510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K172461

B. Purpose for Submission:

New Device

C. Measurand:

Anti-Neutrophil Cytoplasmic Antibodies (ANCA)

D. Type of Test:

Qualitative and semi-quantitative, indirect immunofluorescence

E. Applicant:

AESKU Diagnostics GmbH & Co. KG

F. Proprietary and Established Names:

AESKUSLIDES® ANCA Ethanol
AESKUSLIDES® ANCA Formalin

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5660 – Multiple autoantibodies immunological test system

2. Classification:

   Class II

3. Product code:

   MOB, Test system, antineutrophil cytoplasmic antibodies (ANCA)

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use:

   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 for use:

   Same as intended use

3. Special conditions for use statements:

   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:

Each kit of AESKUSLIDES ANCA Ethanol and AESKUSLIDES ANCA Formalin contains:

- Slides, each containing 6 or 12 wells coated with human neutrophils (ethanol fixation) or 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, 10x, containing PBS

Not provided in the kit:

- Evans Blue 0.2%, 1 x 3 mL, not ready for use
- HELIOS AUTOMATED IFA SYSTEM (K153117) or manual miscroscope

J. Substantial Equivalence Information:

1. Predicate device name:

   NOVA Lite ANCA

2. Predicate 510(k) number:

   K961340

3. Comparison with predicate:

Similarities		
Item	Device: AESKUSLIDES ANCA	Predicate: NOVA Lite ANCA
Intended Use	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.	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.
Methodology	Same	Immunofluorescence assay (IFA)
Procedure	Same	Standard IFA technique
Reported Result	Same	Qualitative and semi-

Similarities		
Item	Device: AESKUSLIDES ANCA	Predicate: NOVA Lite ANCA
		quantitative
Sample Matrix	Same	Serum
Analyte	Same	Anti-neutrophil cytoplasmic autoantibodies (ANCA)
Antigen	Same	Ethanol-fixed human neutrophils and formalin-fixed human neutrophils
Flurescence Marker	Same	FITC
Controls	Same	cANCA Positive, pANCA Positive and one negative control
Conjugate	Same	Anti-Human IgG Conjugate
Screening Dilution	Same	1:20
Storage	Same	2–8 °C
Slides	Same	6 or 12 wells coated with antigen
Shelf-Life	24 months for ANCA Ethanol 18 months for ANCA Formalin	18 months
Manual Interpretation of Results	Manual fluorescence microscopy or HELIOS AUTOMATED IFA SYSTEM with trained operator verification	Manual fluorescence microscopy

Differences		
Item	Device: AESKUSLIDES ANCA	Predicate: NOVA Lite ANCA
Manual Interpretation of Results	Manual fluorescence microscopy or HELIOS AUTOMATED IFA SYSTEM with trained operator verification	Manual fluorescence microscopy

K. Standard/Guidance Document Referenced:

1. EP07-A2, Interference Testing in Clinical Chemistry
2. EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures
3. EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents
4. EP28-A3c, Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory
5. EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures A Statistical Approach

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

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 the HELIOS AUTOMATED IFA SYSTEM.

Slides coated with human neutrophil granulocytes for autoantibody detection are fixed 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, i.e., 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

*Anti-nuclear antibody (ANA)

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

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

Manual interpretation of AESKUSLIDES ANCA (Ethanol):

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 proteinase 3 (PR3).
- P-ANCA presents as perinuclear staining with or without nuclear extension. This pattern is usually characteristic for antibodies reacting with myeloperoxidase (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 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 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 than 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.

Qualitative Evaluation

A serum dilution is considered negative for ANCA antibodies if the cells exhibit < 1+ fluorescence in the cytoplasm or nucleus. Likewise, a serum dilution is considered positive for ANCA antibodies if the cells exhibit ≥ 1+ fluorescence in 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 by the software:

After slides are processed by the HELIOS AUTOMATED IFA SYSTEM, digital images of representative fields of view in the well are captured and stored on the computer system. The HELIOS AUTOMATED IFA SYSTEM 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 AUTOMATED IFA SYSTEM software must be confirmed within the Result Confirmation tool by a trained operator.

The HELIOS PATTERN RECOGNITON software tool identifies the following Immunofluorescence patterns:

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:

The results of all the studies met the Manufacturer's pre-specified acceptance criteria.

1. Analytical performance:

   To establish the analytical performance parameters of the device, the following 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

a. Precision/Reproducibility:

All runs have been performed according to the respective instructions for use (IFUs). One positive and one negative control (kit controls) were included in each run. Samples were tested at 1:20 dilution. Results were analyzed by two independent readers. The samples were characterized using three different methods: method A (HELIOS), method B (HELIOS User Evaluation) and method C (Manual AESKSULIDES ANCA). Positive/negative classification and pattern was recorded for each sample in each run and for each method. Fluorescence intensity (FI) agreement was determined for method C. Pattern suggestion in method A was provided by the Vasculitis Pattern Plus software tool. FI % agreement across all samples was evaluated only for method C and was calculated as the number of samples that did not differ from expected by more than ±1 fluorescene intensity divided by the number of total samples.

Within-Lab Precision:

To assess the precision performance of the AESKUSLIDES ANCA Ethanol and Formalin, 10 samples were tested over five days, two runs per day, three replicates per sample run, resulting in 30 data points for each sample. For ANCA Ethanol, eight samples were positive, four with P-ANCA pattern, of which two were high positive and two medium positive, and four with C-ANCA pattern, of which two were borderline positive, one was medium positive and one was high positive. Two samples were negative. For ANCA Formalin, eight samples were positive (all C-ANCA pattern), of which four were high positive, three were medium positive and one was low positive. There were two negative samples. Below are the results of the within-lab precision study.

AESKUSLIDES ANCA (Ethanol) kit qualitative results for repeatability:

Sample ID	N (Method A)	Method A		N (Method B/C)	Method B		Method C	
		% Negative	% Positive		% Negative	% Positive	% Negative	% Positive
P-ANCA; high +	30	0	100	60	0	100	0	100
P-ANCA; high +	30	13.3	86.7	60	0	100	0	100
P-ANCA; medium +	30	3.3	96.7	60	0	100	0	100
P-ANCA; medium +	30	6.7	93.3	60	0	100	0	100
C-ANCA; borderline	30	77	23	60	76.7	23.3	76.7	23.3
C-ANCA; high +	30	10	90	60	3.3	96.7	0	100
C-ANCA; borderline	30	93	7	60	48.3	51.7	33.3	66.7
C-ANCA; medium +	30	7	93	60	10	90	0	100
negative	30	86.7	13.3	60	90	10	100	0
negative	30	83.3	16.7	60	100	0	100	0

For AESKUSLIDES ANCA Formalin kit, agreement for positive samples was 100% for all methods. For negative samples, agreement was >90% for all methods.

Between-Site Reproducibility:

There were 10 samples 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 U.S., while one was in Germany, with one HELIOS AUTOMATED IFA SYSTEM per study site. Results were analyzed by two different readers per study site, resulting in a total of 90 data points per sample. For ANCA Ethanol, there were four P-ANCA patterns, with two high positive and two medium positive, and four C-ANCA patterns, with one high positive, one medium positive and two borderline samples. For ANCA Formalin, there were eight C-ANCA patterns, with four high positive, three medium and one low positive sample. There were also two negative samples included in the Ethanol and Formalin sample sets. Below are the results of the between-site reproducibility study.

ANCA Ethanol Method C, site-to-site reproducibility results:

When borderline samples were excluded, positive-, negative-, overall-, pattern-, and FI-agreements for method C ranged from 99.6% to 100.0%. When borderline samples were included, the agreements for method C are presented in the table below:

	% 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)	99.0 (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)

ANCA Ethanol Method B, site-to-site reproducibility results:

When borderline samples were included, the agreements for method B are presented in the table below:

	% 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)
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	Not tested	Not tested	Not tested	Not tested

When borderline samples were excluded, the agreements for method B are presented in the table below:

	% 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)
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	Not tested	Not tested	Not tested	Not tested

ANCA Ethanol Method A, site-to-site reproducibility results:

When borderline samples were included, the agreements for method A are presented in the table below:

	% 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	85.0 (77.5 – 90.3)	92.5 (86.4 – 96)	92.5 (86.4 – 96)	90.0 (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)
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	Not tested	Not tested	Not tested	Not tested

When borderline samples were excluded, the agreements for method A are presented in the table below:

	% Agreement (95% CI)			
	Site 1 vs Site 2	**Site 2 vs Site 3**	**Site 1 vs Site 3**	**All Sites**
Positive Agreement	95.0 (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)
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	Not tested	Not tested	Not tested	Not tested

ANCA Formalin, site-to-site reproducibility results:

For Method C, positive-, negative-, overall-, pattern- and FI-agreements ranged from 96.6% to 100%.

The agreements for method B are presented in the table below:

	% Agreement (95% CI)			
	Site 1 vs Site 2	**Site 2 vs Site 3**	**Site 1 vs Site 3**	**All Sites**
Positive Agreement	97.0 (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	Not tested	Not tested	Not tested	Not tested

The agreements for method A are presented in the table below:

	% Agreement (95% CI)			
	Site 1 vs Site 2	**Site 2 vs Site 3**	**Site 1 vs Site 3**	**All Sites**
Positive Agreement	96.0 (93.9 – 97.5)	95 (92.7 – 96.6)	99.0 (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	Not tested	Not tested	Not tested	Not tested

Between-Operator Comparison:

For this study, sample set and study design was the same as described above for Between-Site Reproducibility. There were two operators per site. Only methods B and C were evaluated, considering that method A does not require reader involvement. The results are presented below.

ANCA Ethanol Method C, operator-to-operator reproducibility results:

When borderline samples were included, the agreements for method C are presented in the table below:

	% 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)

When borderline samples were excluded, positive, negative, and overall agreement, pattern agreement, and FI agreement for method C were all 97.7%–100.0%.

ANCA Ethanol Method B, operator-to-operator reproducibility results:

When borderline samples were included, the agreements for method B are presented in the table below:

	% 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)
Pattern Agreement	82.3 (78.6 – 85.4)	92.1 (89.3 – 94.2)	94.8 (92.4 – 96.4)
FI Agreement	Not tested	Not tested	Not tested

When borderline samples were excluded, positive, negative, and overall agreement, pattern agreement, and FI agreement for method B were all 91.7%–100.0%.

ANCA Formalin, operator-to-operator reproducibility results:

The agreements for method C are presented in the table below:

	% 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)
Pattern 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)

The agreements for method B are presented in the table below:

	% 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	Not tested	Not tested	Not tested

Lot-to-Lot Comparison:

A lot-to-lot reproducibility study was performed using three reagent lots each for AESKUSLIDES ANCA Ethanol and ANCA Formalin. For ANCA Ethanol, 12 sera were tested: four P-ANCA patterns, with two high and two medium positive samples, four C-ANCA patterns, with two high, one medium and one low positive sample, two A-ANCA patterns, with one high and one medium positive sample, and two negative samples. For ANCA Formalin, 12 sera were tested: eight C-ANCA patterns, with four high, three medium, and one low positive sample, and four negative samples. For both ANCA Ethanol and ANCA Formalin 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.

ANCA Ethanol Lot-to-Lot Reproducibility:

% 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)

ANCA Formalin Lot-to-Lot Reproducibility:

% 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)	99.0 (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)

b. Linearity/assay reportable range:

Endpoint Titer Estimate Study:

Five serum samples with pre-characterized endpoint titers between 1:10 and 1:320

were assayed to determine the precision of the endpoint titer parameter. This study was performed using method B (HELIOS User Evaluation) and method C (Manual AESKUSLIDES ANCA) 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. This study was conducted at three different study sites to allow the calculation of Between-Site reproducibility. All runs have been performed according to their respective IFUs. One positive and one negative kit controls were included in each run. 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. Two results were determined to be in agreement if the reported titers were ±1 titer from the expected value.

Samples used for this study are displayed in the following table:

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

The results of the endpoint titer estimate study are below.

ANCA Ethanol Percent Titer Agreement Between Study Sites:

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

ANCA Formalin Percent Titer Agreement Between Study Sites:

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

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

Traceability:

A recognized standard for anti-neutrophil cytoplasmic antibodies is not available.

Stability:

Accelarated Stability Study:

Three lots of complete kits (including slides, controls, conjugate and sample buffer) of AESKUSLIDES ANCA Ethanol and Formalin were stored at 37°C for 6 weeks. At t=0, one week, two weeks, three weeks, four weeks, five weeks, and six weeks, a kit was manually tested on a set of eight samples. For ANCA Ethanol, the following samples were assayed: three C-ANCA (high-, medium- and low-positive), three P-ANCA (high-, medium- and low-positive), one A-ANCA (medium-positive), and one negative sample. For ANCA Formalin, four C-ANCA samples were tested (one high-, two medium-, one low-, and one borderline-positive), and three negative samples. The following parameters were assessed: positive-, negative-, overall-, pattern- and FI-agreements. For AESKUSLIDES ANCA Ethanol all acceptance criteria were met for a time span of 6 weeks at 37°C. The Sponsor claims a shelf-life of at least 24 months at 2–8°C. For AESKUSLIDES ANCA Formalin all acceptance criteria were met for a time span of four weeks at 37°C. The Sponsor claims a shelf-life of at least 18 months at 2–8°C.

Real-time Stability Study:

In order to confim the results of the Accelareted Stability Study, a real-time stability study is being conducted over a period of 27 months. ANCA Ethanol and Formalin kits will be stored at 2–8°C for the aforementioned period. The following time points are being tested: t = 0, 3, 6, 12, 18, 24 and 27 months. The following samples are being tested: for ANCA Ethanol, three C-ANCA (high-, medium-, low-positive), three P-ANCA (high-, medium-, low-positive), one A-ANCA (medium-positive), and one negative sample; for ANCA Formalin: five C-ANCA (one high-, two medium-, and two low-positive), and three negative samples. Each serum sample is being tested in triplicate per run. At t = 0, three runs are being performed for each of the three different kit lots to give a total of nine repetitions per serum per lot. Two runs are being conducted for each of three different kit lots to give a total of six repetitions per serum per lot. Positive-, negative, overall-, pattern- and FI-agreements will be evaluated. Current results support ANCA Ethanol and ANCA Formalin real-time stability of at least three months at 2–8°C. This study is ongoing.

In-use Stability Study:

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: t = 0, one, two, four, and six weeks for each reagent lot. After the first test at time point t= 0, kits were incubated at 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 t = 0. Eight serum samples have been tested at each time point: for ANCA Ethanol, three C-ANCA (high-, medium-, and low-positive), three P-ANCA (high-, medium-, and low-positive), one A-ANCA (high-positive), and one negative sample; for ANCA Formalin, six C-ANCA (one high-, two medium-, one low-, and two borderline-positive), and two negative samples. Slides were processed manually and analyzed at the microscope by two independent readers. Positive-, negative-, overall-, pattern- and FI-agreements were evaluated. The data support in-use stability of six weeks at 2–8°C for both ANCA Ethanol and ANCA Formalin.

Stability data for the diluted wash buffer support a time frame of one week at 2–8°C.

Serum Freeze-Thaw Study:

Serum stability was evaluated by testing 10 serum samples. For ANCA Ethanol, the following samples were tested: three P-ANCA (two high- and one medium-positive), three C-ANCA (high-, medium- and low-positive), and one A-ANCA medium-positive sample, and three negative samples. For ANCA Formalin, there were six C-ANCA samples tested: three high-, and three medium-positive samples. For each serum, one aliquot was prepared as a control (no freeze-thaw). A second aliquot for each serum was prepared that underwent four freeze-thaw cycles. Slides were read by two independent readers. Each freeze/thaw sample was compared to the respective control sample. Results were assessed by determining positive-, negative-, overall-, pattern-, and FI-agreements. All agreements were 100% and support the manufacturer’s claim that samples can be frozen and thawed up to four times.

Long-term Storage Stability of Sera:

Nine different serum samples were aliquoted and stored at −20°C for a time period of 14 months. For ANCA Ethanol, the following samples were assayed: two C-ANCA, three P-ANCA, one A-ANCA, and three negative samples. For ANCA Formalin, the following samples were assayed: four C-ANCA, one P-ANCA , and four negative samples. Frozen aliquots of different sera were thawed and assayed with ANCA Ethanol and ANCA Formalin kits at the following time points: 3, 6, 8, 10, and 14 months. All tests were performed manually and subsequently analyzed at the microscope. The following parameters were assessed: positive-, negative-, pattern-, and FI-agreements. 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.

d. Detection limit:

Not applicable

e. Analytical specificity:

Interference:

The interference study was performed according to CLSI guideline EP07-A2. Interference by 10 different substances was assessed at two different concentrations. There were a total of 14 samples tested: for ANCA Ethanol, five C-ANCA (one high-, one medium-, and three low-positive), five P-ANCA (one high-, one medium-, and three low-positive), two A-ANCA (one medium- and one low-positive), and two negative samples; for ANCA Formalin, 10 C-ANCA (three high-, two medium-, and five low-medium), and four negative samples.

The following substances were tested at the indicated concentrations:

Interfering Substance	**Minimum final Concentration tested**	**Maximum final Concentration tested**
Bilirubin conjugated	0.1 mg/mL	0.4 mg/mL
Bilirubin unconjugated	0.1 mg/mL	0.4 mg/mL
Hemoglobin	2.5 mg/mL	5.0 mg/mL
Triglycerides	5 mg/mL	20 mg/mL
RF IgM	200 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 were tested in triplicate for each concentration of interferent with AESKUSLIDES ANCA Ethanol and ANCA Formalin. All tests were performed manually, and results were analyzed by two independent readers at the microscope. Positive-, negative-, overall-, pattern and FI-agreements were evaluated.

No interferences were observed with the tested substances at the two tested concentrations.

Cross-reactivity:

The purpose of this study was to demonstrate that Anti-nuclear antibody(ANA)-positive samples may cause interference with AESKUSLIDES ANCA (Ethanol and Formalin), leading to fluorescence patterns on neutrophil granlocytes that can be confused with ANCA patterns. It is known that ANA and other anti-cell antibodies may interfere with ANCA detection by causing nuclear and/or cytoplasmic staining. It is assumed that the main cause of interference shown in the population below in tables under Method Comparison to Predicate Device is the result of ANA-interference in the samples.

In this study, 25 analytically characterzed ANA-positive samples with homogenous, speckled, centromere, nucleolar, and nuclear dot patterns were tested with ANCA Ethanol and Formalin kits in triplicates. Methods A, B and C were evaluated. One positive and one negative control were included in each run. Samples were tested at 1:20 dilution. The number and percentage of ANCA positive samples were calculated.

In total, 40%, 64% and 60% of ANA positive samples were positive on AESKLUSLIDES ANCA Ethanol with methods A, B and C, respectively.

In total, 20%, 16% and 16% of ANA positive samples were detected positive on AESKLUSLIDES ANCA Formalin methods A, B and C, respectively.

The results show that ANA positive samples representing different ANA specific patterns can also give patterns on neutrophil granulocytes that resemble and can be confused with P- and C-ANCA patterns. Based on the above results, the following limitation is contained in the Package Insert: “Decisions about treatment should not be based solely on ANCA IFA results. ANCA serological test results should be used in conjunction with information available from the clinical evaluation and other diagnostic information. Positive ANCA IFA results should be confirmed by MPO and PR3 ELISA. ANA positive samples may react with fixed neutrophils, generating fluorescence patterns resembling ANCA patterns.”

f. Assay cut-off:

AESKU recommends a screening dilution of 1:20 followed by serial dilutions for semi-quantitative determinations, but suggests that each laboratory establishes their own screening dilution and titration scheme based on their 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.

2. Comparison studies:

a. Method comparison with predicate device:

In order to show comparable performance of AESKUSLIDES ANCA (Ethanol and Formalin) and the predicate assay, a clinical study utilizing 507 serum samples (132 serum samples from patients with AAV and 375 samples from patients with other diseases) was performed. For each assay, the sample set was processed manually and analyzed for positive/negative result and pattern classification using Method C. Positivitiy/negativity was determined in relation to the clinical diagnosis. Samples were screened at a dilution of 1:20.

Below are the results of this study for ANCA Ethanol:

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
		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	1	100
	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	8	100
	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	2	50.0	2	50.0
		Purpura	1	1	100	0	0.0	1	100	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	10	100
		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

The clinical sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for ANCA Ethanol and the predicate device using the available clinical diagnostic data for the samples. The results are presented in the table below:

% Diagnostic Sensitivity and Specificity (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)	%PPV	%NPV
	AAVs (n = 132)	OD (n=375)		
AESKU ANCA Ethanol	48.5 (40.1 – 56.9)	69.3 (64.5 – 73.8)	35.8	79.3
Pedicate Assay	36.4 (28.7 – 44.8)	55.2 (50.1 – 60.2)	22.2	71.1

Qualitative agreement between ANCA Ethanol and the predicate device were calculated and the results are presented in the table below:

Method Comparison Predicate vs AESKUSLIDES ANCA Ethanol	% 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)
Pattern Agreement	68.3 (60.3 – 75.3)
FI Agreement	91.7 (89.0 – 93.8)

Below are the results of this study for ANCA Formalin:

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	6	100
		Primary biliary cholangitis (PBC)	11	0	0.0	11	100	0	0.0	11	100
		Primary sclerosing cholangitis (PSC)	10	2	20.0	8	80.0	0	0.0	10	100
	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
		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	2	10.5	17	89.5
		Myositis	1	0	0.0	1	100	0	0.0	1	100
	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	8	100
	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	2	50.0	2	50.0
		Purpura	1	1	100	0	0.0	1	100	0	0.0
	Leukemia	Lymphoma, Myeloma	15	2	13.3	13	86.7	0	0.0	15	100
	Other diagnosis	Celiac disease	10	0	0.0	10	100	0	0.0	10	100
		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	32	100
Sum			507	101	19.9%	406	80.1	82	16.2	425	83.8

The clinical sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for ANCA Formalin and the predicate device using the available clinical diagnostic data for the samples. The results are presented in the table below:

% Diagnostic Sensitivity and Specificity (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)	%PPV	%NPV
	AAVs (n = 132)	OD (n=375)		
AESKU ANCA Ethanol	50.0 (41.6 – 58.4)	90.7 (87.3 – 93.2)	65.3	83.7
Pedicate Assay	37.9 (30.1 – 46.4)	91.5 (88.2 – 91.5)	61.0	80.7

Qualitative agreement between ANCA Formalin and the predicate device were calculated and the results are presented in the table below:

Method Comparison Predicate vs AESKUSLIDES ANCA Formalin	% Agreement (95% CI)
Positive Agreement	80.5 (70.6 – 87.6)
Negative Agreement	91.8 (88.8 – 94.0)
Overall Agreement	89.9 (87.0 – 92.3)
Pattern Agreement	87.9 (77.9 – 93.7)
FI Agreement	93.7 (91.2 – 95.5)

b. Method comaprison between methods A, B and C:

A clinical study using 630 serum samples (135 serum samples from patients with AAV, 120 characterized MPO/PR3/ANCA positive sera, and 375 samples from patients with other diseases) was performed at two U.S. sites and one German site. This sample set is identical to that used above in the method comparison to the predicate, with the exception of three additional AAV samples (two Wegener's Granulomatosis and one Churg-Strauss Syndrome), and with the exclusion of 120 characterized MPO/PR3/ANCA positive sera. Each site had two readers.

Positive, negative, and overall agreements for ANCA Ethanol across methods and sites are presented in the table below:

		% 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)	99.0 (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)	99.0 (98 – 99.5)	97.4 (96.1 – 98.3)
	Total Agreement	95.3 (94 – 96.4)	96.0 (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 (90.0 – 93.1)	92.9 (91.4 – 94.2)	90.0 (88.2 – 91.5)

Pattern agreements across methods and sites for ANCA Ethanol are presented in the table below:

	% Agreement (95% CI)		
	Method C vs B	**Method B vs A**	**Method C vs A**
Site 1	87.0 (85 – 88.7)	81.0 (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)	80.0 (78.7 – 81.2)

Positive, negative, and overall agreements for ANCA Formalin across methods and sites are presented in the table below:

		% 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 – 78.0)
	Negative Agreement	95.2 (93.7 – 96.4)	98.8 (97.8 – 99.3)	95.0 (93.4 – 96.2)
	Total Agreement	86.9 (82.6 – 90.3)	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 – 97.0)	93.6 (91.9 – 95.0)
	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 – 92.0)	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)	93.8 (81.6 – 85.7)	78.2 (75.9 – 80.4)

Pattern agreements across methods and sites for ANCA Ethanol are presented in the table below:

	% 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)

c. Matrix comparison:

Not applicable

3. Clinical studies:

A clinical study with 510 serum samples (135 serum samples from patients with AAV, and 375 samples from patients with other diseases) was performed at two U.S. sites and one German site. This sample set is identical to that described above under method comparison to predicate study, with the exception of three additional AAV samples (two Wegener's Granulomatosis and one Churg-Strauss Syndrome). At each study site the samples were evaluated using methods A, B and C.

a. Clinical Sensitivity and Specificity:

Clinical senstivity and specificity for ANCA Ethanol Kit is presented in the table below:

AAV	Non-healthy Controls
% Sensitivity (95% CI)	% Specificity (95% CI)
Method C	
30.1 (27.1 – 33.4)	74.8 (73 – 76.6)
Method B	
30.4 (27.3 – 33.6)	76.9 (75.1 – 78.6)
Method A	
26.4 (22.4 – 30.9)	81.7 (79.3 – 83.8)

Clinical senstivity and specificity for ANCA Formalin Kit is presented in the table below:

AAV	Non-healthy Controls
% Sensitivity (95% CI)	% Specificity (95% CI)
Method C	
41.1 (37 – 45.3)	92 (90.5 – 93.3)
Method B	
44.4 (40.3 – 48.7)	91.8 (90.3 – 93.1)
Method A	
43.7 (37.9 – 49.7)	94.8 (93 – 96.2)

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

Not applicable

4. Clinical cut-off**:**

See analytical cut-off

5. Expected values/Reference range:

Expected values for AESKUSLIDES ANCA were analyzed with a panel of 150 sera from healthy donors: 100 from Germany and 50 from the the U.S. Samples were tested at 1:20 dilution and slides were processed manually and subsequently analyzed at the microscope by two independent readers.

Results of the reference range study are as follows:

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

N. Proposed Labeling:

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

O. Conclusion:

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