79.6 - 100)
Overall Agreement	93.3 (87.4 - 96.6)	100 (96.9 - 100)	96.7 (91.7 - 98.7)
Pattern Agreement	93.3 (86.9 - 96.7)	100 (96.5 - 100)	95.2 (89.3 - 97.9)
FI Agreement	99.2 (95.4 - 99.9)	100 (96.9 - 100)	100 (96.9 - 100)

FI = Fluorescence Intensity

Table 96: Real time Stability AESKUSLIDES ANCA Ethanol (Combined Readers) – %Agreement:

% Agreement (95% CI)	Combined Readers		
	Lot 1	Lot 2	Lot 3
Positive Agreement	94.8 (90.9 - 97.1)	100 (98.2 - 100)	98.1 (95.2 - 99.3)
Negative Agreement	96.7 (83.3 - 99.4)	100 (88.6 - 100)	100 (88.6 - 100)
Overall Agreement	95 (91.5 - 97.1)	100 (98.4 - 100)	98.3 (95.8 - 99.4)
Pattern Agreement	94.8 (90.9 - 97.1)	100 (98.2 - 100)	97.1 (93.9 - 98.7)
FI Agreement	99.2 (97 - 99.8)	100 (98.4 - 100)	100 (98.4 - 100)

FI = Fluorescence Intensity

Agreement calculations for AESKUSLIDES ANCA Ethanol included all data points of the test over a time period of 3 months at 2-8°C:

All agreements fulfilled the above criteria.

Positive agreement for AESKUSLIDES ANCA Ethanol for both readers ranged from 94.8% to 100% for all 3 reagent lots.

Negative agreement for ANCA Ethanol for both readers ranged from 96.7% to 100% for all 3 reagent lots.

Overall agreement for ANCA Ethanol for both readers ranged from 95.0% to 100% for all 3 reagent lots.

Pattern agreement for ANCA Ethanol for both readers ranged from 94.8% to 100% for all 3 reagent lots.

FI agreement for ANCA Ethanol for both readers ranged from 99.2% to 100% for all 3 reagent lots.

Just one deviation of fluorescence intensity greater than +/-1 from the initial value has been observed for each reader.

Table 97: Real time Stability AESKUSLIDES ANCA Formalin (Reader 1) – Number of Samples:

Number of Samples	Reader 1								
	Lot 1			Lot 2			Lot 3		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	72	3	75	71	4	75	67	8	75
Negative Agreement	45	0	45	45	0	45	44	1	45
Overall Agreement	117	3	120	116	4	120	111	9	120
Pattern Agreement	72	3	75	71	4	75	67	8	75
FI Agreement	120	0	120	120	0	120	120	0	120

FI = Fluorescence Intensity

Table 98: Real time Stability AESKUSLIDES ANCA Formalin (Reader 1) – Number of Samples:

Number of Samples	Reader 2								
	Lot 1			Lot 2			Lot 3		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	64	11	75	64	11	75	65	10	75
Negative Agreement	45	0	45	45	0	45	44	1	45
Overall Agreement	109	11	120	109	11	120	109	11	120
Pattern Agreement	64	11	75	64	11	75	65	10	75
FI Agreement	120	0	120	119	1	120	120	0	120

FI = Fluorescence Intensity

Table 99: Real time Stability AESKUSLIDES ANCA Formalin (Combined Readers) – Number of Samples:

Number of Samples	Combined Readers								
	Lot 1			Lot 2			Lot 3		
	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers	Found Correctly	Found Incorrectly	Expected Numbers
Positive Agreement	136	14	150	135	15	150	132	18	150
Negative Agreement	90	0	90	90	0	90	88	2	90
Overall Agreement	226	14	240	225	15	240	220	20	240
Pattern Agreement	136	14	150	135	15	150	132	18	150
FI Agreement	240	0	240	239	1	240	240	0	240

FI = Fluorescence Intensity

Table 100: Real time Stability AESKUSLIDES ANCA Formalin (Reader 1) – %Agreement:

% Agreement (95% CI)	Reader 1		
	Lot 1	Lot 2	Lot 3
Positive Agreement	96 (88.9 - 98.6)	94.7 (87.1 - 97.9)	89.3 (80.3 - 94.5)
Negative Agreement	100 (92.1 - 100)	100 (92.1 - 100)	97.8 (88.4 - 99.6)
Overall Agreement	97.5 (92.9 - 99.1)	96.7 (91.7 - 98.7)	92.5 (86.4 - 96)
Pattern Agreement	96 (88.9 - 98.6)	94.7 (87.1 - 97.9)	89.3 (80.3 - 94.5)
FI Agreement	100 (96.9 - 100)	100 (96.9 - 100)	100 (96.9 - 100)

FI = Fluorescence Intensity

Table 101: Real time Stability AESKUSLIDES ANCA Formalin (Reader 2) – %Agreement:

% Agreement (95% CI)	Reader 2		
	Lot 1	Lot 2	Lot 3
Positive Agreement	85.3 (75.6 - 91.6)	85.3 (75.6 - 91.6)	86.7 (77.2 - 92.6)
Negative Agreement	100 (92.1 - 100)	100 (92.1 - 100)	97.8 (88.4 - 99.6)
Overall Agreement	90.8 (84.3 - 94.8)	90.8 (84.3 - 94.8)	90.8 (84.3 - 94.8)
Pattern Agreement	85.3 (75.6 - 91.6)	85.3 (75.6 - 91.6)	86.7 (77.2 - 92.6)
FI Agreement	100 (96.9 - 100)	99.2 (95.4 - 99.9)	100 (96.9 - 100)

FI = Fluorescence Intensity

Table 102: Real time Stability AESKUSLIDES ANCA Formalin (Combined Readers) – %Agreement:

% Agreement (95% CI)	Combined Readers		
	Lot 1	Lot 2	Lot 3
Positive Agreement	90.7 (84.9 - 94.4)	90 (84.2 - 93.8)	88 (81.8 - 92.3)
Negative Agreement	100 (95.9 - 100)	100 (95.9 - 100)	97.8 (92.3 - 99.4)
Overall Agreement	94.2 (90.4 - 96.5)	93.8 (89.9 - 96.2)	91.7 (87.5 - 94.5)
Pattern Agreement	90.7 (84.9 - 94.4)	90 (84.2 - 93.8)	88 (81.8 - 92.3)
FI Agreement	100 (98.4 - 100)	99.6 (97.7 - 99.9)	100 (98.4 - 100)

FI = Fluorescence Intensity

Agreement calculations for AESKUSLIDES ANCA Formalin included all data points of the test over a time period of 3 months at 2-8°C:

All agreements fulfilled the above criteria.

Positive agreement for AESKUSLIDES ANCA Formalin for both readers ranged from 88.0% to 90.7% for all 3 reagent lots.

Negative agreement for ANCA Formalin for both readers ranged from 97.8% to 100% for all 3 reagent lots.

Overall agreement for ANCA Formalin for both readers ranged from 91.7% to 94.2% for all 3 reagent lots.

Pattern agreement for ANCA Formalin for both readers ranged from 88.0% to 90.7% for all 3 reagent lots.

FI agreement for ANCA Formalin for both readers ranged from 99.6% to 100% for all 3 reagent lots.

Just one deviation of fluorescence intensity greater than +/-1 from the initial value was observed for reader 2.

Conclusion:
All acceptance criteria were met. All agreements were found to be > 85%. The presented data support stability for AESKUSLIDES ANCA Ethanol and ANCA Formalin for at least 3 months at 2-8°C, so far. Other test time points will follow to support the claimed shelf life of 24 months (based on the accelerated stability study of AESKUSLIDES ANCA described elsewhere).

In Use Stability Report

In Use stability testing is designed to predict the period of time that a product remains viable once placed into use. Therefore, In Use stability of AESKUSLIDES ANCA was tested over a period of 6 weeks.

Procedure:

Three different reagent lots were stored at 2-8°C over a time period of 6 weeks. Tests have been performed at the following time points: t0, t1, t2, t4, t6 (t1= after 1 week, t2= after 2 weeks, t4= after 4 weeks, t6 after 6 weeks) for each reagent lot. After the first test at time point t0, kits were incubated at the indicated temperature (2-8°C). Each kit component (with the exception of slide foils) had been opened and all buffers (except wash buffer) had been prepared at time point t0. Eight serum samples have been tested at each time point:

Sample ID	AESKUSLIDES ANCA Ethanol			AESKUSLIDES ANCA Formalin		
	Pos/ Neg	Pattern ANCA Ethanol	Grading	Pos/ Neg	Pattern ANCA Formalin	Grading
1	Pos	C-ANCA	high positive	Pos	C-ANCA	high positive
2	Pos	C-ANCA	medium positive	Pos	C-ANCA	medium positive
3	Pos	C-ANCA	low positive	Pos	C-ANCA	low positive
4	Pos	P-ANCA	high positive	Pos	C-ANCA	medium positive
5	Pos	P-ANCA	medium positive	Pos	C-ANCA	borderline positive
6	Pos	P-ANCA	low positive	Pos	C-ANCA	borderline positive
7	Pos	A-ANCA	high positive	Neg	Neg	negative
8	Neg	Neg	negative	Neg	Neg	negative

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

The stability data is covering the stability of all components of the kit, except for diluted wash buffer and opened slides. Diluted wash buffer is stable for 1 week at 2-8°C, as has been shown in a wash buffer stability study. This is explicitly written in the IFU. Also noted in the IFU is the recommendation to process slides immediately upon opening the silde foil.

Agreements were calculated for individual and combined readers. Results of the kit controls were not included in the agreement calculations.

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	> 85%
Negative Agreement	> 85%
Overall Agreement	> 85%
Total Pattern Agreement	> 85%
Fluorescence Intensity Agreement	> 85%

Results:

Table 103: In Use Stability AESKUSLIDES ANCA Ethanol – Number of Samples:

No of samples (Incorrect/total)	Lot 1			Lot 2			Lot 3		
	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers
Positive Agreement	1 / 70	1 / 70	2 / 140	1 / 70	1 / 70	2 / 140	0 / 70	0 / 70	0 / 140
Negative Agreement	0 / 10	0 / 10	0 / 20	0 / 10	0 / 10	0 / 20	0 / 10	0 / 10	0 / 20
Overall Agreement	1 / 80	1 / 80	2 / 160	1 / 80	1 / 80	2 / 160	0 / 80	0 / 80	0 / 160
Pattern Agreement	1 / 70	1 / 70	2 / 140	4 / 70	4 / 70	8 / 140	0 / 70	1 / 70	1 / 140
FI Agreement	0 / 80	0 / 80	0 / 160	0 / 80	0 / 80	0 / 160	0 / 80	0 / 80	0 / 160

FI = Fluorescence Intensity

Table 104: In Use Stability AESKUSLIDES ANCA Ethanol – % Agreement:

% Agreement (95% CI)	Lot 1			Lot 2			Lot 3		
	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers
Positive Agreement	98.6 (92.3 - 99.7)	98.6 (92.3 - 99.7)	98.6 (94.9 - 99.6)	98.6 (92.3 - 99.7)	98.6 (92.3 - 99.7)	98.6 (94.9 - 99.6)	100 (94.8 - 100)	100 (94.8 - 100)	100 (97.3 - 100)
Negative Agreement	100 (72.2 - 100)	100 (72.2 - 100)	100 (83.9 - 100)	100 (72.2 - 100)	100 (72.2 - 100)	100 (83.9 - 100)	100 (72.2 - 100)	100 (72.2 - 100)	100 (83.9 - 100)
Overall Agreement	98.8 (93.3 - 99.8)	98.8 (93.3 - 99.8)	98.8 (95.6 - 99.7)	98.8 (93.3 - 99.8)	98.8 (93.3 - 99.8)	98.8 (95.6 - 99.7)	100 (95.4 - 100)	100 (95.4 - 100)	100 (97.7 - 100)
Pattern Agreement	98.6 (92.3 - 99.7)	98.6 (92.3 - 99.7)	98.6 (94.9 - 99.6)	94.3 (86.2 - 97.8)	94.3 (86.2 - 97.8)	94.3 (89.1 - 97.1)	100 (94.8 - 100)	98.6 (92.3 - 99.7)	99.3 (96.1 - 99.9)
FI Agreement	100 (95.4 - 100)	100 (95.4 - 100)	100 (97.7 - 100)	100 (95.4 - 100)	100 (95.4 - 100)	100 (97.7 - 100)	100 (95.4 - 100)	100 (95.4 - 100)	100 (97.7 - 100)

FI = Fluorescence Intensity

Agreement calculations for AESKUSLIDES ANCA Ethanol included all data points of the whole test time span of 6 weeks at 2-8°C:

All agreements fulfilled the above acceptance criteria.

Positive agreement for AESKUSLIDES ANCA Ethanol for both readers ranged from 98.6% to 100% for all 3 reagent lots.

Negative agreement for ANCA Ethanol for both readers was 100% for all 3 reagent lots.

Overall agreement for ANCA Ethanol for both readers ranged from 98.8% to 100% for all 3 reagent lots.

Pattern agreement for ANCA Ethanol for both readers ranged from 94.3% to 99.3% for all 3 reagent lots.

FI agreement for ANCA Ethanol for both readers was 100% for all 3 reagent lots.

No deviations of fluorescence intensities greater than +/-1 from the initial value have been observed.

Table 105: In Use AESKUSLIDES ANCA Formalin – Number of Samples (including borderline samples 5 and 6):

No of samples (Incorrect/total)	Lot 1			Lot 2			Lot 3		
	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers
Positive Agreement	13 / 60	21 / 60	34 / 120	11 / 60	16 / 60	27 / 120	11 / 60	19 / 60	30 / 120
Negative Agreement	0 / 20	0 / 20	0 / 40	0 / 20	0 / 20	0 / 40	0 / 20	0 / 20	0 / 40
Overall Agreement	13 / 80	21 / 80	34 / 160	11 / 80	16 / 80	27 / 160	11 / 80	19 / 80	30 / 160
Pattern Agreement	13 / 60	21 / 60	34 / 120	11 / 60	16 / 60	27 / 120	11 / 60	19 / 60	30 / 120
FI Agreement	0 / 80	0 / 80	0 / 160	0 / 80	0 / 80	0 / 160	0 / 80	2 / 80	2 / 160

FI = Fluorescence Intensity

Table 106: In Use Stability AESKUSLIDES ANCA Formalin – % Agreement (including borderline samples 5 and 6):

% Agreement (95% CI)	Lot 1			Lot 2			Lot 3		
	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers
Positive Agreement	78.3 (66.4 - 86.9)	65 (52.4 - 75.8)	71.7 (63 - 79)	81.7 (70.1 - 89.4)	73.3 (61 - 82.9)	77.5 (69.2 - 84.1)	81.7 (70.1 - 89.4)	68.3 (55.8 - 78.7)	75 (66.6 - 81.9)
Negative Agreement	100 (83.9 - 100)	100 (83.9 - 100)	100 (91.2 - 100)	100 (83.9 - 100)	100 (83.9 - 100)	100 (91.2 - 100)	100 (83.9 - 100)	100 (83.9 - 100)	100 (91.2 - 100)
Overall Agreement	83.8 (74.2 - 90.3)	73.8 (63.2 - 82.1)	78.8 (71.8 - 84.4)	86.3 (77 - 92.1)	80 (70 - 87.3)	83.1 (76.6 - 88.1)	86.3 (77 - 92.1)	76.3 (65.9 - 84.2)	81.3 (74.5 - 86.5)
Pattern Agreement	78.3 (66.4 - 86.9)	65 (52.4 - 75.8)	71.7 (63 - 79)	81.7 (70.1 - 89.4)	73.3 (61 - 82.9)	77.5 (69.2 - 84.1)	81.7 (70.1 - 89.4)	68.3 (55.8 - 78.7)	75 (66.6 - 81.9)
FI Agreement	100 (95.4 - 100)	100 (95.4 - 100)	100 (97.7 - 100)	100 (95.4 - 100)	100 (95.4 - 100)	100 (97.7 - 100)	100 (95.4 - 100)	97.5 (91.3 - 99.3)	98.8 (95.6 - 99.7)

FI = Fluorescence Intensity

Table 107: In Use AESKUSLIDES ANCA Formalin – Number of Samples (excluding borderline samples 5 and 6):

No of samples (Incorrect/total)	Lot 1			Lot 2			Lot 3		
	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers
Positive Agreement	0 / 40	2 / 40	2 / 80	0 / 40	1 / 40	1 / 80	0 / 40	2 / 40	2 / 80
Negative Agreement	0 / 20	0 / 20	0 / 40	0 / 20	0 / 20	0 / 40	0 / 20	0 / 20	0 / 40
Overall Agreement	0 / 60	2 / 60	2 / 120	0 / 60	1 / 60	1 / 120	0 / 60	2 / 60	2 / 120
Pattern Agreement	0 / 40	2 / 40	2 / 80	0 / 40	1 / 40	1 / 80	0 / 40	2 / 40	2 / 80
FI Agreement	0 / 60	0 / 60	0 / 120	0 / 60	0 / 60	0 / 120	0 / 60	2 / 60	2 / 120

FI = Fluorescence Intensity

Table 108: In Use Stability AESKUSLIDES ANCA Formalin – % Agreement (excluding borderline samples 5 and 6):

% Agreement (95% CI)	Lot 1			Lot 2			Lot 3		
	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers	Reader 1	Reader 2	both Readers
Positive Agreement	100 (91.2 - 100)	95 (83.5 - 98.6)	97.5 (91.3 - 99.3)	100 (91.2 - 100)	97.5 (87.1 - 99.6)	98.8 (93.3 - 99.8)	100 (91.2 - 100)	95 (83.5 - 98.6)	97.5 (91.3 - 99.3)
Negative Agreement	100 (83.9 - 100)	100 (83.9 - 100)	100 (91.2 - 100)	100 (83.9 - 100)	100 (83.9 - 100)	100 (91.2 - 100)	100 (83.9 - 100)	100 (83.9 - 100)	100 (91.2 - 100)
Overall Agreement	100 (94 - 100)	96.7 (88.6 - 99.1)	98.3 (94.1 - 99.5)	100 (94 - 100)	98.3 (91.1 - 99.7)	99.2 (95.4 - 99.9)	100 (94 - 100)	96.7 (88.6 - 99.1)	98.3 (94.1 - 99.5)
Pattern Agreement	100 (91.2 - 100)	95 (83.5 - 98.6)	97.5 (91.3 - 99.3)	100 (91.2 - 100)	97.5 (87.1 - 99.6)	98.8 (93.3 - 99.8)	100 (91.2 - 100)	95 (83.5 - 98.6)	97.5 (91.3 - 99.3)
FI Agreement	100 (94 - 100)	100 (94 - 100)	100 (96.9 - 100)	100 (94 - 100)	100 (94 - 100)	100 (96.9 - 100)	100 (94 - 100)	96.7 (88.6 - 99.1)	98.3 (94.1 - 99.5)

FI = Fluorescence Intensity

Agreement calculations for AESKUSLIDES ANCA Formalin included all data points of a test time span of 6 weeks at 2-8°C:

The sample set for AESKUSLIDES ANCA Formalin included two samples (sample 5 and 6) that were borderline positive. For these samples we expect either a low positive result (FI=1) or a negative result (FI=0). Therefore, these samples lead to many “false negatives” when they are defined as “positive” for the calculation (or to many “false positives” when they are defined as “negative” for the calculation). These “false negatives” (or “false positives”) have a big impact on positive agreement and on pattern agreement which is due to normal test variances that have a bigger impact on borderline samples than on samples that are clearly negative or clearly positive. Therefore, the lower positive (and pattern) agreements we observed with the agreement calculation for ANCA Formalin are not due to stability issues of the assay.

Therefore, we did an alternative agreement calculation excluding these two borderline positive samples. In this calculation all acceptance criteria have been met:

Positive agreement for AESKUSLIDES ANCA Formalin for both readers ranged from 97.5% to 98.8% for all 3 reagent lots.

Negative agreement for ANCA Formalin for both readers was 100% for all 3 reagent lots.

Overall agreement for ANCA Formalin for both readers ranged from 98.3% to 99.2% for all 3 reagent lots.

Pattern agreement for ANCA Formalin for both readers ranged from 97.5% to 98.8% for all 3 reagent lots.

FI agreement for ANCA Formalin for both readers ranged from 98.3% to 100% for all 3 reagent lots. No deviations of fluorescence intensities greater than +/-1 from the initial value have been observed.

<u>Conclusion:</u>
For AESKUSLIDES ANCA Ethanol all acceptance criteria were met for a time span of 6 weeks at 2-8°C. Therefore, we claim an In Use Stability of 6 weeks at 2-8°C storage temperature.

For AESKUSLIDES ANCA Formalin all acceptance criteria were met for a time span of 6 weeks at 2-8°C. Therefore, we claim an In Use Stability of 6 weeks at 2-8°C storage temperature.

Transport Stability Report

To determined transport stability of AESKUSLIDES ANCA, data were taken from Accelerated Stability Report, as these conditions used in the study simulate a worst case scenario of transport of the kits.

The kit components were incubated at 37°C over a period of 6 weeks.

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	> 85%
Negative Agreement	> 85%
Overall Agreement	> 85%
Total Pattern Agreement	> 85%
Fluorescence Intensity Agreement	> 85%

Conclusion:
All criteria were fulfilled in each test. Therefore, we claim that the kit can resist a temperature of 37°C for at least 2 weeks.

For more information please see at “Accelerated Stability Report”.

2. *Comparison studies*

Method comparison with predicate device:

To show comparable performance of AESKUSLIDES ANCA (Ethanol and Formalin) and the predicate assay NOVA Lite ANCA (Ethanol and Formalin) of INOVA.

Procedure:

A method comparison study with 507 serum samples (comprising 132 serum samples from patients with AAV and 375 samples from patients with other diseases) was performed in house. A detailed description of the study sample set can be found on page 12-79, table 110.

Healthy donor samples have been excluded from this study.

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

All assays were performed according to the respective IFUs. Sample were screened at a dilution of 1:20.

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

Furthermore, diagnostic sensitivity and specificity were calculated for both assays, and the cross reactivities for different diagnoses are shown in a table.

Results:

Results Predicate comparison ANCA Ethanol

Diagnostic Sensitivity and Specificity

Table 109a: Method comparison with predicate device_ANCA Ethanol_Diagnostic Sensitivity and Specificity Number of positive and negative results:

Diagnostic Sensitivity & Specificity		Diagnosis		
		AAVs	Other Diseases	Total
AESKUSLIDES ANCA Ethanol	Pos	64	115	179
	Neg	68	260	328
	Total	132	375	507
Predicate Assay	Pos	48	168	216
	Neg	84	207	291
	Total	132	375	507

Table 109b: Method comparison with predicate device_ANCA Ethanol_Diagnostic Sensitivity and Specificity PPV/NPV:

% 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)
	AAVs (n= 132)	OD (n=375)		
AESKUSLIDES ANCA Ethanol	48.5 (40.1 - 56.9)	69.3 (64.5 - 73.8)	35.8%	79.3%
Predicate Assay	36.4 (28.7 - 44.8)	55.2 (50.1 - 60.2)	22.2%	71.1%

Sensitivity of AESKUSLIDES ANCA Ethanol is higher (48.5%) than that calculated for the predicate assay (36.4%). Specificity for AESKUSLIDES ANCA Ethanol was 69.3%, whereas for the predicate assay a specificity of 55.2% was calculated. Values reported in literature are variable and spread over a wide range, depending on the serum panel used in the study. The ANCA titer may be associated with disease activity and may also disappear upon treatment. The AAV cohort tested in this study was not selected for disease activity or treatment.

The higher sensitivity and specificity of AESKUSLIDES ANCA Ethanol go along with a higher PPV (35.0% compared to 22.2% for the predicate) and NPV (79.3% vs 71.1%).

The results for both assays are shown in detail in the cross-reactivity table below. With AESKUSLIDES ANCA Ethanol fewer cross-reactivity are seen. Cross-reactivity could be detected in autoimmune liver diseases (up to 50%), inflammatory bowel diseases (up to 50.7%) and also other rheumatic diseases (SLE, RA, Scleroderma: up to 47.4%). The predicate assay detected positives in similar diagnoses but with higher number of positives.

Table 110: Method comparison with predicate device_ANCA Ethanol. Number and percent positive samples in the different tested diagnosis

Diagnosis			n	AESKUSLIDES ANCA Ethanol				Predicate			
				n Pos	%Pos	n Neg	%Neg	n Pos	%Pos	n Neg	%Neg
Target Diagnosis	ANCA associated Vasculitis (AAV)	Wegener's Granulomatosis	78	33	42.3%	45	57.7%	20	25.6%	58	74.4%
		MPA	29	22	75.9%	7	24.1%	20	69.0%	9	31.0%
		Churg-Strauss Syndrome	25	9	36.0%	16	64.0%	8	32.0%	17	68.0%
Control diagnosis	Autoimmune Liver Diseases	Autoimmune Hepatitis (AIH)	8	3	37.5%	5	62.5%	5	62.5%	3	37.5%
		Autoimmune Hepatitis/ Primary biliary cholangitis	6	3	50.0%	3	50.0%	6	100.0%	0	0.0%
		Primary biliary cholangitis (PBC)	11	2	18.2%	9	81.8%	8	72.7%	3	27.3%
		Primary sclerosing cholangitis (PSC)	10	5	50.0%	5	50.0%	5	50.0%	5	50.0%
	Inflammatory bowel diseases	Ulcerative Colitis	71	36	50.7%	35	49.3%	42	59.2%	29	40.8%
		Crohn's disease	40	16	40.0%	24	60.0%	26	65.0%	14	35.0%
		Inflammatory bowel disease (IBD)	9	4	44.4%	5	55.6%	5	55.6%	4	44.4%
	Other rheumatic diseases	Rheumatoid Arthritis (RA)	12	4	33.3%	8	66.7%	9	75.0%	3	25.0%
		Systemic Lupus Erythematosus (SLE)	30	14	46.7%	16	53.3%	22	73.3%	8	26.7%
		Scleroderma	19	9	47.4%	10	52.6%	13	68.4%	6	31.6%
		Myositis	1	0	0.0%	1	100.0%	0	0.0%	1	100.0%
	Infections	Hepatitis C Virus (HCV)	16	3	18.8%	13	81.3%	5	31.3%	11	68.8%
		Hepatitis B Virus (HBV)	8	0	0.0%	8	100.0%	0	0.0%	8	100.0%
	Vasculitides NOT associated with ANCA	Polymyalgia rheumatica (PMR)	18	3	16.7%	15	83.3%	5	27.8%	13	72.2%
		Giant cell artertitis (GCA)	4	0	0.0%	4	100.0%	2	50.0%	2	50.0%
		Purpura	1	1	100.0%	0	0.0%	1	100.0%	0	0.0%
	Leukemia	Lymphoma, Myeloma	15	3	20.0%	12	80.0%	4	26.7%	11	73.3%
	Other diagnosis	Celiac disease	10	0	0.0%	10	100.0%	0	0.0%	10	100.0%
		Chronic kidney disease	32	2	6.3%	30	93.8%	3	9.4%	29	90.6%
		Sinusitis	22	4	18.2%	18	81.8%	4	18.2%	18	81.8%
		Asthma	32	3	9.4%	29	90.6%	3	9.4%	29	90.6%
Sum			507	179	35.3%	328	64.7%	216	42.6%	291	57.4%

Relative sensitivity and specificity

Table 110a: Method comparison with predicate device_ANCA Ethanol_Relative Sensitivity and Specificity Number of positive and negative results and % agreements:

Method Comparison		Predicate		
		Pos	Neg	Total
AESKU ANCA Ethanol	Pos	145	34	179
	Neg	71	257	328
	Total	216	291	507

Method Comparison Predicate vs AESKU	% Agreement (95% CI)
Positive Agreement	67.1 (60.6 - 73)
Negative Agreement	88.3 (84.1 - 91.5)
Overall Agreement	79.3 (75.5 - 82.6)

Positive, negative and overall agreement between both assays were acceptable (67.1%, 88.3% and 79.3%). The rather low positive agreement was due to the fact, that the predicate assay detects less positive results.

Conclusions

AESKUSLIDES ANCA Ethanol showed higher diagnostic sensitivity (48.5%) and specificity (69.3%) compared to the predicate assay NOVA Lite provided by INOVA (36.4%, 55.2%). This is due to the fact, that AESKU assay detects more positives in the AAV cohort, and less positives in the other disease groups.

This study shows that the AESKU assay is comparable or better than the predicate device.

Results Predicate comparison ANCA Formalin

Diagnostic Sensitivity and Specificity

Table 111: Method comparison with predicate device_ANCA Formalin_Diagnostic Sensitivity and Specificity Number of positive and negative results:

Diagnostic Sensitivity & Specificity		Diagnosis		
		AAVs	Other Diseases	Total
AESKUSLIDES ANCA Formalin	Pos	66	35	101
	Neg	66	340	406
	Total	132	375	507
Predicate Assay	Pos	50	32	82
	Neg	82	343	425
	Total	132	375	507

Table 112: Method comparison with predicate device_ANCA Formalin_Diagnostic Sensitivity and Specificity PPV/NPV:

% 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)
	AAVs (n= 132)	OD (n=375)		
AESKUSLIDES ANCA Formalin	50 (41.6 - 58.4)	90.7 (87.3 - 93.2)	65.3%	83.7%
Predicate Assay	37.9 (30.1 - 46.4)	91.5 (88.2 - 93.9)	61.0%	80.7%

Sensitivity of AESKUSLIDES ANCA Formalin is higher (50.0%) than that calculated for the predicate assay (37.9%) in this study. Specificities for both assays were comparable (90.7% vs 91.5%). PPVs and NPVs of both assays were comparable. However, values for AESKUSLIDES were slightly higher.

The cross-reactivity table shows equivalent results for both assays. Cross-reactivity could be detected in AIH (25.0%), PSC (20.0%), Ulcerative Colitis (16.9% and SLE (20.0%)

Table 113: Method comparison with predicate device_ANCA Formalin. Number and percent positive samples in the different tested diagnosis

Diagnosis			n	AESKUSLIDES ANCA Formalin				Predicate			
				n Pos	%Pos	n Neg	%Neg	n Pos	%Pos	n Neg	%Neg
Target Diagnosis	ANCA associated Vasculitis (AAV)	Wegener's Granulomatosis	78	45	57.7%	33	42.3%	37	47.4%	41	52.6%
		MPA	29	15	51.7%	14	48.3%	12	41.4%	17	58.6%
		Churg-Strauss Syndrome	25	6	24.0%	19	76.0%	1	4.0%	24	96.0%
Control diagnosis	Autoimmune Liver Diseases	Autoimmune Hepatitis (AIH)	8	2	25.0%	6	75.0%	1	12.5%	7	87.5%
		Autoimmune Hepatitis/ Primary biliary cholangitis	6	0	0.0%	6	100.0%	0	0.0%	6	100.0%
		Primary biliary cholangitis (PBC)	11	0	0.0%	11	100.0%	0	0.0%	11	100.0%
		Primary sclerosing cholangitis (PSC)	10	2	20.0%	8	80.0%	0	0.0%	10	100.0%
	Inflammatory bowel diseases	Ulcerative Colitis	71	12	16.9%	59	83.1%	10	14.1%	61	85.9%
		Crohn's disease	40	1	2.5%	39	97.5%	0	0.0%	40	100.0%
		Inflammatory bowel disease (IBD)	9	1	11.1%	8	88.9%	1	11.1%	8	88.9%
	Other rheumatic diseases	Rheumatoid Arthritis (RA)	12	1	8.3%	11	91.7%	1	8.3%	11	91.7%
		Systemic Lupus Erythematosus (SLE)	30	6	20.0%	24	80.0%	8	26.7%	22	73.3%
		Scleroderma	19	0	0.0%	19	100.0%	2	10.5%	17	89.5%
		Myositis	1	0	0.0%	1	100.0%	0	0.0%	1	100.0%
	Infections	Hepatitis C Virus (HCV)	16	2	12.5%	14	87.5%	3	18.8%	13	81.3%
		Hepatitis B Virus (HBV)	8	0	0.0%	8	100.0%	0	0.0%	8	100.0%
	Vasculitides NOT associated with ANCA	Polymyalgia rheumatica (PMR)	18	2	11.1%	16	88.9%	1	5.6%	17	94.4%
		Giant cell artertitis (GCA)	4	0	0.0%	4	100.0%	2	50.0%	2	50.0%
		Purpura	1	1	100.0%	0	0.0%	1	100.0%	0	0.0%
	Leukemia	Lymphoma, Myeloma	15	2	13.3%	13	86.7%	0	0.0%	15	100.0%
	Other diagnosis	Celiac disease	10	0	0.0%	10	100.0%	0	0.0%	10	100.0%
		Chronic kidney disease	32	2	6.3%	30	93.8%	1	3.1%	31	96.9%
		Sinusitis	22	1	4.5%	21	95.5%	1	4.5%	21	95.5%
		Asthma	32	0	0.0%	32	100.0%	0	0.0%	32	100.0%
Sum			507	101	19.9%	406	80.1%	82	16.2%	425	83.8%

Relative sensitivity and specificity

Table 114: Method comparison with predicate device_ANCA Formalin_Relative Sensitivity and Specificity Number of positive and negative results and % agreements:

Method Comparison		Predicate		
		Pos	Neg	Total
AESKUSLIDES ANCA Formalin	Pos	66	35	101
	Neg	16	390	406
	Total	82	425	507

Method Comparison ANCA Formalin	% Agreement (95% CI)
Positive Agreement	80.5 (70.6 - 87.6)
Negative Agreement	91.8 (88.8 - 94)
Overall Agreement	89.9 (87 - 92.3)

Overall agreement between both assays was good (89.9%). The same holds true for the negative agreement (91.8%). The relatively low positive agreement (80.5%) is due to the fact, that the AESKU assay detects more samples as positive.

Conclusions

AESKUSLIDES ANCA Formalin showed a diagnostic sensitivity (50.0%) compared to the predicate assay NOVA Lite provided by INOVA (37.9%). However, the diagnostic sensitivity was comparable between the two (90.7% vs 91.5%).

This study shows that the AESKU assay is comparable or better than the predicate device.

Method Comparison of Method A, B, and C and clinical study

To show comparable performance of AESKUSLIDES ANCA Ethanol and ANCA Formalin when processed manually and on HELIOS.

Procedure:
A clinical study with the same 630 serum samples (comprising 135 serum samples from patients with AAV, 120 characterized MPO/PR3/ANCA positive sera and 375 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 12-87/88, table 118.

At each study site the sample set was processed

- manually and analyzed (positive/negative classification) at the microscope by two independent readers (Method C)
- on HELIOS and HELIOS images were analyzed by two independent readers at the PC (Method B)
- on HELIOS and positive/negative classification was done by the HELIOS Vasculitis Pattern Plus Software (ANCA) (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. For the calculation of diagnostic sensitivity and specificity the 120 characterized MPO/PR3/ANCA positive sera have been excluded.

Acceptance Criteria:

Positive, Negative, and Overall Agreements between the different Methods should be >85%.

Positive, Negative, Overall and Pattern Agreements between the different methods were set to be >80%. This is due to the fact that unknown clinical samples often results in mixed patterns, which are not easy to characterize. For example, in general one reader may report a sample with unspecific fluorescence as positive undefined, whereas another evaluates it as negative. Thus, the evaluation of ANCA slides is very subjective and strongly dependent on the reader's experience. Therefore, we expect and accept variances up to 15% in this clinical study.

For Method A we accept a lower agreement (>70%). Comparing Methods B vs A and C vs A. In general, method A (HELIOS Vasculitis Pattern Plus software) detects less positive samples. In addition, positive samples are mostly classified as positive undefined. This can be explained by the complex patterns, which can be distinguished by the readers at the microscope or at the screen, but to limited extent by the software. Because of this, all results generated by the HELIOS (Method A) must be confirmed by trained personnel.

Results:

Results Clinical study and method comparison ANCA Ethanol

Table 115: Method Comparison of Method A, B and C_ANCA Ethanol_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	86.5 (80.9 - 90.6)	85.9 (80.2 - 90.1)	79 (72.3 - 84.5)	79 (72.3 - 84.5)	70.8 (64 - 76.8)	70.7 (63.9 - 76.7)
	Negative Agreement	99.8 (98.7 - 100)	99.3 (98 - 99.8)	98.3 (96.6 - 99.1)	98.3 (96.6 - 99.1)	99.1 (97.7 - 99.6)	98.9 (97.4 - 99.5)
	Total Agreement	95.7 (93.8 - 97)	95.2 (93.3 - 96.6)	93.2 (90.9 - 94.9)	93.2 (90.9 - 94.9)	90.5 (87.9 - 92.5)	90.3 (87.8 - 92.4)
Site 2	Positive Agreement	88.5 (83.4 - 92.1)	92.8 (88.4 - 95.6)	92.6 (88 - 95.6)	86.9 (81.6 - 90.8)	83.2 (77.5 - 87.6)	81.6 (75.8 - 86.3)
	Negative Agreement	98.6 (96.9 - 99.3)	96.7 (94.5 - 98)	98.6 (97.1 - 99.4)	99.3 (97.9 - 99.8)	97.9 (96 - 98.9)	96.9 (94.8 - 98.2)
	Total Agreement	95.2 (93.3 - 96.6)	95.4 (93.5 - 96.8)	96.8 (95.1 - 97.9)	95.2 (93.3 - 96.6)	93 (90.8 - 94.8)	91.9 (89.5 - 93.8)
Site 3	Positive Agreement	90.2 (85.6 - 93.5)	85.1 (79.5 - 89.4)	79.5 (73.6 - 84.3)	83.8 (78.2 - 88.2)	76 (70 - 81.1)	79 (72.7 - 84.1)
	Negative Agreement	96 (93.7 - 97.6)	91.3 (88.2 - 93.6)	99 (97.5 - 99.6)	98.4 (96.6 - 99.2)	98.3 (96.5 - 99.2)	94.5 (91.9 - 96.3)
	Total Agreement	94 (91.8 - 95.6)	89.4 (86.7 - 91.5)	92.2 (89.9 - 94.1)	93.7 (91.5 - 95.3)	90.3 (87.8 - 92.4)	89.7 (87.1 - 91.8)

Table 116: Method Comparison of Method A, B and C_ANCA Ethanol_Combined Reader Agreements

% Agreement (95% CI)		Method C vs B	Method B vs A	Method C vs A
Site 1	Positive Agreement	86.2 (82.3 - 89.3)	79 (74.4 - 83.1)	70.8 (66 - 75.1)
	Negative Agreement	99.5 (98.8 - 99.8)	98.3 (97.2 - 98.9)	99 (98.1 - 99.5)
	Total Agreement	95.5 (94.2 - 96.5)	93.2 (91.6 - 94.4)	90.4 (88.6 - 91.9)
Site 2	Positive Agreement	90.6 (87.4 - 93)	89.6 (86.3 - 92.3)	82.4 (78.5 - 85.8)
	Negative Agreement	97.6 (96.4 - 98.5)	99 (98 - 99.5)	97.4 (96.1 - 98.3)
	Total Agreement	95.3 (94 - 96.4)	96 (94.8 - 97)	92.5 (90.9 - 93.8)
Site 3	Positive Agreement	87.9 (84.4 - 90.6)	81.6 (77.6 - 85)	77.4 (73.1 - 81.1)
	Negative Agreement	93.6 (91.7 - 95)	98.7 (97.7 - 99.3)	96.3 (94.8 - 97.4)
	Total Agreement	91.7 (90 - 93.1)	92.9 (91.4 - 94.2)	90 (88.2 - 91.5)

Table 117: Method Comparison of Method A, B and C – Pattern Agreements

% Agreement (95% CI)	Method C vs B	Method B vs A	Method C vs A
Site 1	87 (85 - 88.7)	81 (78.8 - 82.9)	77.8 (75.4 - 79.9)
Site 2	89.2 (87.5 - 90.7)	84.7 (82.6 - 86.5)	81.8 (79.7 - 83.8)
Site 3	82.5 (80.5 - 84.2)	85.6 (83.7 - 87.4)	80.3 (78.2 - 82.3)
All sites	86.2 (85.2 - 87.2)	83.8 (82.6 - 84.8)	80 (78.7 - 81.2)

Table 118: Method comparison. Number and percent positive samples in the different tested diagnosis_ANCA Ethanol

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	
		Reader 1		Reader 2		Reader 1		Reader 2				Reader 3		Reader 4		Reader 3		Reader 4				Reader 5		Reader 6		Reader 5		Reader 6			
Diagnosis	n	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
Wegener's	80	13	16.3	13	16.3	13	16.3	13	16.3	11	13.8	20	25.0	22	27.5	17	21.2	21	26.2	17	21.2	19	23.7	21	26.2	20	25	22	27.5	17	21.2
MPA	29	18	62.1	18	62.1	16	55.2	16	55.2	14	48.3	19	65.5	18	62.0	20	68.9	20	68.9	20	68.9	19	65.5	19	65.5	19	65.5	19	65.5	18	62.0
Churg-Strauss	26	2	7.7	2	7.7	2	7.7	2	7.7	0	0.0	5	19.2	4	15.3	5	19.2	5	19.2	6	23.0	6	23.0	6	23.0	8	30.7	8	30.7	4	15.3
C-ANCA	20	14	70.0	14	70.0	13	65.0	13	65.0	11	55.0	17	85.0	17	85	16	80	16	80	14	70	14	70	13	65	13	65	14	70	13	65
P-ANCA	50	24	48.0	23	46.0	24	48.0	24	48.0	25	50.0	23	46.0	23	46	23	46	25	50	24	48	25	50	25	50	25	50	26	52	25	50
MPO	25	18	72.0	18	72.0	17	68.0	17	68.0	18	72.0	23	92.0	22	88	19	76	22	88	18	72	22	88	20	80	20	80	19	76	18	72
PR3	25	5	20.0	5	20.0	3	12.0	3	12.0	3	12.0	9	36.0	7	28	8	32	7	28	7	28	15	60	12	48	10	40	10	40	11	44
AIH	8	5	62.5	5	62.5	5	62.5	5	62.5	4	50.0	3	37.5	3	37.5	2	25	3	37.5	2	25	2	25	1	12.5	3	37.5	1	12.5	1	12.5
AIH/PBC	6	4	66.7	4	66.7	4	66.7	4	66.7	3	50.0	3	50.0	3	50	3	50	3	50	3	50	2	33.3	1	16.6	4	66.6	3	50	2	33.3
PBC	11	3	27.3	3	27.3	3	27.3	3	27.3	2	18.2	2	18.2	2	18.1	3	27.2	4	36.3	1	9.09	4	36.3	2	18.1	4	36.3	3	27.2	1	9.0
PSC	10	5	50.0	5	50.0	5	50.0	5	50.0	3	30.0	5	50.0	5	50	5	50	5	50	5	50	4	40	1	10	4	40	4	40	3	30
Ulcerative Colitis	71	28	39.4	28	39.4	16	22.5	16	22.5	15	21.1	30	42.3	30	42.2	28	39.4	29	40.8	28	39.4	32	45.0	27	38.0	34	47.8	25	35.2	27	38.0
Crohn`s	40	10	25.0	9	22.5	8	20.0	8	20.0	2	5.0	13	32.5	11	27.5	9	22.5	7	17.5	6	15	13	32.5	12	30	12	30	10	25	9	22.5
IBD	9	1	11.1	1	11.1	1	11.1	1	11.1	0	0.0	4	44.4	4	44.4	4	44.4	4	44.4	3	33.3	5	55.5	3	33.3	5	55.5	3	33.3	3	33.3
RA	12	1	8.3	1	8.3	1	8.3	1	8.3	1	8.3	1	8.3	2	16.6	0	0	1	8.33	0	0	4	33.3	4	33.3	1	8.3	1	8.3	1	8.3
SLE	30	16	53.3	16	53.3	15	50.0	15	50.0	12	40.0	14	46.7	14	46.6	14	46.6	14	46.6	13	43.3	16	53.3	12	40	14	46.6	12	40	10	33.3
Myositis	1	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	0	0	0	0	0	0	0	0	0
Scleroderma	19	10	52.6	10	52.6	10	52.6	10	52.6	6	31.6	5	26.3	8	42.1	4	21.0	9	47.3	5	26.3	8	42.1	0	0	10	52.6	9	47.3	5	26.3

HBV	8	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	1	12.5	1	12.5	0	0	0	0	0	0
HCV	16	3	18.8	3	18.8	2	12.5	2	12.5	3	18.8	3	18.8	3	18.7	3	18.7	3	18.7	3	18.7	3	18.7	3	18.7	3	18.7	3	18.7	2	12.5
GCA	4	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	0	0	0	0	0	0	0	0	0
PMR	18	4	22.2	4	22.2	3	16.7	3	16.7	3	16.7	2	11.1	2	11.1	0	0	1	5.5	0	0	3	16.6	3	16.6	2	11.1	2	11.1	1	5.5
Purpura	1	1	100	1	100	0	0.0	0	0.0	0	0.0	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100
Leukemia	15	3	20.0	4	26.7	2	13.3	2	13.3	1	6.7	2	13.3	2	13.3	2	13.3	2	13.3	2	13.3	3	20	2	13.3	3	20	2	13.3	2	13.3
Celiac disease	10	1	10.0	1	10.0	1	10.0	1	10.0	1	10.0	0	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CKD	32	1	3.1	1	3.1	1	3.1	1	3.1	1	3.1	1	3.1	1	3.1	1	3.1	1	3.1	0	0	1	3.1	3	9.3	1	3.1	3	9.3	1	3.1
Asthma	32	1	3.1	1	3.1	1	3.1	1	3.1	0	0.0	1	3.1	1	3.1	1	3.1	1	3.1	2	6.2	1	3.1	1	3.1	1	3.1	2	6.2	1	3.1
Sinusitis	22	1	4.5	1	4.5	1	4.5	1	4.5	1	4.5	2	9.1	2	9.0	2	9.0	2	9.0	2	9.0	2	9.0	2	9.0	2	9.0	2	9.0	2	9.0

Table 119: Diagnostic Sensitivity and Specificity_ANCA Ethanol

% Diagnostic Sensitivity & Specificity (95% CI)			% Sensitivity (95% CI)	% Specificity (95% CI)		% Sensitivity (95% CI)	% Specificity (95% CI)
			AAV (n= 135)	OD (n=375)		AAV (n= 135)	OD (n=375)
Site 1	Manual	Reader 1	24.4 (18 - 32.3)	73.9 (69.2 - 78.1)	Combined Readers	24.4 (19.7 - 29.9)	73.9 (70.6 - 76.9)
		Reader 2	24.4 (18 - 32.3)	73.9 (69.2 - 78.1)			
	Reader Confirmation at HELIOS	Reader 1	23 (16.7 - 30.7)	78.9 (74.5 - 82.8)	Combined Readers	23 (18.3 - 28.3)	78.9 (75.9 - 81.7)
		Reader 2	23 (16.7 - 30.7)	78.9 (74.5 - 82.8)			
	HELIOS		18.5 (12.9 - 25.9)	84.5 (80.5 - 87.8)			
Site 2	Manual	Reader 3	32.6 (25.3 - 40.9)	75.5 (70.9 - 79.5)	Combined Readers	32.6 (27.3 - 38.4)	75.2 (72 - 78.2)
		Reader 4	32.6 (25.3 - 40.9)	74.9 (70.3 - 79.1)			
	Reader Confirmation at HELIOS	Reader 3	31.1 (23.9 - 39.4)	78.1 (73.7 - 82)	Combined Readers	32.6 (27.3 - 38.4)	77.1 (73.9 - 79.9)
		Reader 4	34.1 (26.6 - 42.4)	76 (71.4 - 80)			
	HELIOS		31.9 (24.6 - 40.1)	79.7 (75.4 - 83.5)			
Site 3	Manual	Reader 5	32.6 (25.3 - 40.9)	72 (67.3 - 76.3)	Combined Readers	33.3 (28 - 39.2)	75.5 (72.3 - 78.4)
		Reader 6	34.1 (26.6 - 42.4)	78.9 (74.5 - 82.8)			
	Reader Confirmation at HELIOS	Reader 5	34.8 (27.3 - 43.2)	72.3 (67.5 - 76.6)	Combined Readers	35.6 (30.1 - 41.4)	74.7 (71.4 - 77.6)
		Reader 6	36.3 (28.7 - 44.7)	77.1 (72.5 - 81)			
	HELIOS		28.9 (21.9 - 37)	80.8 (76.5 - 84.5)			

Note: The 120 characterized MPO/PR3/ANCA positive sera have been excluded from the calculation of diagnostic sensitivity and specificity.

Summary of Results:

Positive, Negative, Overall and Pattern Agreements for Method Comparison between Method C (Manual) and Method B (Reader Confirmation of HELIOS images) were > 80%. All acceptance criteria were met.

Positive Agreement of Method C vs B ranged from 85.1% to 92.8%.

Negative Agreement of Method C vs B ranged 91.3% to 99.8%.

Overall Agreement of Method C vs B ranged from 89.4% to 95.7%.

Pattern Agreement of Method C vs B ranged from 82.5% to 89.2%.

The results show that Methods C (Manual) and B (Reader Confirmation of HELIOS images) give equivalent results. Furthermore, the results of the 3 study site were comparable with each other.

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

Positive Agreement of Method B vs A ranged from 79.0% to 92.6%.

Negative Agreement of Method B vs A ranged from 98.3% to 99.3%.

Overall Agreement of Method B vs A ranged from 92.2% to 96.8%.

Pattern Agreement of Method B vs A ranged from 81.0% to 85.6%.

According to this, acceptance criteria for comparison of Method B vs A were met. 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 70.7% to 83.2%.

Negative Agreement of Method C vs A ranged from 94.5% to 99.1%.

Overall Agreement of Method C vs A ranged from 89.7% to 93.0%.

Pattern Agreement of Method C vs A ranged from 77.8% to 81.8%.

Overall, the crossreactivity table (table No.96) shows few crossreactivities for Method A, B and C. The most crossreactivities could be seen in the autoimmune liver disease group (AIH, PBC, PSC: up to 66.7%) and in inflammatory bowel diseases (Ulcerative Colitis, Crohn’s disease: up to 45.0%). However, these values are in accordance with literature, where it is stated that ANCAs could also be associated with inflammatory bowel disease and autoimmune liver disease.

Results Clinical study and method comparison ANCA Formalin

Table 120: Method Comparison of Method A, B and C_ANCA Formalin_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	87.5 (80.9 - 92)	85.7 (79.1 - 90.5)	84.9 (78.2 - 89.8)	82.2 (75.2 - 87.5)	75 (67.1 - 81.5)	71.4 (63.7 - 78.1)
	Negative Agreement	94.5 (92.2 - 96.2)	95.9 (93.7 - 97.3)	99.2 (97.9 - 99.7)	98.3 (96.8 - 99.2)	94.7 (92.4 - 96.4)	95.2 (93 - 96.8)
	Total Agreement	93 (90.8 - 94.8)	93.5 (91.3 - 95.2)	95.9 (94 - 97.2)	94.6 (92.6 - 96.1)	90.5 (87.9 - 92.5)	89.7 (87.1 - 91.8)
Site 2	Positive Agreement	86.8 (79.9 - 91.6)	87 (81 - 91.4)	88.7 (81.9 - 93.2)	73.6 (66.7 - 79.5)	79.8 (72.1 - 85.9)	71 (63.6 - 77.4)
	Negative Agreement	97.6 (95.9 - 98.6)	92.1 (89.3 - 94.2)	94.1 (91.6 - 95.8)	98 (96.3 - 98.9)	92.6 (90 - 94.6)	94.6 (92.2 - 96.3)
	Total Agreement	95.4 (93.5 - 96.8)	90.8 (88.3 - 92.8)	93 (90.7 - 94.7)	91.1 (88.6 - 93.1)	90 (87.4 - 92.1)	88.6 (85.8 - 90.8)
Site 3	Positive Agreement	89.6 (83.8 - 93.5)	88.6 (83.2 - 92.4)	99.4 (96.6 - 99.9)	98.7 (96.1 - 99.5)	98.7 (95.4 - 99.6)	92.9 (88.3 - 95.8)
	Negative Agreement	94.9 (92.6 - 96.6)	86.3 (82.8 - 89.2)	72.2 (67.9 - 76)	82.7 (78.7 - 86.1)	70.7 (66.5 - 74.6)	73 (68.7 - 76.9)
	Total Agreement	93.6 (91.5 - 95.3)	87 (84.1 - 89.4)	79.2 (75.8 - 82.2)	88.4 (85.7 - 90.7)	77.6 (74.2 - 80.7)	78.9 (75.5 - 81.9)

Table 121: Method Comparison of Method A, B and C_ANCA Formalin_Combined Reader Agreements

% Agreement (95% CI)		Method C vs B	Method B vs A	Method C vs A
Site 1	Positive Agreement	86.6 (82.1 - 90.1)	83.6 (78.9 - 87.4)	73.1 (67.7 - 78)
	Negative Agreement	95.2 (93.7 - 96.4)	98.8 (97.8 - 99.3)	95 (93.4 - 96.2)
	Total Agreement	93.3 (91.7 - 94.5)	95.2 (93.9 - 96.3)	90.1 (88.3 - 91.6)
Site 2	Positive Agreement	86.9 (82.6 - 90.3)	79.8 (74.9 - 83.9)	74.9 (69.6 - 79.5)
	Negative Agreement	94.9 (93.4 - 96.1)	95.9 (94.5 - 97)	93.6 (91.9 - 95)
	Total Agreement	93.1 (91.5 - 94.4)	92.1 (90.4 - 93.4)	89.3 (87.4 - 90.9)
Site 3	Positive Agreement	89.1 (85.3 - 92)	99 (97.4 - 99.6)	95.6 (92.8 - 97.3)
	Negative Agreement	90.8 (88.7 - 92.5)	77.1 (74.2 - 79.7)	71.8 (68.9 - 74.7)
	Total Agreement	90.3 (88.5 - 91.8)	83.8 (81.6 - 85.7)	78.2 (75.9 - 80.4)

Table 122: Method Comparison of Method A, B and C_ANCA Formalin_Pattern Agreements

% Agreement (95% CI)	Method C vs B	Method B vs A	Method C vs A
Site 1	82.1 (79.9 - 84.2)	90.8 (89.1 - 92.3)	77.9 (75.5 - 80.1)
Site 2	87.5 (85.5 - 89.2)	84 (81.9 - 86)	79.6 (77.3 - 81.7)
Site 3	82.1 (79.8 - 84.1)	76.1 (73.7 - 78.4)	69.7 (67.1 - 72.2)
All sites	83.9 (82.7 - 85)	83.7 (82.4 - 84.8)	75.7 (74.3 - 77.1)

Table 123: Method comparison. Number and percent positive samples in the different tested diagnosis_ANCA Formalin

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	
		Reader 1		Reader 2		Reader 1		Reader 2				Reader 3		Reader 4		Reader 3		Reader 4				Reader 5		Reader 6		Reader 5		Reader 6			
Diagnosis	n	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
Wegener's	80	39	48.8	42	52.5	45	56.3	43	53.8	41	51.3	36	45.0	39	48.8	31	38.8	37	46.3	37	46.3	38	47.5	51	63.8	38	47.5	50	62.5	56	70.0
MPA	29	13	44.8	12	41.4	15	51.7	14	48.3	14	48.3	11	37.9	16	55.2	13	44.8	17	58.6	14	48.3	12	41.4	13	44.8	13	44.8	14	48.3	19	65.5
Churg-Strauss	26	2	7.7	3	11.5	7	26.9	4	15.4	6	23.1	4	15.4	5	19.2	5	19.2	9	34.6	6	23.1	1	3.8	1	3.8	2	7.7	4	15.4	11	42.3
C-ANCA	20	14	70.0	15	75.0	14	70.0	15	75.0	14	70.0	15	75.0	17	85.0	15	75.0	15	75.0	13	65.0	19	95.0	19	95.0	19	95.0	19	95.0	20	100
P-ANCA	50	20	40.0	20	40.0	18	36.0	19	38.0	17	34.0	17	34.0	18	36.0	16	32.0	23	46.0	20	40.0	22	44.0	25	50.0	20	40.0	25	50.0	32	64.0
MPO	25	8	32.0	7	28.0	8	32.0	7	28.0	3	12.0	7	28.0	15	60.0	7	28.0	17	68.0	12	48.0	19	76.0	19	76.0	16	64.0	19	76.0	24	96.0
PR3	25	14	56.0	16	64.0	14	56.0	14	56.0	15	60.0	13	52.0	16	64.0	13	52.0	16	64.0	17	68.0	19	76.0	20	80.0	15	60.0	20	80.0	23	92.0
AIH	8	4	50.0	2	25.0	4	50.0	2	25.0	2	25.0	3	37.5	3	37.5	5	62.5	4	50.0	3	37.5	2	25.0	2	25.0	3	37.5	3	37.5	4	50.0
AIH/PBC	6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	16.7	0	0.0	1	16.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	4	66.7
PBC	11	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	18.2	0	0.0	2	18.2	0	0.0	0	0.0	2	18.2	2	18.2	2	18.2	3	27.3	4	36.4
PSC	10	0	0.0	1	10.0	0	0.0	1	10.0	0	0.0	3	30.0	4	40.0	4	40.0	3	30.0	0	0.0	3	30.0	3	30.0	3	30.0	7	70.0	8	80.0
Ulcerative Colitis	71	6	8.5	8	11.3	4	5.6	8	11.3	4	5.6	5	7.0	7	9.9	4	5.6	4	5.6	5	7.0	7	9.9	9	12.7	14	19.7	27	38.0	37	52.1
Crohn`s Disease	40	0	0.0	2	5.0	0	0.0	2	5.0	0	0.0	1	2.5	2	5.0	0	0.0	2	5.0	0	0.0	0	0.0	3	7.5	2	5.0	11	27.5	13	32.5
IBD	9	0	0.0	1	11.1	0	0.0	1	11.1	0	0.0	0	0.0	1	11.1	0	0.0	1	11.1	0	0.0	0	0.0	1	11.1	0	0.0	1	11.1	1	11.1
RA	12	1	8.3	1	8.3	1	8.3	1	8.3	1	8.3	0	0.0	0	0.0	0	0.0	0	0.0	1	8.3	0	0.0	1	8.3	0	0.0	0	0.0	0	0.0
SLE	30	5	16.7	4	13.3	5	16.7	3	10.0	3	10.0	5	16.7	7	23.3	5	16.7	7	23.3	5	16.7	2	6.7	4	13.3	5	16.7	7	23.3	6	20.0
Myositis	1	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	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Scleroderma	19	3	15.8	3	15.8	4	21.1	2	10.5	3	15.8	0	0.0	1	5.3	1	5.3	4	21.1	2	10.5	4	21.1	3	15.8	4	21.1	3	15.8	8	42.1
HBV	8	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	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
HCV	16	3	18.8	2	12.5	3	18.8	2	12.5	1	6.3	1	6.3	2	12.5	1	6.3	2	12.5	3	18.8	2	12.5	4	25.0	2	12.5	4	25.0	6	37.5
GCA	4	1	25.0	1	25.0	1	25.0	1	25.0	1	25.0	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	0.0	1	25.0
PMR	18	1	5.6	1	5.6	1	5.6	1	5.6	1	5.6	3	16.7	3	16.7	1	5.6	2	11.1	1	5.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Purpura	1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	100	0	0.0	1	100	0	0.0	1	100	1	100	1	100	1	100	1	100
Leukemia	15	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	6.7	0	0.0	1	6.7	1	6.7	0	0.0	2	13.3	2	13.3	4	26.7	7	46.7
Celiac disease	10	1	10.0	1	10.0	1	10.0	1	10.0	1	10.0	0	0.0	1	10.0	0	0.0	3	30.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	30.0
CKD	32	0	0.0	3	9.4	0	0.0	3	9.4	0	0.0	1	3.1	2	6.3	0	0.0	3	9.4	0	0.0	1	3.1	1	3.1	0	0.0	1	3.1	2	6.3
Asthma	32	1	3.1	1	3.1	1	3.1	1	3.1	1	3.1	0	0.0	0	0.0	0	0.0	1	3.1	0	0.0	0	0.0	0	0.0	1	3.1	1	3.1	1	3.1
Sinusitis	22	0	0.0	1	4.5	0	0.0	1	4.5	0	0.0	1	4.5	1	4.5	0	0.0	6	27.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

Table 124: Diagnostic Sensitivity and Specificity_ANCA Formalin

% Diagnostic Sensitivity & Specificity (95% CI)			% Sensitivity (95% CI)	% Specificity (95% CI)		% Sensitivity (95% CI)	% Specificity (95% CI)
			AAV (n= 135)	OD (n=375)		AAV (n= 135)	OD (n=375)
Site 1	Manual	Reader 1	40 (32.1 - 48.4)	93.1 (90 - 95.2)	Combined Readers	41.1 (35.4 - 47.1)	92.3 (90.1 - 94)
		Reader 2	42.2 (34.2 - 50.7)	91.5 (88.2 - 93.9)			
	Reader Confirmation at HELIOS	Reader 1	49.6 (41.3 - 58)	93.3 (90.3 - 95.4)	Combined Readers	47.4 (41.5 - 53.4)	92.7 (90.6 - 94.3)
		Reader 2	45.2 (37 - 53.6)	92 (88.8 - 94.3)			
	HELIOS		45.2 (37 - 53.6)	95.2 (92.5 - 96.9)			
Site 2	Manual	Reader 3	37.8 (30 - 46.2)	93.1 (90 - 95.2)	Combined Readers	41.1 (35.4 - 47.1)	91.7 (89.5 - 93.5)
		Reader 4	44.4 (36.3 - 52.9)	90.4 (87 - 93)			
	Reader Confirmation at HELIOS	Reader 3	36.3 (28.7 - 44.7)	93.6 (90.7 - 95.7)	Combined Readers	41.5 (35.8 - 47.4)	90.9 (88.7 - 92.8)
		Reader 4	46.7 (38.5 - 55.1)	88.3 (84.6 - 91.1)			
	HELIOS		42.2 (34.2 - 50.7)	94.4 (91.6 - 96.3)			
Site 3	Manual	Reader 5	37.8 (30 - 46.2)	93.6 (90.7 - 95.7)	Combined Readers	43 (37.2 - 48.9)	92 (89.8 - 93.7)
		Reader 6	48.1 (39.9 - 56.5)	90.4 (87 - 93)			
	Reader Confirmation at HELIOS	Reader 5	39.3 (31.4 - 47.7)	89.6 (86.1 - 92.3)	Combined Readers	44.8 (39 - 50.8)	85.1 (82.3 - 87.4)
		Reader 6	50.4 (42 - 58.7)	80.5 (76.2 - 84.2)			
	HELIOS		63.7 (55.3 - 71.3)	71.7 (67 - 76.1)			

Note: The 120 characterized MPO/PR3/ANCA positive sera have been excluded from the calculation of diagnostic sensitivity and specificity.

Summary of Results:

Positive, Negative, Overall and Pattern Agreements for Method Comparison between Method C (Manual) and Method B (Reader Confirmation of HELIOS images) were > 80%. All acceptance criteria were met.

Positive Agreement of Method C vs B ranged from 85.7% to 89.6%.

Negative Agreement of Method C vs B ranged 86.3% to 97.6%.

Overall Agreement of Method C vs B ranged from 87.0% to 95.4%.

Pattern Agreement of Method C vs B ranged from 82.1% to 87.5%.

The results show that Methods C (Manual) and B (Reader Confirmation of HELIOS images) give equivalent results. Furthermore, the results of the 3 study site were comparable with each other.

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

Positive Agreement of Method B vs A ranged from 73.6% to 99.4%.

Negative Agreement of Method B vs A ranged from 72.2% to 99.2%.

Overall Agreement of Method B vs A ranged from 79.2% to 95.9%.

Pattern Agreement of Method B vs A ranged from 76.1% to 90.8%.

According to this, acceptance criteria for comparison of Method B vs A were met.

The comparison of Method C (Manual) vs A (HELIOS suggested results) gave good results within the acceptance criteria for positive, negative and overall agreement. In the case of pattern agreement, 69.7% was reached. This is just below the acceptance criteria and we tolerate the deviation since this is a slight and unique occurrence.

Positive Agreement of Method C vs A ranged from 71.0% to 98.7%.

Negative Agreement of Method C vs A ranged from 70.7% to 95.2%.

Overall Agreement of Method C vs A ranged from 77.6% to 90.5%.

Pattern Agreement of Method C vs A ranged from 69.7% to 79.6%.

The Cross Reactivity Table (table no.: 101) shows that there were only few crossreactivities for Method A, B and C, which is also reflected by the high specificity of this methods (90%). The most crossreactivities could be seen in the Autoimmune Liver Disease Group (AIH, PBC, and PCS: up to 62.5%), in the inflammatory bowel disease group (Crohn' Disease, Ulcerative Colitis, IBD: up to 11.3%) and in SLE (up to 23%). These results reflect values reported in the literature. Method A (site 3) has a lower specificity as described and therefore, higher crossreactivities can be seen.

Conclusion:

Method C and Method B show substantial equivalence. AESKUSLIDES ANCA Ethanol and Formalin perform 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 we found a lower agreement compared to Method B and C. This can be explained by the complex patterns, which can be distinguished by the readers at the microscope or at the screen, but to limited extent by the software.

Our results show that results generated at HELIOS must always be confirmed by trained readers. The Vasculitis Pattern Plus software can support the reader to preselect positive samples, regardless of the detected pattern. According to the international recommendations on ANCA testing and the literature, it is recommended to further characterize ANCA positive IFA results by another method, for example PR3- and/or MPO-ELISA.

Endpoint Titer comparison:

To evaluate Within-Lab Precision and Between-Lab Precision of endpoint titer determination on AESKUSLIDES ANCA Ethanol and ANCA Formalin processed on HELIOS and processed manually, based on CLSI Guideline EP12-A2.

Procedure:
Five serum samples with endpoint titers between 1:10 and 1:320 were diluted serially, ranging from 1:10 up to 1:1280 sample dilution, depending on the expected enpoint titer displayed in the table below. For each sample at least 4 different dilutions around the expected endpoint titer were made. Those serum dilutions were assayed on AESKUSLIDES ANCA Ethanol and Formalin and analyzed on HELIOS (Method B, Reader Confirmation) and manually at the microscope (Method C) by two independent readers. For each of the two methods, each serum dilution was assayed on five days, with two runs per day and three replicates per run, resulting in 30 repetitions per serum dilution per reader.

Sample ID	Pos/Neg	Pattern ANCA Ethanol	Endpoint titer ANCA Ethanol	Pattern ANCA Formalin	Endpoint titer ANCA Formalin
1	Pos	C-ANCA	10	C-ANCA	20
2	Pos	C-ANCA	160	C-ANCA	160
3	Pos	C-ANCA	160	C-ANCA	320
4	Pos	P-ANCA	80	C-ANCA	20
5	Pos	P-ANCA	320	C-ANCA	40

All runs have been performed according to the respective IFUs. 1 positive and 1 negative control (kit controls) were included in each run.

The described study has been conducted at three different study sites to allow the calculation of Between-Lab Precision.

Data Analysis

Positive/Negative Classification and Pattern were recorded for each sample dilution in each run and for each Method. For Method C (Manual performance) fluorescence intensity was also reported.

Determination of endpoint titer:

For each replicate of the dilution series endpoint titer was reported as the reciprocal of the highest dilution at which the sample was classified as positive.

After pairwise comparison of the datapoints Within-lab precision was calculated for Methods B and C, for each of the three study sites.

Data will be presented as

- percentage of samples that differ maximum ±1 titer level from each other and
- percentage of samples that are > ±1 titer level away from each other.

For Between-Lab Precision the titer agreements (including 95% CI) between the three study sites were determined for each Method B and C (site 1 vs site 2, site 2 vs site 3, site 1 vs site 3).

Acceptance Criteria

- For each serum reported endpoint titers are allowed to differ maximum ±1 titer level from each other
- Calculations should meet the following criteria:

Calculations	Method B	Method C
Percentage of samples that differ maximum ±1 titer level	≥ 90%	≥ 90%
Titer Agreement	≥ 80%	≥ 80%

Results – Within-Lab Precision of endpoint titer determination

Results of endpoint titer determination for each of the three replicates per sample dilution can be found in the appendix.

For calculation of Within-Lab Precision the percentages of samples that are within and outside +/-1 titer level were calculated for the two readers separately (30 replicates for each sample dilution) and for all readers combined. The results of Within-Lab Precision for study sites 1, 2 and 3 are presented in the tables below.

AESKUSLIDES ANCA Ethanol

Table 125a: Number and percentages of samples that are within and outside +/-1 titer level – Method B and C

AESKUSLIDES ANCA Ethanol - Method B		Number of samples within +/-1 titer level	Number of samples > +/-1 titer level	Total number	Percentage of samples that are +/-1 titer level	Percentage of samples > +/-1 titer level
Site 1	Reader 1	147	3	150	98.0%	2.0%
	Reader 2	139	11	150	92.7%	7.3%
Site 2	Reader 3	139	11	150	92.7%	7.3%
	Reader 4	138	12	150	92.0%	8.0%
Site 3	Reader 5	149	1	150	99.3%	0.7%
	Reader 6	144	6	150	96.0%	4.0%
All readers combined		856	44	900	95.1%	4.9%

AESKUSLIDES ANCA Ethanol - Method C		Number of samples within +/-1 titer level	Number of samples > +/-1 titer level	Total number	Percentage of samples that are +/-1 titer level	Percentage of samples > +/-1 titer level
Site 1	Reader 1	143	7	150	95.3%	4.7%
	Reader 2	144	6	150	96.0%	4.0%
Site 2	Reader 3	143	7	150	95.3%	4.7%
	Reader 4	142	8	150	94.7%	5.3%
Site 3	Reader 5	146	4	150	97.3%	2.7%
	Reader 6	141	9	150	94.0%	6.0%
All readers combined		859	41	900	95.4%	4.6%

Acceptance criteria for Within-Lab Precision of endpoint titer determination on ANCA Ethanol were met. Percentage of samples that were within +/-1 titer level was 95.1% for Method B and 95.4% for Method C, for combined readers from all study sites. Considering all three study sites and readers separately, percentage of samples that were within +/-1 titer level ranged from 92.0% to 99.3% for Method B (Reader Confirmation) and from 94.0% to 97.3% for Method C (Manual). Results for Methods B and C were comparable.

Pos/Neg classification and pattern determination were consistent for 100% of the replicates.

AESKUSLIDES ANCA Formalin

Table 125b: Number and percentages of samples that are within and outside +/-1 titer level – Method B and C

AESKUSLIDES ANCA Formalin - Method B		Number of samples within +/-1 titer level	Number of samples > +/-1 titer level	Total number	Percentage of samples that are +/-1 titer level	Percentage of samples > +/-1 titer level
Site 1	Reader 1	145	5	150	96.7%	3.3%
	Reader 2	145	5	150	96.7%	3.3%
Site 2	Reader 3	147	3	150	98.0%	2.0%
	Reader 4	135	15	150	90.0%	10.0%
Site 3	Reader 5	145	5	150	96.7%	3.3%
	Reader 6	135	15	150	90.0%	10.0%
All readers combined		852	48	900	94.7%	5.3%

AESKUSLIDES ANCA Formalin - Method C		Number of samples within +/-1 titer level	Number of samples > +/-1 titer level	Total number	Percentage of samples that are +/-1 titer level	Percentage of samples > +/-1 titer level
Site 1	Reader 1	145	5	150	96.7%	3.3%
	Reader 2	148	2	150	98.7%	1.3%
Site 2	Reader 3	145	5	150	96.7%	3.3%
	Reader 4	144	6	150	96.0%	4.0%
Site 3	Reader 5	149	1	150	99.3%	0.7%
	Reader 6	134	16	150	89.3%	10.7%
All readers combined		865	35	900	96.1%	3.9%

Acceptance criteria for Within-Lab Precision of endpoint titer determination on ANCA Formalin were met. Percentage of samples that were within +/-1 titer level was 94.7% for Method B and 96.1% for Method C, for combined readers from all study sites.

Considering all three study sites and readers separately, percentage of samples that were within +/-1 titer level ranged from 90.0% to 98.0% for Method B (Reader Confirmation) and from 89.3% to 99.3 % for Method C (Manual). Only for reader 6 in Method C the percentage of samples was slightly below our acceptance criterion (89.3% instead of 90%). We tolerate this deviation, since this only concerns one reader out of six. However, this result shows that positive/negative classification of samples near its endpoint titer is subjective and highly depends on the experience of the reader.

Results for Methods B and C were comparable.

Pos/Neg classification and pattern determination were consistent for 100% of the replicates.

Results – Between-Lab Precision of endpoint titer determination

In order to evaluate Between-Lab Precision of endpoint titer determination, titer agreements (including 95% CIs) were determined between the three sites (site 1 vs site 2, site 2 vs site 3, site 1 vs site 3) for each Method B and C. Results are displayed in the following tables.

AESKUSLIDES ANCA Ethanol

Table 126a: % Titer agreement between three study sites – Method B and C

% Titer agreement (95% CI)	Site 1 vs. Site 2	Site 1 vs. Site 3	Site 2 vs. Site 3
Method B (Reader Confirmation)	93.3 (89.9 - 95.6)	86.7 (82.4 - 90.1)	82.3 (77.6 - 86.2)
Method C (Manual)	98.3 (96.2 - 99.3)	88.7 (84.6 - 91.8)	87 (82.7 - 90.3)

For Between-lab Precision of endpoint titer determination on ANCA Ethanol, all acceptance criteria were met. Titer agreements comparing the three study sites ranged from 82.3% to 93.3% for Method B (Reader Confirmation) and from 87.0% to 98.3% for Method C (Manual). With manual reading of the slides (Method C) slightly higher titer agreements have been achieved.

AESKUSLIDES ANCA Formalin

Table 126b: % Titer agreement between three study sites – Method B and C

% Titer agreement (95% CI)	Site 1 vs. Site 2	Site 1 vs. Site 3	Site 2 vs. Site 3
Method B (Reader Confirmation)	86.7 (82.4 - 90.1)	79 (74 - 83.2)	82.7 (78 - 86.5)
Method C (Manual)	93.7 (90.3 - 95.9)	80.3 (75.5 - 84.4)	82.7 (78 - 86.5)

Titer agreement between the three study sites ranged from 79.0% to 86.7% for Method B (Reader Confirmation) and from 80.3% to 93.7% for Method C (Manual). For Between-Lab Precision of endpoint titer determination on ANCA Formalin, all acceptance criteria were met, except for Method B site 1 vs. site 3. Here, titer agreement was slightly below the acceptance criterion (79.0% instead of 80%). However, we tolerate this deviation, since at study site 3, consistently higher endpoint titers have been obtained than at study sites 1 and 2. This is also reflected by the generally lower agreements when comparing site 3 to the other study sites. This study shows that the evaluation of sample titer is subjective and can vary from laboratory to laboratory, from microscope to microscope. Thus, we suggest for each laboratory to perform a titer control using the positive control or other well-known samples to balance variations in endpoint titer determination.

Results for Methods B and C were comparable when contrasting Site 1 vs Site 3 and Site 2 vs Site 3. When comparing Site 1 vs Site 2 titer agreements were slightly higher for Method C (Manual).

Conclusion
Pre-determined acceptance criteria were met. Method B (Reader Confirmation) as well as Method C (Manual) showed a good and acceptable repeatability (Within-Lab Precision) and reproducibility (Between-Lab Precision) for enpoint titer determination.

3. ***Clinical cut-off:***

See assay cut-off

4. ***Expected values/Reference range***

Expected values for ANCA were analyzed with a panel of 150 sera from healthy donors: 100 from Germany (56 females,44 males, mean age 31 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 ANCA Ethanol, there were 6 positive results for reader 1 and 3 positive results for reader 2, and with AESKUSLIDES ANCA Formalin, there were 6 positive results for reader 1 and 4 positive results for reader 2.

Table 127: Normal Range Study, number and percent positive and negative samples:

Normal Range Study	ANCA Ethanol			ANCA Formalin		
	Pos (%)	Neg (%)	Total	Pos (%)	Neg (%)	Total
Reader 1	6 (4)	144 (96)	150	6 (4)	144 (96)	150
Reader 2	3 (2)	147 (98)	150	4 (2.7)	146 (97.3)	150

Table 128: Normal Range Study, positive samples:

Normal Range Study		ANCA Ethanol						ANCA Formalin					
Sample ID	Sample Origin	Reader 1			Reader 2			Reader 1			Reader 2		
		Pos/Neg	ANCA Pattern	FI	Pos/Neg	ANCA Pattern	FI	Pos/Neg	ANCA Pattern	FI	Pos/Neg	ANCA Pattern	FI
NC 008	USA	Pos	A	2	Pos	A	2	Pos	C	1	Pos	C	1
NC 010	USA	Neg	Neg	0	Neg	Neg	0	Neg	Neg	0	Pos	C	1
NC 028	USA	Pos	P	1	Pos	P	1	Neg	Neg	0	Neg	Neg	0
NC 085	Germany	Pos	A	1	Pos	A	1	Neg	Neg	0	Neg	Neg	0
NC 109	Germany	Neg	Neg	0	Neg	Neg	0	Pos	C	1	Pos	C	1
NC 123	Germany	Pos	C	1	Neg	Neg	0	Pos	C	1	Neg	Neg	0
NC 126	Germany	Neg	Neg	0	Neg	Neg	0	Pos	C	1	Neg	Neg	0
NC 127	Germany	Pos	P	1	Neg	Neg	0	Neg	Neg	0	Neg	Neg	0
NC 139	Germany	Pos	C	1	Neg	Neg	0	Pos	C	1	Neg	Neg	0
NC 144	Germany	Neg	Neg	0	Neg	Neg	0	Pos	C	1	Pos	C	1

FI = Fluorescence Intensitiy

Only one sample (NC 008) has been found positive in both ANCA Ethanol (FI = 2) and ANCA Formalin (FI = 1) assays by both readers. 2 samples (NC 123, NC 139) have been found positive in both assays (FI=1) by reader 1 and negative by reader 2. All other samples have been found positive either in the ANCA Ethanol or in the ANCA Formalin assay with an FI of 1.

Conclusion:
The low number of positive samples we found with AESKUSLIDES ANCA Ethanol and ANCA Formalin 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