510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY

I Background Information:

A 510(k) Number

K243776

B Applicant

ZEUS Scientific

C Proprietary and Established Names

Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-fixed)
Anti-Neutrophil Cytoplasmic Antibodies (Formalin-fixed)

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MOB	Class II	21 CFR 866.5660 - Multiple Autoantibodies Immunological Test System	IM - Immunology
PIV	Class II	21 CFR 866.4750 - Automated Indirect Immunofluorescence Microscope and Software-Assisted System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

New assay on a previously cleared instrument

B Measurand:

Anti-Neutrophil Cytoplasmic Antibodies (ANCA)

C Type of Test:

Qualitative and/or semi-quantitative indirect immunofluorescence assay (IFA); manual or semi-automated

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-fixed) test system is an indirect immunofluorescence assay (IFA) for the qualitative and semi-quantitative determination of anti-neutrophil cytoplasmic antibodies (ANCA) of the IgG isotype in human serum by manual fluorescence microscopy or with dlFine. The presence of ANCA in conjunction with other clinical and laboratory findings can be used to aid in the diagnosis of ANCA associated vasculitis (AAV). All suggested results obtained with dlFine must be confirmed by a trained operator.

The Anti-Neutrophil Cytoplasmic Antibodies (Formalin-fixed) test system is an indirect immunofluorescence assay (IFA) for the qualitative and semi-quantitative determination of anti-neutrophil cytoplasmic antibodies (ANCA) of the IgG isotype in human serum by manual fluorescence microscopy or with dlFine. The presence of ANCA in conjunction with other clinical and laboratory findings can be used to aid in the diagnosis of ANCA associated vasculitis (AAV). All suggested results obtained with dlFine' must be confirmed by a trained operator.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

- For *In Vitro* diagnostic use.
- The device is for use by a trained operator in a clinical laboratory setting.
- All software-aided results must be confirmed by the trained operator.

D Special Instrument Requirements:

Zeus dIFine or manual fluorescence microscope

IV Device/System Characteristics:

A Device Description:

The Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-fixed) and the Anti-Neutrophil Cytoplasmic Antibodies (Formalin-fixed) test systems each contain the following components:

- ANCA Substrate Slides: Ten, 10-well Slides with absorbent blotter and desiccant pouch
- Conjugate: 2 x 3 mL, Goat anti-human IgG labeled with FITC in phosphate buffer with BSA and counterstain

- p-ANCA Positive Control: 1 x 0.5 mL, p-ANCA positive human serum with preservatives.
- c-ANCA Positive Control: 1 x 0.5 mL, c-ANCA positive human serum with preservatives.
- Negative Control: 1 x 0.5 mL, human serum negative for ANCA with preservatives. 0.5mL
- Cover Glass: 24 x 60 mm, Thickness #1
- Phosphate-buffered-saline (PBS): 2 packets, each buffer packet for one liter of distilled or deionized water, pH 7.2 ± 0.2
- Mounting Media (Buffered Glycerol): 1 x 12 mL (in 15 mL bottle), ready to use

Materials that are required but not provided with the kit:

- dIFine instrument or manual fluorescence microscope
- General laboratory materials (e.g., pipettors, disposable pipette tips, test tubes, dilutions plates, slide washer, distilled or deionized water, etc.)

B Principle of Operation:

The ANCA IFA test systems are designed to detect the presence of ANCA in human serum. Each test system employs either ethanol-fixed or formalin-fixed human granulocyte substrate, optimized goat anti-human IgG fluorescein isothiocyanate (FITC) conjugate solution, and wash solution. For either product, the reaction occurs as follows:

1. Step one is the sample incubation where ANCA present in the patient sample may bind to the cell substrate, forming an antigen-antibody complex. Other serum components are subsequently washed away.
2. Step two is the Conjugate incubation where the anti-human IgG FITC conjugate reacts with any human IgG bound to the substrate during the sample incubation. This will form a stable antigen-antibody-Conjugate complex at the location where the initial patient antibody bound to the cell substrate. Excess Conjugate is subsequently washed away.
3. The results of the assay can be visualized using a properly equipped fluorescent microscope or dIFine. Any positive reactions will appear as apple-green, fluorescent staining within the cell. If the sample did not have ANCA present, there will be no distinct nuclear or cytoplasmic staining of the cell.

Manual Interpretation:

The interpretation of the results depends on the pattern observed as well as the titer of the autoantibody present in the specimen. A positive reaction is the presence of any pattern of nuclear apple-green staining observed at a 1:20 dilution based on a 1+ to 4+ scale of staining intensity where 1+ is considered a weak reaction and 4+ a strong reaction. Zeus recommends sera positive at 1:20 should be titered to endpoint dilution by making 1:40, 1:80, etc. serial dilutions. The endpoint titer is the highest dilution that produces a 1+ positive reaction.

dIFine Interpretation:

When slides are analyzed by dIFine, digital images of representative fields of view of the well are captured. The default scanning area is composed of 12 fields with each field approximately 610 µm x 510 µm using the 20X objective. All images are taken through a fluorescein isothiocyanate (FITC) filter. The dIFine System reads the slides and measures the FITC light

intensity of the cell substrate that are included in the region. Then, the dIFine System reports the measured average fluorescence intensity as an index percentage and recommends a qualitative result. To facilitate the interpretation, the dIFine System presents acquired digital images of the slide and the following information for human analysis: Negative, Positive or Uncertain classification as well as a suggested pattern (c-ANCA, p-ANCA, or Others).

Trained operators then review the images taken by dIFine System. During the review process, further options include:

- Navigation of digitized well using virtual microscope tools (zooming/browsing images at different magnifications).
- Enlargement of images to examine detail.
- Image Atlas to assist with identification of patterns.

The trained operator can confirm the results by clicking the "Validate" button on the screen and accepting the classification (negative/positive and pattern) suggested by the dIFine System, or they can revise the suggested dIFine classification (negative to positive and vice versa or from Uncertain to either positive or negative), add comments (if any), change the pattern (if necessary) and eventually click the "Validate" button on the screen.

V Substantial Equivalence Information:

A Predicate Device Name(s):

NOVA Lite DAPI ANCA Ethanol Kit
NOVA Lite DAPI ANCA Formalin Kit

B Predicate 510(k) Number(s):

K161258

C Comparison with Predicate(s):

Device & Predicate Device(s):	K243776 (Candidate)	K161258 (Predicate)
Device Trade Name	Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-fixed) Anti-Neutrophil Cytoplasmic Antibodies (Formalin-fixed)	NOVA Lite DAPI ANCA (Ethanol) NOVA Lite DAPI ANCA (Formalin)
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-fixed) and Anti-Neutrophil Cytoplasmic Antibodies (Formalin-fixed) test systems are indirect immunofluorescence assays (IFA) for the qualitative and semi-quantitative determination of anti-neutrophil cytoplasmic antibodies (ANCA) of the IgG isotype in human serum by manual fluorescence microscopy or with dIFine. The presence of ANCA in conjunction with other clinical and laboratory findings can be used to aid in the diagnosis of ANCA associated vasculitis (AAV). All suggested results obtained with dIFine must be confirmed by a trained operator.	NOVA Lite DAPI ANCA (Ethanol) Kit or NOVA Lite DAPI ANCA (Formalin) Kit is indirect immunofluorescence assay for the qualitative detection and semi-quantitative determination of anti-neutrophil cytoplasmic antibodies (ANCA) of IgG isotypes in human serum by manual fluorescence microscopy or with NOVA View Automated Fluorescence Microscope. The presence of ANCA, in conjunction with other serological, radiological, histological, and clinical findings, aids in the diagnosis of ANCA associated vasculitis. A trained operator must confirm results when generated with the NOVA View device.
Solid Phase (Substrate)	Ethanol-fixed or Formalin-fixed human neutrophils	Same
Technology	Indirect Immunofluorescence Assay	Same
Sample	Human Serum	Same
Positive Control	Positive pANCA human control Positive cANCA human control	Same
Negative control	Human Serum with no ANCA activity	Same
Sample Diluent	PBS Buffer	Same
Wash Solution	PBS Buffer	Same
Screening Dilution	1:20 dilution in PBS	Same
Staining Pattern Observed	pANCA, cANCA, Other, Uncertain	Same
General Device Characteristic Differences		
Conjugate	Anti-human IgG labeled with FITC	FITC-labeled anti-human IgG (Fc) Conjugate with DAPI
Mounting Media	Buffered Glycerol	Unknown
Interpretation Method	Manual microscope or dIFine automated microscope	Manual microscope or NOVA View automated microscope

VI Standards/Guidance Documents Referenced:

The following Clinical and Laboratory Standards Institute (CLSI) guidelines were used:

- CLSI EP05-A3 Evaluation of Precision Performance of Quantitative Measurement Methods, Approved Guideline - 3rd Edition
- CLSI EP06, 2nd ed. Evaluation of the Linearity of Quantitative Measurement Procedures – Second Edition
- CLSI EP07-A2 Interference Testing in Clinical Chemistry, 2nd Edition
- CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition
- CLSI EP28-A3c Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

When establishing performance characteristics of the ANCA Ethanol-fixed IFA test system or ANCA Formalin-fixed IFA test system, slides were interpreted using three different methods as outlined below:

Method	Processing	Imaging	Reading/Evaluation of Slides
A	Manual	Manual	Manual (read of microscope field)
B	Automated	Automated	Manual (read of digital image)
C	Automated	Automated	Automated (software interpretation)

Data analysis was performed to determine the agreement based on the qualitative results and the staining pattern results:

- The qualitative agreement is the output read by each interpretation method to conclude if a sample is positive or negative.
- The staining pattern agreement defines if the positive sample belongs to c-ANCA (cytoplasmic ANCA) pattern or p-ANCA (perinuclear ANCA) pattern.

1. Precision/Reproducibility:

   a. **Repeatability: 20-day Within-Laboratory Precision Study**

   A panel of samples including: two low positive samples (~1:20-1:40 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), two mid positive samples (~1:80-1:160 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), and two high positive samples (> 1:160 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), and two negative samples, were assayed in triplicate, at a 1:20

screening dilution, once per day, on 20 different days, resulting a total of 60 results per sample for each of the Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-fixed) and the Anti-Neutrophil Cytoplasmic Antibodies (Formalin-fixed) test systems. Qualitative results and pattern results were interpreted by two technicians for Methods A and B, and by a single dIFine instrument for Method C. For each test system, the summary results for within-method and between method agreements (for both qualitative and patten) with combined results from two technicians (a total of 120 results per sample) are presented in the tables below:

Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-Fixed)

Within-Method Agreement - Qualitative Result

Sample	Agreement% (95% CI)		
	Method A	Method B	Method C
Low Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Low Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Mid Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Mid Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
High Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
High Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Negative 1	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Negative 2	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)

Within-Method Agreement - Pattern Result

Sample	Agreement% (95% CI)		
	Method A	Method B	Method C
Low Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	98.3% (94.1–99.5%)
Low Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	96.7% (91.7–98.7%)
Mid Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Mid Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
High Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	96.7% (91.7–98.7%)
High Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)

Between-Method Agreement - Qualitative Result

Sample	Agreement% (95% CI)		
	Method A	Method B	Method C
Low Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Low Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Mid Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Mid Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
High Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
High Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Negative 1	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Negative 2	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)

Between-Method Agreement - Pattern Result

Sample	Agreement% (95% CI)		
	Method A	Method B	Method C
Low Positive – c-ANCA	100% (96.9–100.0%)	98.3% (94.1–99.5%)	98.3% (94.1–99.5%)
Low Positive – p-ANCA	100% (96.9–100.0%)	96.7% (91.7–98.7%)	96.7% (91.7–98.7%)
Mid Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Mid Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
High Positive – c-ANCA	100% (96.9–100.0%)	96.7% (91.7–98.7%)	96.7% (91.7–98.7%)
High Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)

Anti-Neutrophil Cytoplasmic Antibodies (Formalin-Fixed)

Within-Method Agreement - Qualitative Result

Sample	Agreement% (95% CI)		
	Method A	Method B	Method C
Low Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Low Positive – p-ANCA	96.7% (91.7–98.7%)	96.7% (91.7–98.7%)	88.3% (81.4–92.9%)
Mid Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Mid Positive – p-ANCA	98.3% (94.1–99.5%)	98.3% (94.1–99.5%)	91.7% (85.3–95.4%)
High Positive – c-ANCA	98.3% (94.1–99.5%)	98.3% (94.1–99.5%)	98.3% (94.1–99.5%)
High Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	96.7% (91.7–98.7%)
Negative 1	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Negative 2	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)

Within-Method Agreement - Pattern Result

Sample	Agreement% (95% CI)		
	Method A	Method B	Method C
Low Positive – c-ANCA	100% (96.9–100.0%)	98.3% (94.1–99.5%)	98.3% (94.1–99.5%)
Low Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	96.7% (91.7–98.7%)
Mid Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Mid Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
High Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	98.3% (94.1–99.5%)
High Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	94.9% (89.4–97.7%)

Between-Method Agreement - Qualitative Result

Sample	Agreement% (95% CI)		
	Method A	Method B	Method C
Low Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Low Positive – p-ANCA	100% (96.9–100.0%)	91.7% (85.3–95.4%)	91.7% (85.3–95.4%)
Mid Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Mid Positive – p-ANCA	100% (96.9–100.0%)	93.3% (87.4–96.6%)	93.3% (87.4–96.6%)
High Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
High Positive – p-ANCA	100% (96.9–100.0%)	96.7% (91.7–98.7%)	96.7% (91.7–98.7%)
Negative 1	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Negative 2	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)

Between-Method Agreement - Pattern Result

Sample	Agreement% (95% CI)		
	Method A	Method B	Method C
Low Positive – c-ANCA	100% (96.9–100.0%)	98.3% (94.1–99.5%)	98.3% (94.1–99.5%)
Low Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Mid Positive – c-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
Mid Positive – p-ANCA	100% (96.9–100.0%)	100% (96.9–100.0%)	100% (96.9–100.0%)
High Positive – c-ANCA	100% (96.9–100.0%)	98.3% (94.1–99.5%)	98.3% (94.1–99.5%)
High Positive – p-ANCA	100% (96.9–100.0%)	94.9% (89.4–97.7%)	94.9% (89.4–97.7%)

b. **Lot-to-Lot Reproducibility**

A panel of 10 samples includes: two low positive samples (~1:20-1:40 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), two mid positive samples (~1:80-1:160 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), two high positive samples (> 1:160 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), and four negative samples, were each assayed at a 1:20 screening dilution using three different lots of the Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-fixed) test system or the Anti-Neutrophil Cytoplasmic Antibodies (Formalin-fixed) test system. The six positive samples were also serially diluted and assayed using the same three lots of each test system. Results were interpreted by all three methods noted above. A total of 44 results per lot from the diluted positive samples and the 4 negative samples were analyzed for qualitative agreement and pattern agreement analyses. Endpoint dilutions were also determined for the serially diluted samples. The results are summarized in the tables below:

Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-Fixed)

Within-Lot Agreement - Qualitative Results between Three Methods

Interpretation Methods	Agreement% (95% CI)		
	Lot 1	Lot 2	Lot 3
A vs B	100.0% (92.0–100.0%)	97.7% (88.2–99.6%)	95.5% (84.9–98.7%)
A vs C	86.4% (73.3–93.6%)	79.6% (65.5–88.9%)	77.3% (63.0–87.1%)
B vs C	90.9% (78.8–96.4%)	81.8% (68.0–90.5%)	81.8% (68.0–90.5%)

Within-Lot Agreement – Pattern Results between Three Methods

Interpretation Methods	Agreement% (95% CI)		
	Lot 1	Lot 2	Lot 3
A vs B	100.0% (85.7–100.0%)	100.0% (85.1–100.0%)	100.0% (85.7–100.0%)
A vs C	100.0% (85.7–100.0%)	86.4% (66.7–95.3%)	82.6% (62.9–93.0%)
B vs C	96.0% (80.5–99.3%)	86.4% (66.7–95.3%)	84.0% (65.4–93.6%)

Between Lot Agreement - Qualitative Results between Three Methods

Interpretation Methods	Agreement% (95% CI)		
	Lot 1 vs. Lot 2	Lot 1 vs. Lot 3	Lot 2 vs. Lot 3
A vs A	97.7% (88.2–99.6%)	95.5% (84.9–98.7%)	97.7% (88.2–99.6%)
B vs B	95.5% (84.9–98.7%)	100.0% (92.0–100.0%)	95.5% (84.9–98.7%)
C vs C	84.1% (70.6–92.1%)	86.4% (73.3–93.6%)	79.6% (65.5–88.9%)
A vs B	95.5% (84.9–98.7%)	95.5% (84.9–98.7%)	93.2% (81.8–97.7%)
A vs C	77.3% (63.0–87.2%)	77.3% (63.0–87.2%)	75.0% (60.6–85.4%)
B vs C	79.6% (65.5–88.9%)	81.8% (68.0–90.5%)	77.3% (63.0 - 87.2%)
B vs A	93.2% (81.8–97.7%)	95.5% (84.9–98.7%)	95.5% (84.9–98.7%)
C vs A	84.1% (70.6–92.1%)	86.4% (73.3–93.6%)	79.6% (65.5–88.9%)
C vs B	86.4% (73.3–93.6%)	90.9% (78.8–96.4%)	79.6% (65.5–88.9%)

Between Lot Agreement – Pattern Results between Three Methods

Interpretation Methods	Agreement% (95% CI)		
	Lot 1 vs. Lot 2	Lot 1 vs. Lot 3	Lot 2 vs. Lot 3
A vs A	100.0% (85.1–100.0%)	100.0% (85.1–100.0%)	100.0% (85.1–100.0%)
B vs B	100.0% (85.1–100.0%)	100.0% (86.7–100.0%)	100.0% (85.7–100.0%)
C vs C	83.3% (64.2–93.3%)	80.8% (62.1–91.5%)	80.0% (60.9–91.1%)
A vs B	100.0% (85.1–100.0%)	100.0% (85.7–100.0%)	100.0% (85.1–100.0%)
A vs C	86.4% (66.7–95.3%)	82.6% (62.9–93.0%)	86.4% (66.7–95.3%)
B vs C	82.6% (62.9- 93.0%)	84.0% (65.4–93.6%)	87.0% (67.9–95.5%)
B vs A	100.0% (85.1–100.0%)	100.0% (85.7–100.0%)	100.0% (85.1–100.0%)
C vs A	100.0% (85.1–100.0%)	95.7% (79.0–99.2%)	86.4% (66.7–95.3%)
C vs B	100.0% (85.1–100.0%)	96.0% (80.5–99.3%)	87.0% (67.9–95.5%)

Endpoint Titers Agreement:

Sample		Method A	Method B	Method C	
				UNC as (-)	UNC as (+)
Low Positive – c-ANCA	Lot 1	1:40	1:40	1:40	1:40
	Lot 2	1:40	1:40	1:160	1:320
	Lot 3	1:40	1:40	1:80	1:160
Low Positive – p-ANCA	Lot 1	1:40	1:40	1:80	1:160
	Lot 2	1:40	1:40	1:80	1:160
	Lot 3	1:40	1:40	1:80	1:80
Mid Positive – c-ANCA	Lot 1	1:80	1:80	1:80	1:80
	Lot 2	1:80	1:80	1:80	1:160
	Lot 3	1:80	1:80	1:160	1:320
Mid Positive – p-ANCA	Lot 1	1:80	1:160	1:160	1:320
	Lot 2	1:80	1:160	1:160	1:320
	Lot 3	1:80	1:160	1:160	1:320
High Positive – c-ANCA	Lot 1	1:1280	1:2560	1:2560	1:5120
	Lot 2	1:1280	1:1280	1:1280	1:2560
	Lot 3	1:2560	1:2560	1:2560	1:5120
High Positive – p-ANCA	Lot 1	1:640	1:640	1:640	1:640
	Lot 2	1:320	1:320	1:320	1:320
	Lot 3	1:320	1:640	1:640	1:1280

Anti-Neutrophil Cytoplasmic Antibodies (Formalin-Fixed)

Within-Lot Agreement - Qualitative Results between Three Methods

Interpretation Methods	Agreement% (95% CI)		
	Lot 1	Lot 2	Lot 3
A vs B	100.0% (92.0–100.0%)	100.0% (92.0–100.0%)	100.0% (92.0–100.0%)
A vs C	88.6% (76.0–95.1%)	86.4% (73.3–93.6%)	81.8% (68.0–90.5%)
B vs C	88.6% (76.0–95.1%)	86.4% (73.3–93.6%)	81.8% (68.0–90.5%)

Within-Lot Agreement – Pattern Results between Three Methods

Interpretation Methods	Agreement% (95% CI)		
	Lot 1	Lot 2	Lot 3
A vs B	100.0% (86.2–100.0%)	100.0% (85.7–100.0%)	100.0% (85.7–100.0%)
A vs C	65.0% (43.3–81.9%)	79.0% (56.7–91.5%)	100.0% (83.9–100.0%)
B vs C	65.0% (43.3–81.9%)	79.0% (56.7–91.5%)	100.0% (83.9–100.0%)

Between Lot Agreement - Qualitative Results between Three Methods

Interpretation Methods	Agreement% (95% CI)		
	Lot 1 vs. Lot 2	Lot 1 vs. Lot 3	Lot 2 vs. Lot 3
A vs A	97.7% (88.2–99.6%)	97.7% (88.2–99.6%)	100.0% (92.0–100.0%)
B vs B	97.7% (88.2–99.6%)	97.7% (88.2–99.6%)	100.0% (92.0–100.0%)
C vs C	79.6% (65.5–88.9%)	79.6% (65.5–88.9%)	79.6% (65.5–88.9%)
A vs B	97.7% (88.2–99.6%)	97.7% (88.2–99.6%)	100.0% (92.0–100.0%)
A vs C	84.1% (70.6–92.1%)	79.6% (65.5–88.9%)	81.8% (68.0–90.5%)
B vs C	84.1% (70.6–92.1%)	79.6% (65.5–88.9%)	81.8% (68.0–90.5%)
B vs A	97.7% (88.2–99.6%)	97.7% (88.2–99.6%)	100.0% (92.0–100.0%)
C vs A	88.6% (76.0–95.1%)	88.6% (76.0–95.1%)	86.4% (73.3–93.6%)
C vs B	88.6% (76.0–95.1%)	88.6% (76.0–95.1%)	86.4% (73.3–93.6%)

Between Lot Agreement - Pattern Results between Three Methods

Interpretation Methods	Agreement% (95% CI)		
	Lot 1 vs. Lot 2	Lot 1 vs. Lot 3	Lot 2 vs. Lot 3
A vs A	100.0% (85.7–100.0%)	100.0% (85.7–100.0%)	100.0% (85.7–100.0%)
B vs B	100.0% (85.7–100.0%)	100.0% (85.7–100.0%)	100.0% (85.7–100.0%)
C vs C	70.6% (46.9–86.7%)	66.7% (43.8–83.7%)	82.4% (59.0–93.8%)
A vs B	100.0% (85.7–100.0%)	100.0% (85.7–100.0%)	100.0% (85.7–100.0%)
A vs C	79.0% (56.7–91.5%)	100.0% (83.9–100.0%)	100.0% (83.9–100.0%)
B vs C	79.0% (56.7–91.5%)	100.0% (83.9–100.0%)	100.0% (83.9–100.0%)
B vs A	100.0% (85.7–100.0%)	100.0% (85.7–100.0%)	100.0% (85.7–100.0%)
C vs A	65.0% (43.3–81.9%)	65.0% (43.3–81.9%)	79.0% (56.7–91.5%)
C vs B	65.0% (43.3–81.9%)	65.0% (43.3–81.9%)	79.0% (56.7–91.5%)

Endpoint Titers Agreement:

Sample		Method A	Method B	Method C	
				UNC as (-)	UNC as (+)
Low Positive – c-ANCA	Lot 1	1:40	1:40	1:40	1:80
	Lot 2	1:40	1:40	1:20	1:80
	Lot 3	1:40	1:40	1:40	1:80
Low Positive – p-ANCA	Lot 1	1:40	1:40	1:40	1:40
	Lot 2	1:40	1:40	1:40	1:80
	Lot 3	1:40	1:40	1:80	1:160
Mid Positive – c-ANCA	Lot 1	1:80	1:80	1:80	1:80
	Lot 2	1:80	1:80	1:80	1:160
	Lot 3	1:80	1:80	1:40	1:160
Mid Positive – p-ANCA	Lot 1	1:160	1:160	1:80	1:160
	Lot 2	1:160	1:160	1:80	1:160
	Lot 3	1:160	1:160	1:160	1:320
High Positive – c-ANCA	Lot 1	1:1280	1:1280	1:640	1:1280
	Lot 2	1:640	1:640	1:160	1:640
	Lot 3	1:640	1:640	1:320	1:640
High Positive – p-ANCA	Lot 1	1:640	1:640	1:160	1:640
	Lot 2	1:640	1:640	1:640	1:640
	Lot 3	1:640	1:640	1:320	1:640

c. **Multi-Site Reproducibility Study**

To assess the multi-site reproducibility of the Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-fixed) and the Anti-Neutrophil Cytoplasmic Antibodies (Formalin-fixed), a panel of 8 samples including two low positive samples (~1:20-1:40 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), two mid positive samples (~1:80-1:160 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), two high positive samples (> 1:160 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), and two negative samples were each assayed in triplicate at a 1:20 screening dilution twice per day, on five different days, by three different technicians at three different laboratories using each test system. Qualitative and pattern results were interpreted by two technicians for Methods A and B, and by a single dIFine instrument for Method C (six total technicians and three total dIFine instruments). The results (30 results per sample per method per technician) were analyzed for each site and for combined. The combined results (a total of 240 results for each method per technician for qualitative analysis and a total of 180 results for each method per technician for pattern analysis) are summarized in the tables below:

Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-Fixed)

Site-to-Site and Technician-to-Technician Agreement – Qualitative Results

Method A		Site 1		Site 2		Site 3	
		Technician 1	Technician 2	Technician 1	Technician 2	Technician 1	Technician 2
Site 1	Technician 1		100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
	Technician 2			100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
Site 2	Technician 1				100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
	Technician 2					100% (98.4–100.0%)	100% (98.4–100.0%)
Site 3	Technician 1						100% (98.4–100.0%)
	Technician 2						

Method B		Site 1		Site 2		Site 3	
		Technician 1	Technician 2	Technician 1	Technician 2	Technician 1	Technician 2
Site 1	Technician 1		100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
	Technician 2			100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
Site 2	Technician 1				100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
	Technician 2					100% (98.4–100.0%)	100% (98.4–100.0%)
Site 3	Technician 1						100% (98.4–100.0%)
	Technician 2						

Method C	Site 1	Site 2	Site 3
Site 1		99.6% (97.7–99.9%)	99.6% (97.7–99.9%)
Site 2			99.2% (97.0–99.8%)
Site 3			

There was 100% qualitative pattern agreement between sites and between technicians for Method A and Method B. While there was high qualitative agreement for Method C between sites dIFine was not able to identify low positive p-ANCA sample at Site 2 for one of the replicates across days and runs and low positive p-ANCA sample at Site 3 for couple of replicates across days. Additionally, dIFine did not correctly recognize a negative sample at Site 3 for one of the replicates across days and runs.

Site-to-Site and Technician-to-Technician Agreement – Pattern Results

Method A		Site 1		Site 2		Site 3	
		Technician 1	Technician 2	Technician 1	Technician 2	Technician 1	Technician 2
Site 1	Technician 1		100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
	Technician 2			100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
Site 2	Technician 1				100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
	Technician 2					100% (98.4–100.0%)	100% (98.4–100.0%)
Site 3	Technician 1						100% (98.4–100.0%)
	Technician 2						

Method B		Site 1		Site 2		Site 3	
		Technician 1	Technician 2	Technician 1	Technician 2	Technician 1	Technician 2
Site 1	Technician 1		100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
	Technician 2			100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
Site 2	Technician 1				100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
	Technician 2					100% (98.4–100.0%)	100% (98.4–100.0%)
Site 3	Technician 1						100% (98.4–100.0%)
	Technician 2						

Method C	Site 1	Site 2	Site 3
Site 1		92.7% (88.0–95.7%)	94.4% (90.1–97.0%)
Site 2			91.6% (86.6–94.9%)
Site 3			

There was 100% pattern agreement between sites and technicians for Method A and Method B. While there was high pattern agreement for Method C between sites dIFine was not able to identify low positive p-ANCA sample and mid positive c-ANCA sample at Site 1 across days and runs. Additionally, at Site 2 dIFine did not correctly recognize pattern for low positive p-ANCA sample, low positive c-ANCA sample and mid positive c-ANCA sample for few of the replicates across days and runs. At site 3, dIFine did not accurately identify patterns for low positive and mid positive p-ANCA samples at few replicates across days and runs.

Anti-Neutrophil Cytoplasmic Antibodies (Formalin-Fixed)

Site-to-Site and Technician-to-Technician Agreement – Qualitative Results

Method A		Site 1		Site 2		Site 3	
		Technician 1	Technician 2	Technician 1	Technician 2	Technician 1	Technician 2
Site 1	Technician 1		100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
	Technician 2			100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
Site 2	Technician 1				100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
	Technician 2					100% (98.4–100.0%)	100% (98.4–100.0%)
Site 3	Technician 1						100% (98.4–100.0%)
	Technician 2						

Method B		Site 1		Site 2		Site 3	
		Technician 1	Technician 2	Technician 1	Technician 2	Technician 1	Technician 2
Site 1	Technician 1		100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
	Technician 2			100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
Site 2	Technician 1				100% (98.4–100.0%)	100% (98.4–100.0%)	100% (98.4–100.0%)
	Technician 2					100% (98.4–100.0%)	100% (98.4–100.0%)
Site 3	Technician 1						100% (98.4–100.0%)
	Technician 2						

Method C	Site 1	Site 2	Site 3
Site 1		97.5% (94.7–98.9%)	99.2% (97.0–99.8%)
Site 2			97.9% (95.2–99.1%)
Site 3			

There was 100% qualitative result agreement in all cases across sites and technicians for Method A and Method B. While there was high qualitative agreement for Method C between sites, dIFine was not able to identify low positive c-ANCA sample at Sites 1 and 2 and low positive p-ANCA sample at Site 3 for couple of replicates across days.

Site-to-Site and Technician-to-Technician Agreement – Pattern Results

Method A		**Site 1**		**Site 2**		**Site 3**	
		Technician 1	**Technician 2**	**Technician 1**	**Technician 2**	**Technician 1**	**Technician 2**
Site 1	**Technician 1**		99.4% (96.9–99.9%)	100% (97.9–100.0%)	100% (97.9–100.0%)	100% (97.9–100.0%)	100% (97.9–100.0%)
	Technician 2			99.4% (96.9–99.9%)	99.4% (96.9–99.9%)	99.4% (96.9–99.9%)	99.4% (96.9–99.9%)
Site 2	**Technician 1**				100% (97.9–100.0%)	100% (97.9–100.0%)	100% (97.9–100.0%)
	Technician 2					100% (97.9–100.0%)	100% (97.9–100.0%)
Site 3	**Technician 1**						100% (97.9–100.0%)
	Technician 2						

Method B		**Site 1**		**Site 2**		**Site 3**	
		Technician 1	**Technician 2**	**Technician 1**	**Technician 2**	**Technician 1**	**Technician 2**
Site 1	**Technician 1**		99.4% (96.9–99.9%)	100% (97.9–100.0%)	100% (97.9–100.0%)	100% (97.9–100.0%)	100% (97.9–100.0%)
	Technician 2			99.4% (96.9–99.9%)	99.4% (96.9–99.9%)	99.4% (96.9–99.9%)	99.4% (96.9–99.9%)
Site 2	**Technician 1**				100% (97.9–100.0%)	100% (97.9–100.0%)	100% (97.9–100.0%)
	Technician 2					100% (97.9–100.0%)	100% (97.9–100.0%)
Site 3	**Technician 1**						100% (97.9–100.0%)
	Technician 2						

Method C	**Site 1**	**Site 2**	**Site 3**
Site 1		98.3% (95.0–99.4%)	98.3% (95.2–99.4%)
Site 2			98.9% (95.9–99.7%)
Site 3			

While there was 100% pattern agreement in most cases across sites and technicians for Method A and Method B, there was one discrepant result for mid-positive p-ANCA sample for Technician 2 where the pattern was wrongly identified for one of the three replicates at Site 1. While there was high pattern agreement for Method C between sites dIFine was not able to recognize pattern for low positive c-ANCA sample at Site 2 for few of the replicates across days.

2. Linearity:

Six samples including: two low positive samples (~1:20-1:40 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), two mid positive samples (~1:80-1:160 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), and two high positive samples (> 1:160 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), were each assayed at a 1:20 screening dilution, as well as at various corresponding serial dilutions. For Methods A and B, the fluorescence intensity was recorded at each dilution using a scale of "4" (very intense) and "0" (no fluorescence). Qualitative results and pattern results were interpreted by all three methods. For each the Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-fixed) and the Anti-Neutrophil Cytoplasmic Antibodies (Formalin-fixed) test system, the endpoints for each sample and each method, and the sample dilutions and the associated fluorescence intensities for Method A and Method B (Method C cannot provide an intensity grade, only a positive, negative, or uncertain call) are summarized in the tables below:

Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-Fixed)

Endpoint Titers across Methods				
Sample	**Method A**	**Method B**	**Method C**	
			UNC as (-)	**UNC as (+)**
Low Positive PR3 (c-ANCA)	1:40	1:40	1:40	1:40
Low Positive MPO (p-ANCA)	1:40	1:40	1:80	1:160
Medium Positive PR3 (c-ANCA)	1:80	1:80	1:80	1:80
Medium Positive MPO (p-ANCA)	1:80	1:160	1:160	1:320
High Positive PR3 (c-ANCA)	1:1280	1:2560	1:2560	1:5120
High Positive MPO (p-ANCA)	1:640	1:640	1:640	1:640

The sample dilutions and associated intensity grade results

Sample		**1:20**	**1:40**	**1:80**	**1:160**	**1:320**	**1:640**	**1:1280**	**1:2560**	**1:5120**
Low Positive c-ANCA	Method A	2+	1+	0	0	0				
	Method B	2+	1+	0	0	0				
Low Positive p-ANCA	Method A	2+	1+	0	0	0				
	Method B	2+	1+	0	0	0				
Mid Positive c-ANCA	Method A	3+	2+	1+	0	0				
	Method B	3+	2+	1+	0	0				
Mid Positive p-ANCA	Method A	3+	2+	1+	0	0				
	Method B	4+	3+	2+	1+	0				
High Positive c-ANCA	Method A	4+	4+	4+	4+	3+	2+	1+	0	0
	Method B	4+	4+	4+	4+	4+	3+	2+	1+	0
High Positive p-ANCA	Method A	4+	4+	4+	3+	2+	1+	0	0	0
	Method B	4+	4+	4+	3+	2+	1+	0	0	0

Anti-Neutrophil Cytoplasmic Antibodies (Formalin-Fixed)

Endpoint Titers across Methods				
Sample	**Method A**	**Method B**	**Method C**	
			UNC as (-)	**UNC as (+)**
Low Positive PR3 (c-ANCA)	1:40	1:40	1:40	1:80
Low Positive MPO (p-ANCA)	1:40	1:40	1:40	1:40
Medium Positive PR3 (c-ANCA)	1:80	1:80	1:80	1:80
Medium Positive MPO (p-ANCA)	1:160	1:160	1:80	1:160
High Positive PR3 (c-ANCA)	1:1280	1:1280	1:640	1:1280
High Positive MPO (p-ANCA)	1:640	1:640	1:160	1:640

The sample dilutions and associated intensity grade results

Sample		**1:20**	**1:40**	**1:80**	**1:160**	**1:320**	**1:640**	**1:1280**	**1:2560**	**1:5120**
Low Positive c-ANCA	Method A	2+	1+	0	0	0				
	Method B	2+	1+	0	0	0				
Low Positive p-ANCA	Method A	2+	1+	0	0	0				
	Method B	2+	1+	0	0	0				
Mid Positive c-ANCA	Method A	3+	2+	1+	0	0				
	Method B	3+	2+	1+	0	0				
Mid Positive p-ANCA	Method A	4+	3+	2+	1+	0				
	Method B	4+	3+	2+	1+	0				
High Positive c-ANCA	Method A	4+	4+	4+	4+	3+	2+	1+	0	0
	Method B	4+	4+	4+	4+	3+	2+	1+	0	0
High Positive p-ANCA	Method A	4+	4+	4+	3+	2+	1+	0	0	0
	Method B	4+	4+	4+	3+	2+	1+	0	0	0

3. Analytical Specificity/Interference:

The panel of samples include: two low positive samples (~1:20-1:40 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA); two mid positive samples (~1:80-1:160 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA); two high positive samples (> 1:160 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA); and two negative samples, were spiked with two different concentrations (Low Concentration and High Concentration) of 19 different interferents as outlined in the table below. All specimens were assayed in triplicate by each of the Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-fixed) and the Anti-Neutrophil Cytoplasmic Antibodies (Formalin-fixed) at a 1:20 dilution and interpreted by all three methods. Qualitative results and pattern results were interpreted by two technicians for Methods A and B, and by a single dIFine instrument for Method C.

Endogenous Substances		
Substance	**Low Concentration**	**High Concentration**
Bilirubin (Unconjugated)	0.02 mg/mL	0.15 mg/mL
Cholesterol (total)	1.5 mg/mL	2.2 mg/mL
Triglycerides (total)	1.0 mg/mL	2.5 mg/mL
Albumin	35.0 mg/mL	52.0 mg/mL
Hemoglobin	100.0 mg/mL	200.0 mg/mL
RF	200.0 U/mL	400.0 U/mL

Exogenous Substances		
Substance	**Low Concentration**	**High Concentration**
Intralipids	2.0 mg/mL	20.0 mg/mL
Cyclophosphamide	0.183 mg/mL	0.549mg/mL
Ibuprofen	0.073 mg/mL	0.219 mg/mL
Hydroxychloroquine	0.006 mg/mL	0.024 mg/mL
Simvastatin	0.0000277 mg/mL	0.000083 mg/mL
Prednisone	0.000033 mg/mL	0.000099 mg/mL
Azathioprine	0.00086 mg/mL	0.00258 mg/mL
Diltiazem	0.0003 mg/mL	0.0009 mg/mL
Mycophenolate mofetil	0.012 mg/mL	0.048 mg/mL
Rituximab	0.5 mg/mL	2.0 mg/mL
Belimumab	2.0 mg/mL	8.0 mg/mL
Methotrexate	0.454 mg/mL	1.36 mg/mL
Naproxen	0.12 mg/mL	0.36 mg/mL

For both Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-fixed) and the Anti-Neutrophil Cytoplasmic Antibodies (Formalin-fixed) test systems, none of the interferents affected the expected qualitative results for any samples when read by Methods A and B. Method C yielded qualitatively correct results for the Anti-Neutrophil Cytoplasmic Antibodies (Formalin-fixed) test systems, however there were few instances of discrepant results for Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-fixed) as noted below:

- The low positive PR3 (c-ANCA) sample showed effect of interference for Diltiazem spiked at a low concentration.
- The low positive PR3 (c-ANCA) sample showed effect of interference for cholesterol spiked at a low concentration.
- The low positive PR3 (c-ANCA) sample showed effect of interference for Prednisone spiked at a low concentration.
- The low positive MPO (p-ANCA) sample showed effect of interference for hemoglobin spiked at a high concentration.

4. Assay Reportable Range:

   Not Applicable.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   a. Traceability:

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

   b. Stability

      Sample Stability:
      Three serum samples including one negative sample, one low positive c-ANCA sample and one low positive p-ANCA sample were assayed using the ZEUS ANCA kits (Ethanol-fixed and Formalin-fixed) to assess the stability of ANCAs in serum samples

stored for various conditions. Multiple aliquots of the three serum samples were prepared and one set of aliquot served as the baseline or time T_0 time-point. The remaining aliquots were placed at room temperature and -20°C. An aliquot from each storage temperature was assayed in triplicate, at a 1:20 screening dilution by one technician using Methods A and B and one dIFine instrument for Method C, at the following time points:

- Room temperature and 2-8°C storage: 1 day, 3 days, 7 days, and 14 days
- -20°C storage (frozen): 7 days, 14 days, and 21 days

For the samples stored at room temperature, there was no change in qualitative outcome or pattern over the 14-day testing period, for Methods A and B on both the Ethanol-fixed and Formalin-fixed slides. Likewise, for the frozen samples, there was no change in the qualitative outcome, or the pattern observed over the 21-day period, regardless of the method interpretation on both the Ethanol-fixed and Formalin-fixed slides. This study demonstrates that the ANCA antibody in the human serum samples are stable for a minimum of 7 days at room temperature and 14 days when frozen.

Reagent Stability:

The reagent stability was assessed based on the real-time stability study in which a panel of samples includes: two low positive samples (~1:20-1:40 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), two mid positive samples (~1:80-1:160 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), two high positive samples (> 1:160 endpoint) with one anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), and two negative samples, were assayed in singlicate at a 1:20 screening dilution to represent the baseline or time-point T_0. Different lots of ANCA Ethanol-fixed or ANCA Formalin-fixed reagent kits stored at 2-8°C were tested at different time-points. The data were analyzed for the qualitative results and patterns agreement results comparing to the baseline. The results support that the reagent kits of the Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-Fixed) and Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-Fixed) are stable up to 7 months when stored at 2-8°C.

6. Detection Limit:

   Not Applicable.

7. Assay Cut-Off:

   It is recommended to perform the test using a screening dilution of 1:20 followed by serial dilutions for semi-quantitative determinations.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   In order to show comparable performance between the ANCA Ethanol-fixed or Formalin-fixed IFA test system and the predicate ANCA Ethanol-Fixed or Formalin-fixed IFA test system, 583 clinically characterized serum specimens that included 223 serum specimens with ANCA Associated Vasculitis (AAV) and 360 serum specimens with control diseases were tested. These 583 clinically characterized specimens included in the study are outlined in the table below.

Diseases		N
ANCA Associated Vasculitis	Microscopic Polyangiitis (MPA)	123
	Granulomatosis with Polyangiitis (GPA)	92
	Eosinophilic Granulomatosis with Polyangiitis (eGPA)	8
Control Diseases	Non-ANCA Associated Vasculitis	30
	Autoimmune Thyroid	30
	Rheumatoid Arthritis	30
	Chronic Kidney Disease	30
	Systemic Sclerosis	30
	Systemic Lupus Erythematosus	30
	Dermatomyositis/Polymyositis	30
	Inflammatory Bowel Disease	30
	Sinusitis/Allergic Rhinitis	30
	Infectious Disease	30
	Asthma/COPD	30
	Cancer	30
Total		**583**

All 583 samples were tested by three different technicians at a 1:20 screening dilution using the candidate device, i.e., the Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-fixed) or Anti-Neutrophil Cytoplasmic Antibodies (Formalin-fixed) test system. The samples were also tested by a single technician using the predicate device, i.e., NOVA Lite DAPI ANCA (Ethanol) or NOVA Lite DAPI ANCA (Formalin). Qualitative and pattern results for the candidate assay were interpreted by two technicians at three different sites for Methods A and B, and by a single dIFine instrument at each laboratory for Method C (6 total technicians and 3 total dIFine instruments) while qualitative and pattern results for the predicate were interpreted at a single laboratory for Method A. The results are summarized in the tables below.

Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-Fixed)

ANCA (Ethanol-fixed) vs NOVA Lite DAPI ANCA (Ethanol) – Qualitative Results

	% Positive Agreement (95% CI)	% Negative Agreement (95% CI)	% Total Agreement (95% CI)
Method A vs Predicate	92.3% (91.1-93.4%)	88.5% (86.8-90.1%)	90.8% (89.8-91.7%)
Method B vs Predicate	93.0% (91.8-94.0%)	87.7% (85.9-89.3%)	90.8% (89.8-91.8%)
Method C vs Predicate (UNC as positive)	94.4% (92.8-95.6%)	83.5% (80.6-96.1%)	90.0% (88.5-91.3%)
Method C vs Predicate (UNC as negative)	91.8% (90.0-93.3%)	90.0% (87.6-92.0%)	91.1% (89.7-92.3%)

ANCA (Ethanol-fixed) vs NOVA Lite DAPI ANCA (Ethanol) – Pattern Results

	% Agreement (95% CI)
Method A vs Predicate	79.7% (78.4-81.1%)
Method B vs Predicate	79.6% (78.2-80.9%)
Method C vs Predicate	78.4% (76.4-80.2%)

Between-Method Agreement of the ANCA (Ethanol-fixed) - Qualitative Results

	% Positive Agreement (95% CI)	% Negative Agreement (95% CI)	% Total Agreement (95% CI)
Method A vs Method B	99.4% (98.9-99.6%)	97.3% (86.8-90.1%)	98.5% (98.1-98.9%)
Method A vs Method C (UNC as positive)	99.3% (98.9-99.6%)	91.0% (89.4-92.4%)	96.0% (95.2-96.6%)
Method A vs Method C (UNC as negative)	96.7% (95.8-97.4%)	97.4% (96.4-98.1%)	97.0% (96.4-97.5%)
Method B vs Method C (UNC as positive)	99.5% (99.1-99.7%)	93.0% (91.4-94.1%)	96.9% (96.2-97.4%)
Method B vs Method C (UNC as negative)	96.1% (95.1-96.8%)	98.2% (97.4-98.8%)	96.9% (96.3-97.4%)

Between-Methods Agreement of the ANCA (Ethanol-fixed) – Pattern Results

	% Agreement (95% CI)
Method A vs Method B	95.5% (94.5-96.3%)
Method A vs Method C	85.5% (83.9–87.0%)
Method B vs Method C	86.5% (85.0-87.9%)

Anti-Neutrophil Cytoplasmic Antibodies (Formalin-Fixed)

ANCA (Formalin-fixed) vs NOVA Lite DAPI ANCA (Formalin) – Qualitative Results

	% Positive Agreement (95% CI)	% Negative Agreement (95% CI)	% Total Agreement (95% CI)
Method A vs Predicate	92.0% (90.5-93.3%)	95.6% (94.6-96.4%)	94.8% (92.6-96.2%)
Method B vs Predicate	91.9% (90.3-93.2%)	95.5% (94.5-96.3%)	94.0% (93.2-94.8%)
Method C vs Predicate (UNC as positive)	88.9% (86.4-91.1%)	97.7% (96.6-98.5%)	94.2% (93.0-95.2%)
Method C vs Predicate (UNC as negative)	84.9% (82.1-87.4%)	98.0% (97.0-98.7%)	92.8% (91.5-93.9%)

ANCA (Formalin-fixed) vs NOVA Lite DAPI ANCA (Formalin) – Qualitative Results

	% Agreement (95% CI)
Method A vs Predicate	93.2% (92.3-94.0%)
Method B vs Predicate	93.3% (92.4-94.1%)
Method C vs Predicate	91.5% (90.1-92.7%)

Between-Method of the ANCA (Formalin-fixed) - Qualitative Results

	% Positive Agreement (95% CI)	% Negative Agreement (95% CI)	% Total Agreement (95% CI)
Method A vs Method B	99.2% (98.6-99.6%)	99.5% (99.1-99.7%)	99.4% (99.1-99.6%)
Method A vs Method C (UNC as positive)	92.9% (91.4-94.1%)	99.5% (99.1-99.7%)	96.9% (96.3-97.4%)
Method A vs Method C (UNC as negative)	88.8% (87.0-90.4%)	99.8% (99.5-99.9%)	95.5% (94.7-96.1%)
Method B vs Method C (UNC as positive)	93.1% (91.6-94.3%)	99.7% (99.3-99.8%)	97.1% (96.5-97.6%)
Method B vs Method C (UNC as negative)	88.9% (87.2-90.5%)	99.9% (99.7-100.0%)	95.6% (94.9-96.2%)

Between-Methods of the ANCA (Ethanol-fixed) – Pattern Results

	% Agreement (95% CI)
Method A vs Method B	95.5% (94.5-96.3%)
Method A vs Method C	85.5% (83.9–87.0%)
Method B vs Method C	86.5% (85.0-87.9%)

2. Matrix Comparison:

Not Applicable.

C Clinical Studies:

1. Clinical Sensitivity and Clinical Specificity:

The samples tested in the Method Comparison study (see Section above) were evaluated for clinical performance of each of the Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-fixed) and the Anti-Neutrophil Cytoplasmic Antibodies (Formalin-fixed) test systems. A total of 583 samples including 223 AAV samples and 360 control disease samples were tested with each test system at three sites. The clinical performance of the candidate devices is summarized in the tables below:

Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-Fixed)

Percent positives at each site and each technician by different interpretation methods are presented in the tables below:

Site 1							
ANCA (Ethanol-Fixed)		**Method A**		**Method B**		**Method C**	
		Tech A	**Tech B**	**Tech A**	**Tech B**	**UNC (-)**	**UNC (+)**
Diagnosis	**N**	**% Pos**	**% Pos**	**% Pos**	**% Pos**	**% Pos**	**% Pos**
Target Disease (ANCA Associated Vasculitis)							
MPA	123	97.6%	98.4%	98.4%	98.4%	98.4%	99.2%
GPA	92	92.4%	92.4%	92.4%	92.4%	89.1%	92.4%
eGPA	8	87.5%	100.0%	87.5%	87.5%	87.5%	100.0%
Control Disease							
Non-AAV*	30	40.0%	40.0%	40.0%	40.0%	43.3%	50.0%
Autoimmune Thyroid	30	36.7%	36.7%	40.0%	36.7%	40.0%	46.7%
Rheumatoid Arthritis	30	53.3%	53.3%	53.3%	53.3%	50.0%	56.7%
Chronic Kidney Disease	30	46.7%	43.3%	46.7%	43.3%	36.7%	50.0%
Systemic Sclerosis	30	56.7%	56.7%	56.7%	56.7%	56.7%	56.7%
SLE*	30	83.3%	83.3%	83.3%	83.3%	86.7%	90.0%
DM/PM*	30	26.7%	26.7%	26.7%	26.7%	23.3%	30.0%
IBD*	30	40.0%	40.0%	40.0%	40.0%	40.0%	40.0%
Sinusitis/Allergic Rhinitis	30	13.3%	13.3%	13.3%	13.3%	20.0%	20.0%
Infectious Disease	30	26.7%	30.0%	26.7%	26.7%	26.7%	46.7%
Asthma/COPD	30	13.3%	13.3%	13.3%	13.3%	13.3%	13.3%
Cancer	30	16.7%	13.3%	16.7%	16.75	13.3%	23.3%
Total	**583**						

* Non-AAV: Non-ANCA Associated Vasculitis; SLE: Systemic Lupus Erythematosus; DM/PM: Dermatomyositis/Polymyositis; IBD: Inflammatory Bowel Disease

Site 2							
ANCA (Ethanol-Fixed)		**Method A**		**Method B**		**Method C**	
		Tech A	**Tech B**	**Tech A**	**Tech B**	**UNC (-)**	**UNC (+)**
Diagnosis	**N**	**% Pos**	**% Pos**	**% Pos**	**% Pos**	**% Pos**	**% Pos**
Target Disease (ANCA Associated Vasculitis)							
MPA	123	98.4%	98.4%	98.4%	98.4%	98.4%	99.2%
GPA	92	90.2%	89.1%	90.2%	89.1%	89.1%	90.2%
eGPA	8	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Control Disease							
Non-AAV*	30	40.0%	40.0%	40.0%	43.3%	36.7%	43.3%
Autoimmune Thyroid	30	26.7%	30.0%	33.3%	26.7%	26.7%	36.7%
Rheumatoid Arthritis	30	50.0%	50.0%	50.0%	50.0%	46.7%	50.0%
Chronic Kidney Disease	30	36.7%	40.0%	40.0%	40.0%	36.7%	43.3%
Systemic Sclerosis	30	53.3%	56.7%	56.7%	53.3%	53.3%	60.0%
SLE*	30	80.0%	80.0%	80.0%	80.0%	76.7%	83.3%
DM/PM*	30	13.3%	13.3%	16.7%	13.3%	16.7%	20.0%
IBD*	30	36.7%	36.7%	36.7%	36.7%	40.0%	43.3%
Sinusitis/Allergic Rhinitis	30	6.7%	6.7%	6.7%	6.7%	6.7%	6.7%
Infectious Disease	30	20.0%	20.0%	20.0%	20.0%	20.0%	26.7
Asthma/COPD	30	10.0%	10.0%	10.0%	10.0%	10.0%	10.0%
Cancer	30	16.7%	16.7%	16.7%	16.7%	13.3%	16.7%
Total	**583**						

* Non-AAV: Non-ANCA Associated Vasculitis; SLE: Systemic Lupus Erythematosus;
DM/PM: Dermatomyositis/Polymyositis; IBD: Inflammatory Bowel Disease

Site 3							
ANCA (Ethanol-Fixed)		**Method A**		**Method B**		**Method C**	
		Tech A	**Tech B**	**Tech A**	**Tech B**	**UNC (-)**	**UNC (+)**
Diagnosis	**N**	**% Pos**	**% Pos**	**% Pos**	**% Pos**	**% Pos**	**% Pos**
Target Disease (ANCA Associated Vasculitis)							
MPA	123	99.2%	99.2%	99.2%	99.2%	99.2%	99.2%
GPA	92	95.7%	95.7%	95.7%	94.6%	91.3%	93.5%
eGPA	8	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Control Disease							
Non-AAV*	30	40.0%	40.0%	40.0%	43.3%	36.7%	43.3%
Autoimmune Thyroid	30	26.7%	30.0%	33.3%	26.7%	26.7%	36.7%
Rheumatoid Arthritis	30	50.0%	50.0%	50.0%	50.0%	46.7%	50.0%
Chronic Kidney Disease	30	36.7%	40.0%	40.0%	40.0%	36.7%	43.3%
Systemic Sclerosis	30	53.3%	56.7%	56.7%	53.3%	53.3%	60.0%
SLE*	30	80.0%	80.0%	80.0%	80.0%	76.7%	83.3%
DM/PM*	30	13.3%	13.3%	16.7%	13.3%	16.7%	20.0%
IBD*	30	36.7%	36.7%	36.7%	36.7%	40.0%	43.3%
Sinusitis/Allergic Rhinitis	30	6.7%	6.7%	6.7%	6.7%	6.7%	6.7%
Infectious Disease	30	20.0%	20.0%	20.0%	20.0%	20.0%	26.7%
Asthma/COPD	30	10.0%	10.0%	10.0%	10.0%	10.0%	10.0%
Cancer	30	16.7%	16.7%	16.7%	16.7%	13.3%	16.7%
Total	**583**						

* Non-AAV: Non-ANCA Associated Vasculitis; SLE: Systemic Lupus Erythematosus;
DM/PM: Dermatomyositis/Polymyositis; IBD: Inflammatory Bowel Disease

When the same samples were also run on the predicate device, there is a high correlation for % positive samples in the AAV and the control disease group between the candidate device across sites and technicians when compared with the predicate device.

Clinical Performance Summary for ANCA Ethanol-Fixed

Site	Tech	Interpretation	AAV* (n = 223)	CD** (n = 360)
			% Sensitivity (95% CI)	% Specificity 95% CI
1	A	Method A	95.1% (91.4-97.2%)	62.2% (57.1-67.1%)
		Method B	95.5% (91.9-97.6%)	61.9% (56.8-66.8%)
	B	Method A	96.0% (92.5-97.9%)	62.5% (57.4-67.3%)
		Method B	95.5% (91.9-97.6%)	62.5% (57.4-67.3%)
	dIFine	Method C - UNC as (-)	94.2% (90.3-96.6%)	62.5% (57.4-67.3%)
		Method C - UNC as (+)	96.4% (93.1-98.2%)	56.7% (51.5-61.7%)
2	A	Method A	95.1% (91.4-97.2%)	67.5% (62.5-72.1%)
		Method B	95.1% (91.4-97.2%)	66.1% (61.1-70.8%)
	B	Method A	94.6% (90.8-96.9%)	66.7% (61.6-71.3%)
		Method B	94.6% (90.8-96.9%)	66.9% (61.9-71.6%)
	dIFine	Method C - UNC as (-)	94.6% (90.8-96.9%)	68.1% (63.1-72.7%)
		Method C - UNC as (+)	95.5% (91.9-97.6%)	63.3% (58.2-68.2%)
3	A	Method A	97.8% (94.9-99.0%)	60.0% (54.9-64.9%)
		Method B	97.8% (94.9-99.0%)	57.2 (52.1-62.2%)
	B	Method A	97.8% (94.9-99.0%)	60.3 (55.1-65.2%)
		Method B	97.3% (94.3-98.8%)	56.9 (51.8-62.0%)
	dIFine	Method C - UNC as (-)	96.0% (92.5-97.9%)	61.4 (56.3-66.3%)
		Method C - UNC as (+)	96.9% (93.7-98.5%)	54.2(49.0-59.2%)

Samples were run on predicate manual interpretation method at Site 1 and the clinical performance was evaluated. The clinical performance between the candidate device and the predicate device at the same site using the same technician showed the comparable clinical sensitivity and specificity as shown below.

Interpretation (Site 1, Tech A, **Method A**)	AAV (n = 223)	CD (n = 360)
	Sensitivity% (95% CI)	Specificity% (95% CI)
ANCA (Ethanol-Fixed)	95.1% (91.4-97.2%)	62.2% (57.1-67.1%)
INOVA ANCA (Ethanol)	92.8% (88.7-95.5%)	61.4% (56.3-66.3%)

Anti-Neutrophil Cytoplasmic Antibodies (Formalin-Fixed)

Percent positives at each site and each technician by different interpretation methods are presented in the tables below.

Site 1							
ANCA (Formalin-Fixed)		**Method A**		**Method B**		**Method C**	
		Tech A	**Tech B**	**Tech A**	**Tech B**	**UNC (-)**	**UNC (+)**
Diagnosis	**N**	**% Pos**	**% Pos**	**% Pos**	**% Pos**	**% Pos**	**% Pos**
Target Disease (ANCA Associated Vasculitis)							
MPA	123	89.4%	90.2%	91.1%	90.2%	87.8%	88.6%
GPA	92	83.7%	83.7%	83.7%	82.6%	80.4%	83.7%
eGPA	8	87.5%	87.5%	87.5%	87.5%	87.5%	87.5%
Control Disease							
Non-AAV*	30	3.3%	3.3%	3.3%	3.3%	0.0%	3.3%
Autoimmune Thyroid	30	6.6%	6.7%	6.7%	6.7%	3.3%	3.3%
Rheumatoid Arthritis	30	13.3%	13.3%	13.3%	13.3%	13.3%	13.3%
Chronic Kidney Disease	30	6.7%	6.7%	6.7%	6.7%	0.0%	3.3%
Systemic Sclerosis	30	13.3%	13.3%	13.3%	13.3%	6.7%	10.0%
SLE*	30	43.3%	43.3%	36.7%	36.7%	40.0%	40.0%
DM/PM*	30	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
IBD*	30	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Sinusitis/Allergic Rhinitis	30	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Infectious Disease	30	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Asthma/COPD	30	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Cancer	30	3.3%	3.3%	3.3%	3.3%	3.3%	3.3%
Total	**583**						

* Non-AAV: Non-ANCA Associated Vasculitis; SLE: Systemic Lupus Erythematosus;
DM/PM: Dermatomyositis/Polymyositis; IBD: Inflammatory Bowel Disease

Site 2							
ANCA (Formalin-Fixed)		**Method A**		**Method B**		**Method C**	
		Tech A	**Tech B**	**Tech A**	**Tech B**	**UNC (-)**	**UNC (+)**
Diagnosis	**N**	**% Pos**	**% Pos**	**% Pos**	**% Pos**	**% Pos**	**% Pos**
Target Disease (ANCA Associated Vasculitis)							
MPA	123	87.0%	84.6%	87.0%	84.6%	82.9%	85.4%
GPA	92	85.9%	82.6%	85.9%	80.4%	73.9%	78.3%
eGPA	8	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Control Disease							
Non-AAV*	30	3.3%	3.3%	3.3%	3.3%	3.3%	3.3%
Autoimmune Thyroid	30	6.7%	6.7%	6.7%	6.7%	6.7%	6.7%
Rheumatoid Arthritis	30	10.0%	10.0%	10.0%	10.0%	6.7	10.0%
Chronic Kidney Disease	30	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Systemic Sclerosis	30	10.0%	10.0%	10.0%	10.0%	10.0%	10.0%
SLE*	30	40.0%	30.0%	36.7%	40.0%	33.3%	43.3%
DM/PM*	30	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
IBD*	30	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Sinusitis/Allergic Rhinitis	30	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Infectious Disease	30	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Asthma/COPD	30	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Cancer	30	3.3%	0.0%	3.3%	0.0%	0.0%	0.0%
Total	**583**						

* Non-AAV: Non-ANCA Associated Vasculitis; SLE: Systemic Lupus Erythematosus.
DM/PM: Dermatomyositis/Polymyositis; IBD: Inflammatory Bowel Disease

Site 3							
ANCA (Formalin-Fixed)		**Method A**		**Method B**		**Method C**	
		Tech A	**Tech B**	**Tech A**	**Tech B**	**UNC (-)**	**UNC (+)**
Diagnosis	**N**	**% Pos**	**% Pos**	**% Pos**	**% Pos**	**% Pos**	**% Pos**
Target Disease (ANCA Associated Vasculitis)							
MPA	123	93.5%	95.1%	94.3%	95.1%	83.7%	89.4%
GPA	92	92.4%	92.4%	93.5%	93.5%	82.6%	84.8%
eGPA	8	100.0%	100.0%	100.0%	100.0%	87.5%	87.5%
Control Disease							
Non-AAV*	30	3.3%	0.0%	3.3%	3.3%	0.0%	0.0%
Autoimmune Thyroid	30	13.3%	10.0%	13.3%	13.3%	3.3%	6.7%
Rheumatoid Arthritis	30	23.3%	23.3%	23.3%	23.3%	13.3%	16.7%
Chronic Kidney Disease	30	6.7%	6.7%	6.7%	6.7%	0.0%	0.0%
Systemic Sclerosis	30	20.0%	20.0%	20.0%	20.0%	10.0%	13.3%
SLE*	30	53.3%	53.3%	53.3%	53.3%	40.0%	43.3%
DM/PM*	30	6.7%	10.0%	3.3%	6.7%	0.0%	0.0%
IBD*	30	6.7%	6.7%	6.7%	6.7%	0.0%	0.0%
Sinusitis/Allergic Rhinitis	30	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Infectious Disease	30	6.7%	6.7%	6.7%	6.7%	0.0%	0.0%
Asthma/COPD	30	6.7%	6.7%	6.7%	6.7%	0.0%	0.0%
Cancer	30	6.7%	6.7%	6.7%	6.7%	3.3%	3.3%
Total	**583**						

* Non-AAV: Non-ANCA Associated Vasculitis; SLE: Systemic Lupus Erythematosus.
DM/PM: Dermatomyositis/Polymyositis; IBD: Inflammatory Bowel Disease

When the same samples were run on the predicate device, there is a high correlation for % positive samples in the AAV and the control disease group between the candidate device across sites and technicians when compared with the predicate device.

Clinical Performance for the Anti-Neutrophil Cytoplasmic Antibodies (Formalin-Fixed)

Site	**Tech**	**Interpretation**	**AAV (n = 223)**	**CD (n = 360)**
			Sensitivity% (95% CI)	**Specificity% (95% CI)**
1	A	Method A	87.0% (82.0-91.0%)	92.5% (89.3-94.8%)
		Method B	87.9% (83.0-91.5%)	93.1% (90.0-95.3%)
	B	Method A	87.4% (82.5-91.2%)	92.5% (89.3-94.8%)
		Method B	87.0% (82.0-91.0%)	93.1% (90.0-95.3%)
	dIFine	Method C - UNC as (-)	84.8% (79.5-88.9%)	94.4% (91.6-96.4%)
		Method C - UNC as (+)	86.5% (81.4-90.4%)	93.6% (90.6-95.7%)
2	A	Method A	87.0% (82.0-90.8%)	93.9% (90.9-95.9%)
		Method B	87.0% (82.0-90.8%)	94.2% (91.3-96.2%)
	B	Method A	84.3% (79.0-88.5%)	95.0% (92.2-96.8%)
		Method B	83.4% (78.0-87.7%)	94.2% (91.3-96.2%)
	dIFine	Method C - UNC as (-)	79.8% (74.0-84.6%)	95.0% (92.2-96.8%)
		Method C - UNC as (+)	83.0% (77.5-87.3%)	93.9% (90.9-95.9%)
3	A	Method A	93.3% (89.2-95.9%)	87.2% (83.4-90.3%)
		Method B	94.2% (90.3-96.6%)	87.5% (83.7-90.5%)
	B	Method A	94.2% (90.3-96.6%)	87.5% (83.7-90.5%)
		Method B	94.6% (90.8-96.9%)	87.2% (83.4-90.3%)
	dIFine	Method C - UNC as (-)	83.4% (78.0-87.7%)	94.2% (91.3-96.2%)
		Method C - UNC as (+)	87.4% (82.5-91.2%)	93.1% (90.0-95.3%)

Samples were analyzed using the predicate manual interpretation method at Site 1 and the clinical performance was evaluated. The clinical performance between the candidate device and the predicate device at the same site using the same technician showed comparable clinical sensitivity and specificity as shown below.

Interpretation (Site 1, Tech A, **Method A)**	**AAV (n = 223)** **Sensitivity% (95% CI)**	**CD (n = 360)** **Specificity% (95% CI)**
ANCA (Formalin-Fixed)	88.8% (84.0-92.3%)	90.6% (87.1-93.2%)
INOVA ANCA (Formalin)	87.0% (82.0-91.0%)	92.5% (89.3-94.8%)

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

One hundred and fifty serum samples were acquired from apparently healthy donors within the United States. Each sample was tested at the screening dilution of 1:20, then scanned by ZEUS dIFine and the qualitative results (i.e., Positive, Negative, Uncertain) were determined via Methods A, B, and C. The percent positivity was determined for the 1:20 screening data and the results are summarized below in the table:

Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-Fixed)

Method	**N**	**Number of Positives**	**% Positives**	**Number of Negatives**	**% Negatives**	**Number of Uncertain**	**% Uncertain**
A	150	16*	10.7	134	89.3	NA	NA
B	150	16**	10.7	134	89.3	NA	NA
C	150	19***	12.7	125	83.3	6***	4.0

*6 of 16 ANA positive by HEp-2 IFA; **7 of 19 ANA positive by HEp-2 IFA; ***1 of 6 ANA positive by HEp-2 IFA

Anti-Neutrophil Cytoplasmic Antibodies (Formalin-Fixed)

Method	**N**	**Number of Positives**	**% Positives**	**Number of Negatives**	**% Negatives**	**Number of Uncertain**	**% Uncertain**
A	150	2*	1.3	148	98.7	NA	NA
B	150	2**	1.3	148	98.7	NA	NA
C	150	1	0.7	148	98.7	1**	0.7

*1 of 2 ANA positive by HEp-2 IFA; ** 1 of 1 ANA positive by HEp-2 IFA

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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