EN

Anti-Neutrophil Cytoplasmic Antibodies (Ethanol-fixed)

REF FA9601-1010 **IVD** **Rx Only** Σ 100

INTENDED 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 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.

SUMMARY AND EXPLANATION

Anti-Neutrophil Cytoplasmic Antibodies (ANCA) are autoantibodies targeted against antigens within the cytoplasm of neutrophils and are associated with a group of rare diseases referred to as ANCA-Associated Vasculitis (AAV).[1,2] ANCA are predominantly directed against two proteins, Myeloperoxidase (MPO) and Proteinase 3 (PR3), which are located within cytoplasmic granules of neutrophils; however, other antigens have been described such as, but not limited to, Elastase, Cathepsin G, and Lactoferrin.[3] *In vivo*, MPO and PR3 possess enzymatic activity and play important roles within the innate immune system's defense against pathogens; including production of hypochlorous acid, antimicrobial peptides, and pro-inflammatory cytokines.[2,3] When testing AAV patient samples using Ethanol-fixed ANCA IFA slides, two different staining patterns typically emerge; depending upon whether the autoantibodies are directed against PR3 or MPO.[4,5,6] Anti-MPO ANCA primarily produce a perinuclear staining pattern (p-ANCA), whereas anti-PR3 ANCA produce a cytoplasmic staining pattern (c-ANCA).[7] Samples yielding the p-ANCA pattern result are typically associated with AAV diseases such as Microscopic Polyangiitis (MPA) and Eosinophilic Granulomatosis with Polyangiitis (eGPA).[7] Samples yielding the c-ANCA pattern result are typically associated with Granulomatosis with Polyangiitis (GPA), formerly known as Wegener's Granulomatosis.[7] Staining patterns that closely mimic p-ANCA, and/or atypical patterns, may also be observed due to the presence of anti-nuclear antibodies (ANA) or other cross-reactive antibodies that are sometimes present in serum samples derived from patients diagnosed with non-AAV autoimmune or inflammatory diseases; including Systemic Lupus Erythematosus (SLE), Inflammatory Bowel Disease (IBD), and Rheumatoid Arthritis (RA).[7-10] Consequently, serum samples yielding a p-ANCA result on Ethanol-fixed slides should undergo additional serologic testing, such as Formalin-fixed ANCA IFA, solid phase MPO/PR3 assays, as well as ANA detection assays, towards distinguishing between true positives and false positives.[7,8]

PRINCIPLE OF THE ASSAY

The Sebia ANCA Ethanol-fixed IFA test system is designed to detect the presence of ANCA in human serum. The assay employs an ethanol-fixed human granulocyte substrate, optimized goat anti-human IgG fluorescein isothiocyanate (FITC) conjugate solution, and wash solution. The reaction occurs in two steps:

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. 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.

REAGENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: Conjugate and Controls contain a combination of Proclin (0.05% v/v) and Sodium Azide (<0.1% w/v) as preservatives.**

SLD	1	ANCA Substrate Slides: Ten, 10-well Slides with absorbent blotter and desiccant pouch.
CONJ	2	Conjugate: Goat anti-human IgG labeled with FITC. Contains phosphate buffer with BSA and counterstain. Two, amber bottles, containing 3mL. Ready to use.
CTRL +	3	c-ANCA Positive Control: Contains c-ANCA positive human serum, along with preservatives. One red-capped microvial. 0.5mL, ready to use.
CTRL +	4	p-ANCA Positive Control: Contains p-ANCA positive human serum, along with preservatives. One orange-capped microvial. 0.5mL, ready to use.
CTRL -	5	Negative Control: Contains human serum negative for ANCA, along with preservatives. One green-capped microvial. 0.5mL, ready to use.
COVGLS	6	Cover Glass. Package of twelve, 24 x 60 mm, Thickness #1.

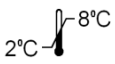
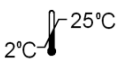
BUF PBS	7	Phosphate-buffered-saline (PBS): pH 7.2 ± 0.2. Empty contents of each buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved. Two packets, sufficient to prepare 2 liters.
MNTMED	8	Mounting Media (Buffered Glycerol): Two, 3 mL, white-capped dripper-tipped bottles. Ready to use

NOTES:
The following components are not kit lot number dependent and may be used interchangeably with the Sebia IFA products, as long as the product numbers are identical: Mounting media (Product #: FA0009S), Negative control (FA2005-IUNC), Cover glass (Product S8007), and PBS (Product #: 0008S).

MATERIALS REQUIRED BUT NOT PROVIDED

1. dIFine® instrument or a properly equipped fluorescence microscope.
2. Pipettor(s) capable of dispensing volumes between 10 and 200 µL.
3. Disposable pipette tips.
4. Small test tubes, dilution plate or similar for preparing sample dilutions.
5. Slide Washer, or a large staining dish with a magnetic stir plate for washing Slides between incubation steps.
6. Distilled or deionized water.
7. 1 Liter Graduated Cylinder.
8. Laboratory timer to monitor incubation steps.
9. Disposal basin and disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite).

STORAGE CONDITIONS

	Unopened Test System is stable until the expiration date provided on the lot-specific labeling.
	After opening for the first time, Mounting Media, Conjugate, Positive Controls, and Negative Control have been demonstrated to be stable for up to 15 months.
	Rehydrated PBS has been shown to be stable for up to 30 days.
	Unopened phosphate-buffered-saline (PBS) packets are stable until the expiration date provided on the lot-specific labeling.

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. This device is for use by a trained operator in a clinical laboratory setting.
3. All software-aided results must be confirmed by the trained operator.
4. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
5. The wells of the Slide do not contain viable cells. However, consider the Slide **potentially bio-hazardous materials** and handle accordingly.
6. The Controls are **potentially bio-hazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Bio-safety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (11).
7. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay**. Return unused reagents to their original containers immediately and follow storage requirements.
8. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual PBS, by blotting, before adding Conjugate. Do not allow the wells to dry out between incubations.
9. Conjugate and Controls contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide. This preservative may be toxic if ingested.
10. Dilution or adulteration of these reagents may generate erroneous results.
11. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
12. Avoid microbial contamination of reagents. Incorrect results may occur.
13. Cross contamination of reagents and/or samples could cause erroneous results.
14. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
15. Avoid splashing or generation of aerosols.
16. Do not expose reagents to strong light during storage or incubation.
17. Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
18. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.:10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.

19. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (Sodium Hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.
20. Do not apply pressure to slide envelope. This may damage the substrate.
21. The components of this Test System are matched for optimum sensitivity and reproducibility. Reagents from other manufacturers should not be interchanged. Follow Package Insert carefully.
22. Evans Blue Counterstain is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.

SPECIMEN COLLECTION

1. It is recommended that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Occupationally Acquired Infectious Diseases. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
2. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures with this assay (12, 13). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
3. It is recommended that serum samples be stored at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, it is recommended that sera may be stored between 2 - 8°C, for up to 48 hours. If a delay in testing is anticipated, store test sera at –20°C or lower. It is also recommended to avoid multiple freeze/thaw cycles which may cause loss of antibody activity. It should be noted however, that internal studies using the Sebia ANCA Ethanol-fixed IFA test system have demonstrated serum samples positive and negative for ANCA are stable for up to 14 days at 2 - 8°C or room temperature. Additional internal studies demonstrated serum samples are stable for at least 4 freeze/thaw cycles. Ultimately, it is the responsibility of each individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (14).

ASSAY PROCEDURE

1. Remove Slides and other kit components from refrigerated storage and allow them to warm to room temperature (20 - 25°C). Tear open the protective envelope and remove Slides. **Do not apply pressure to flat sides of protective envelope.**
2. Prepare a 1:20 dilution (e.g.: 10µL of serum + 190µL of PBS Buffer) of each patient serum. When titrating patient specimens, the initial 1:20 dilution, and all subsequent dilutions, should be made using 1X PBS. **NOTE: The Controls are intended to be used undiluted**.
3. With suitable dispenser, dispense 25µL of each Control and each diluted patient sera to the appropriate wells.

4. Incubate Slides at room temperature (20 - 25°C) for 35 +/- 5 minutes.
5. Gently rinse Slides with PBS. If washing manually **do not direct a stream of PBS into the test wells.**
6. Wash Slides for two, 5-minute intervals, changing PBS between washes.
7. Remove Slides from PBS one at a time. Invert Slide and key wells to holes in blotters provided. Blot Slide by wiping the reverse side with an absorbent wipe. CAUTION: Position the blotter and Slide on a hard, flat surface. Blotting on paper towels may destroy the Slide matrix. **Do not allow the Slides to dry during the test procedure**.
8. Add 25µL of Conjugate to each well.
9. Repeat steps 4 through 7.
10. Apply a liberal amount (several drops or approximately 20 µL per well) of Mounting Media to the entire Slide (on or between the wells) and apply the cover glass removing any excess Mounting Media or bubbles. Examine Slides with an appropriate fluorescence microscope.

QUALITY CONTROL

1. For each batch of assayed slides, the p-ANCA Positive Control, c-ANCA Positive Control, and Negative Control must be included.
2. It is recommended that the Controls be read prior to evaluating the test samples. If the Controls do not appear as described below, results are invalid:
   a. Negative Control - characterized by the absence of specific fluorescence and a red, or dull green, background staining of all cells due to counterstain.
   b. p-ANCA Positive Control - characterized by nuclear or peri-nuclear (p-ANCA) staining pattern exhibiting apple-green fluorescence.
   c. c-ANCA Positive Control - characterized by cytoplasmic (c-ANCA) staining pattern exhibiting apple-green fluorescence.
3. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

INTERPRETATION OF RESULTS

1. Negative - Characterized by the absence of specific fluorescence and a red, or dull green, background staining of all cells due to counterstain.
2. p-ANCA Positive - Characterized by nuclear or peri-nuclear staining pattern exhibiting apple-green fluorescence.
3. c-ANCA Positive - Characterized by cytoplasmic staining pattern exhibiting apple-green fluorescence.
4. Atypical Positive - Characterized by a mixture of nuclear and cytoplasmic staining, accentuated/linear perinuclear staining, or other staining patterns not interpreted as solely c-ANCA or p-ANCA, exhibiting apple-green fluorescence.

LIMITATIONS OF THE ASSAY

1. The ANCA Ethanol-fixed IFA test system is a diagnostic aid. It is therefore imperative that the results be interpreted in conjunction with additional recommended ANCA serologic confirmatory testing, ANA serologic testing, as well as a patient's clinical features, by a medical authority.
2. ANA positive samples may react with Ethanol-fixed ANCA IFA slides to yield positive results with atypical staining patterns and/or staining patterns that mimic p-ANCA.
3. Sample matrices other than serum have not been validated with this test system.
4. All suggested results obtained with dIFine® must be confirmed by a trained operator.

EXPECTED RESULTS

Expected values in a normal population are negative at a 1:20 starting dilution. However, samples containing anti-nuclear antibodies (ANA) may yield false positive results within a normal testing population.

PERFORMANCE CHARACTERISTICS

NOTE: When establishing Performance Characteristics of the ANCA Ethanol-fixed IFA test system, slides were interpreted using three different methods as outlined below:

Interpretation Methods:
Method A. Method A was a completely manual interpretation method. It was accomplished using a traditional fluorescent microscope equipped with objective and ocular lenses. Determining the qualitative outcome was accomplished using trained laboratory technicians.
Method B. Method B was accomplished by scanning the slides using dIFine® and subsequently having a trained laboratory technician interpret the qualitative results using the digital image appearing on the computer monitor.
Method C. Method C is the ***suggested outcome*** predicted by dIFine®; Method C predicts the qualitative and pattern results. If Method C is "UNC" (uncertain), the level of fluorescence measured by dIFine is borderline between positive and negative, or other features within the slide well that prevented a definitive suggestion. Pattern results suggested by dIFine are p-ANCA, c-ANCA, or 'Others'. Method C must be "validated" or accepted by the laboratory technician or modified or invalidated completely. For purposes of this study and the data presented below, **Method C** is logged "AS IS" without any modification by the laboratory technician(s). It is therefore presented for *informational purposes only*.

1. **Analytical Performance Studies:**
   a. **Linearity:**
   The following six samples were assembled: Two low positive samples (~1:20-1:40 endpoint); One anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA). Two mid positive samples (~1:80-1:160 endpoint); One anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA). Two high positive samples (> 1:160 endpoint); One anti-PR3 (c-ANCA) and one anti-

MPO (p-ANCA). The six samples were each assayed at a 1:20 screening dilution, as well as at various serial dilutions. Qualitative and pattern results were interpreted by all three methods noted above. The endpoints for each sample and each method are presented below:

Sample	Method A	Method B	Method C*
PR3 (c-ANCA) Low Positive	1:40	1:40	1:40
MPO (p-ANCA) Low Positive	1:40	1:40	1:80
PR3 (c-ANCA) Medium Positive	1:80	1:80	1:80
MPO (p-ANCA) Medium Positive	1:80	1:160	1:160
PR3 (c-ANCA) High Positive	1:1280	1:2560	1:2560
MPO (p-ANCA) High Positive	1:640	1:640	1:640

***UNC samples counted as Negative**

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

****UNC samples counted as Positive**

For Methods A and B, the fluorescence intensity was recorded at each dilution using a scale of 4 being very intense and 0 indicating no fluorescence. The sample dilutions and the associated fluorescence intensities are summarized in the tables appearing below:

Fluorescence Intensity (4+ to 0) - Method A										
Sample	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240
PR3 (c-ANCA) Low Positive	2+	1+	0	0	0	NT	NT	NT	NT	NT
MPO (p-ANCA) Low Positive	2+	1+	0	0	0	NT	NT	NT	NT	NT
PR3 (c-ANCA) Medium Positive	3+	2+	1+	0	0	NT	NT	NT	NT	NT
MPO (p-ANCA) Medium Positive	3+	2+	1+	0	0	NT	NT	NT	NT	NT
PR3 (c-ANCA) High Positive	4+	4+	4+	4+	3+	2+	1+	0	0	0
MPO (p-ANCA) High Positive	4+	4+	4+	3+	2+	1+	0	0	0	0

NT = Not tested

Fluorescence Intensity (4+ to 0) - Method B										
Sample	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240
PR3 (c-ANCA) Low Positive	2+	1+	0	0	0	NT	NT	NT	NT	NT
MPO (p-ANCA) Low Positive	2+	1+	0	0	0	NT	NT	NT	NT	NT
PR3 (c-ANCA) Medium Positive	3+	2+	1+	0	0	NT	NT	NT	NT	NT
MPO (p-ANCA) Medium Positive	4+	3+	2+	1+	0	NT	NT	NT	NT	NT
PR3 (c-ANCA) High Positive	4+	4+	4+	4+	4+	3+	2+	1+	0	0
MPO (p-ANCA) High Positive	4+	4+	4+	3+	2+	1+	0	0	0	0

NT = Not tested

b. Lot-to-Lot Reproducibility:

The following ten serum samples were assembled: Two low positive samples (~1:20-1:40 endpoint); One anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA). Two mid positive samples (~1:80-1:160 endpoint); One anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA). Two high positive samples (> 1:160 endpoint); One anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), as well as four negative samples. All ten samples were assayed at a 1:20 screening dilution, using three different lots of ANCA Ethanol-fixed IFA test systems. The six positive samples were also serially diluted and assayed using the same three lots of ANCA Ethanol-fixed IFA test systems. Slide wells were interpreted by all three methods noted above. Results from the diluted positive samples and the 4 negative samples, totaled 44 slide wells which to perform qualitative agreement analyses. Pattern agreement analyses were performed for slide wells that were in positive agreement. Endpoint dilutions were also determined for the serially diluted samples.

Results:

i. Qualitative Agreement:

Qualitative Agreement - Within Lot						
Interpretation Methods	Lot 1		Lot 2		Lot 3	
	% Agreement	95% CI	% Agreement	95% CI	% Agreement	95% CI
A vs B	100.00 (44/44)	91.97 - 100.00	97.73 (43/44)	88.19 - 99.60	95.45 (42/44)	84.87 - 98.74
A vs C	86.36 (38/44)	73.29 - 93.60	79.55 (35/44)	65.50 - 88.85	77.27 (34/44)	63.01 - 87.16
B vs C	90.91 (40/44)	78.84 - 96.41	81.82 (36/44)	68.04 - 90.49	81.82 (36/44)	68.04 - 90.49

Qualitative Agreement - Between Lot						
Interpretation Methods	Lot 1 vs Lot 2		Lot 1 vs Lot 3		Lot 2 vs Lot 3	
	% Agreement	95% CI	% Agreement	95% CI	% Agreement	95% CI
A vs A	97.73 (43/44)	88.19 - 99.60	95.45 (42/44)	84.87 - 98.74	97.73 (43/44)	88.19 - 99.60
B vs B	95.45 (42/44)	84.87 - 98.74	100.00 (44/44)	91.97 - 100.00	95.45 (42/44)	84.87 - 98.74
C vs C	84.09 (37/44)	70.63 - 92.07	86.36 (38/44)	73.29 - 93.60	79.55 (35/44)	65.50 - 88.85
A vs B	95.45 (42/44)	84.87 - 98.74	95.45 (42/44)	84.87 - 98.74	93.18 (41/44)	81.77 - 97.65
A vs C	77.27 (34/44)	63.01 - 87.16	77.27 (34/44)	63.01 - 87.16	75.00 (33/44)	60.56 - 85.43
B vs C	79.55 (35/44)	65.50 - 88.85	81.82 (36/44)	68.04 - 90.49	77.27 (34/44)	63.01 - 87.16
B vs A	93.18 (41/44)	81.77 - 97.65	95.45 (42/44)	84.87 - 98.74	95.45 (42/44)	84.87 - 98.74
C vs A	84.09 (37/44)	70.63 - 92.07	86.36 (38/44)	73.29 - 93.60	79.55 (35/44)	65.50 - 88.85
C vs B	86.36 (38/44)	73.29 - 93.60	90.91 (40/44)	78.84 - 96.41	79.55 (35/44)	65.50 - 88.85

ii. Pattern Agreement:

Pattern Agreement- Within Lot						
Interpretation Method	Lot 1		Lot 2		Lot 3	
	% Agreement	95% CI	% Agreement	95% CI	% Agreement	95% CI
A vs B	100.00 (23/23)	85.69 - 100.00	100.00 (22/22)	85.13 - 100.00	100.00 (23/23)	85.69 - 100.00
A vs C	100.00 (23/23)	85.69 - 100.00	86.36 (19/22)	66.67 - 95.25	82.61 (19/23)	62.86 - 93.02
B vs C	96.00 (24/25)	80.46 - 99.29	86.36 (19/22)	66.67 - 95.25	84.00 (21/25)	65.35 - 93.60

Pattern Agreement- Between Lot						
Interpretation Methods	Lot 1 vs Lot 2		Lot 1 vs Lot 3		Lot 2 vs Lot 3	
	% Agreement	95% CI	% Agreement	95% CI	% Agreement	95% CI
A vs A	100.00 (22/22)	85.13 - 100.00	100.00 (22/22)	85.13 - 100.00	100.00 (22/22)	85.13 - 100.00
B vs B	100.00 (23/23)	85.69 - 100.00	100.00 (25/25)	86.68 - 100.00	100.00 (23/23)	85.69 - 100.00
C vs C	83.33 (20/24)	64.15 - 93.32	80.77 (21/26)	62.12 - 91.49	80.00 (20/25)	60.87 - 91.14
A vs B	100.00 (22/22)	85.13 - 100.00	100.00 (23/23)	85.69 - 100.00	100.00 (22/22)	85.13 - 100.00
A vs C	86.36 (19/22)	66.67 - 95.25	82.61 (19/23)	62.86 - 93.02	86.36 (19/22)	66.67 - 95.25
B vs C	82.61 (19/23)	62.86 - 93.02	84.00 (21/25)	65.35 - 93.60	86.96 (20/23)	67.87 - 95.46
B vs A	100.00 (22/22)	85.13 - 100.00	100.00 (23/23)	85.69 - 100.00	100.00 (22/22)	85.13 - 100.00
C vs A	100.00 (22/22)	85.13 - 100.00	95.65 (22/23)	79.01 - 99.23	86.36 (19/22)	66.67 - 95.25
C vs B	100.00 (23/23)	85.69 - 100.00	96.00 (24/25)	80.46 - 99.29	86.96 (20/23)	67.87 - 95.46

iii. Endpoint Titer Agreement:

Endpoint Titers - Lot 1*			
Sample	Method A	Method B	Method C**
PR3 (c-ANCA) Low Positive	1:40	1:40	1:40
MPO (p-ANCA) Low Positive	1:40	1:40	1:80
PR3 (c-ANCA) Medium Positive	1:80	1:80	1:80
MPO (p-ANCA) Medium Positive	1:80	1:160	1:160
PR3 (c-ANCA) High Positive	1:1280	1:2560	1:2560
MPO (p-ANCA) High Positive	1:640	1:640	1:640

*** Lot 1 = same table as linearity study**

**** UNC counted as negative**

Endpoint Titers - Lot 2			
Sample	Method A	Method B	Method C*
PR3 (c-ANCA) Low Positive	1:40	1:40	1:160
MPO (p-ANCA) Low Positive	1:40	1:40	1:80
PR3 (c-ANCA) Medium Positive	1:80	1:80	1:80
MPO (p-ANCA) Medium Positive	1:80	1:160	1:160
PR3 (c-ANCA) High Positive	1:1280	1:1280	1:1280
MPO (p-ANCA) High Positive	1:320	1:320	1:320

*** UNC counted as negative**

Endpoint Titers - Lot 3			
Sample	Method A	Method B	Method C*
PR3 (c-ANCA) Low Positive	1:40	1:40	1:80
MPO (p-ANCA) Low Positive	1:40	1:40	1:80
PR3 (c-ANCA) Medium Positive	1:80	1:80	1:160
MPO (p-ANCA) Medium Positive	1:80	1:160	1:160
PR3 (c-ANCA) High Positive	1:2560	1:2560	1:2560
MPO (p-ANCA) High Positive	1:320	1:640	1:640

*** UNC counted as negative**

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

*** Lot 1 = same table as linearity study**

**** UNC counted as positive**

Endpoint Titers - Lot 2			
Sample	Method A	Method B	Method C*
PR3 (c-ANCA) Low Positive	1:40	1:40	1:320
MPO (p-ANCA) Low Positive	1:40	1:40	1:160
PR3 (c-ANCA) Medium Positive	1:80	1:80	1:160
MPO (p-ANCA) Medium Positive	1:80	1:160	1:320
PR3 (c-ANCA) High Positive	1:1280	1:1280	1:2560
MPO (p-ANCA) High Positive	1:320	1:320	1:320

*** UNC counted as positive**

Endpoint Titers - Lot 3			
Sample	Method A	Method B	Method C*
PR3 (c-ANCA) Low Positive	1:40	1:40	1:160
MPO (p-ANCA) Low Positive	1:40	1:40	1:80
PR3 (c-ANCA) Medium Positive	1:80	1:80	1:320
MPO (p-ANCA) Medium Positive	1:80	1:160	1:320
PR3 (c-ANCA) High Positive	1:2560	1:2560	1:5120
MPO (p-ANCA) High Positive	1:320	1:640	1:1280

*** UNC counted as positive**

c. Reference Range Study:

One hundred and fifty randomly selected serum samples were acquired from healthy donors in the Northeastern United States. The samples were assayed at the 1:20 screening dilution and interpreted via all three methods. The results of the screening test are summarized below:

Sebia ANCA IFA Ethanol - Reference Range (n = 150)			
Sample	**Method A**	**Method B**	**Method C**
Positive	16*	16*	19**
Negative	134	134	125
Uncertain	N/A	N/A	6***

*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

d. Twenty-day Repeatability Study:

The following eight serum samples were assembled: Two low positive samples (~1:20-1:40 endpoint); One anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA). Two mid positive samples (~1:80-1:160 endpoint); One anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA). Two high positive samples (> 1:160 endpoint); One anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), as well as two negative samples. All eight samples were assayed in triplicate, at a 1:20 screening dilution, once per day, on twenty different days, using the ANCA Ethanol-fixed IFA 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. Sample identities were blinded and randomized independently prior to each day of testing.

Results:

i. **Within-Method Qualitative Agreement:**

Within-Method Qualitative Result Agreement (Technician 1)

Sample	Method A Agreement (95% CI)	Method B Agreement (95% CI)	Method C Agreement (95% CI)
PR3 (c-ANCA) Low Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94.0 - 100%)	60/60 - 100% (94.0 - 100%)
MPO (p-ANCA) Low Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
PR3 (c-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
MPO (p-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
PR3 (c-ANCA) High Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
MPO (p-ANCA) High Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
Negative-1	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
Negative-2	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)

Within-Method Qualitative Result Agreement (Technician 2)

Sample	Method A Agreement (95% CI)	Method B Agreement (95% CI)	Method C Agreement (95% CI)
PR3 (c-ANCA) Low Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94.0 - 100%)	60/60 - 100% (94.0 - 100%)
MPO (p-ANCA) Low Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
PR3 (c-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
MPO (p-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
PR3 (c-ANCA) High Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
MPO (p-ANCA) High Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
Negative-1	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
Negative-2	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)

Within-Method Qualitative Result Agreement (Technician 1 & 2 Combined)

Sample	Method A Agreement (95% CI)	Method B Agreement (95% CI)	Method C Agreement (95% CI)
PR3 (c-ANCA) Low Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
MPO (p-ANCA) Low Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
PR3 (c-ANCA) Medium Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
MPO (p-ANCA) Medium Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
PR3 (c-ANCA) High Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
MPO (p-ANCA) High Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
Negative-1	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
Negative-2	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)

ii. **Between-Method Qualitative Agreement:**

Between-Method Qualitative Result Agreement (Technician 1)

Sample	Method A vs Method B Agreement (95% CI)	Method A vs Method C Agreement (95% CI)	Method B vs Method C Agreement (95% CI)
PR3 (c-ANCA) Low Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94.0 - 100%)	60/60 - 100% (94.0 - 100%)
MPO (p-ANCA) Low Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
PR3 (c-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
MPO (p-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
PR3 (c-ANCA) High Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
MPO (p-ANCA) High Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
Negative-1	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
Negative-2	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)

Between-Method Qualitative Result Agreement (Technician 2)

Sample	Method A vs Method B Agreement (95% CI)	Method A vs Method C Agreement (95% CI)	Method B vs Method C Agreement (95% CI)
PR3 (c-ANCA) Low Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94.0 - 100%)	60/60 - 100% (94.0 - 100%)
MPO (p-ANCA) Low Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
PR3 (c-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
MPO (p-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
PR3 (c-ANCA) High Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
MPO (p-ANCA) High Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
Negative-1	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
Negative-2	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)

Between-Method Qualitative Result Agreement (Technician 1 & 2 Combined)

Sample	Method A vs Method B Agreement (95% CI)	Method A vs Method C Agreement (95% CI)	Method B vs Method C Agreement (95% CI)
PR3 (c-ANCA) Low Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
MPO (p-ANCA) Low Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
PR3 (c-ANCA) Medium Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
MPO (p-ANCA) Medium Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
PR3 (c-ANCA) High Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
MPO (p-ANCA) High Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
Negative-1	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
Negative-2	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)

iii. Within-Method Pattern Agreement:

Within-Method Pattern Result Agreement (Technician 1)

Sample	Method A Agreement (95% CI)	Method B Agreement (95% CI)	Method C Agreement (95% CI)
PR3 (c-ANCA) Low Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	59/60 - 98.3% (91.1 - 99.7%)
MPO (p-ANCA) Low Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	58/60 - 96.7% (88.6 - 99.1%)
PR3 (c-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
MPO (p-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
PR3 (c-ANCA) High Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	58/60 - 96.7% (88.6 - 99.1%)
MPO (p-ANCA) High Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)

Within-Method Pattern Result Agreement (Technician 2)

Sample	Method A Agreement (95% CI)	Method B Agreement (95% CI)	Method C Agreement (95% CI)
PR3 (c-ANCA) Low Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	59/60 - 98.3% (91.1 - 99.7%)
MPO (p-ANCA) Low Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	58/60 - 96.7% (88.6 - 99.1%)
PR3 (c-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
MPO (p-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
PR3 (c-ANCA) High Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	58/60 - 96.7% (88.6 - 99.1%)
MPO (p-ANCA) High Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)

Within-Method Pattern Result Agreement (Technician 1 &2 Combined)

Sample	Method A Agreement (95% CI)	Method B Agreement (95% CI)	Method C Agreement (95% CI)
PR3 (c-ANCA) Low Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	118/120 - 98.3% (94.1 - 99.5%)
MPO (p-ANCA) Low Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	116/120 - 96.7% (91.7 - 98.7%)
PR3 (c-ANCA) Medium Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
MPO (p-ANCA) Medium Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
PR3 (c-ANCA) High Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	116/120 - 96.7% (91.7 - 98.7%)
MPO (p-ANCA) High Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)

iv. **Between-Method Pattern Agreement:**

Between-Method Pattern Result Agreement (Technician 1)

Sample	Method A vs Method B Agreement (95% CI)	Method A vs Method C Agreement (95% CI)	Method B vs Method C Agreement (95% CI)
PR3 (c-ANCA) Low Positive	60/60 - 100% (94 - 100%)	59/60 - 98.3% (91.1 - 99.7%)	59/60 - 98.3% (91.1 - 99.7%)
MPO (p-ANCA) Low Positive	60/60 - 100% (94 - 100%)	58/60 - 96.7% (88.6 - 99.1%)	58/60 - 96.7% (88.6 - 99.1%)
PR3 (c-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
MPO (p-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
PR3 (c-ANCA) High Positive	60/60 - 100% (94 - 100%)	58/60 - 96.7% (88.6 - 99.1%)	58/60 - 96.7% (88.6 - 99.1%)
MPO (p-ANCA) High Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)

Between-Method Pattern Result Agreement (Technician 2)

Sample	Method A vs Method B Agreement (95% CI)	Method A vs Method C Agreement (95% CI)	Method B vs Method C Agreement (95% CI)
PR3 (c-ANCA) Low Positive	60/60 - 100% (94 - 100%)	59/60 - 98.3% (91.1 - 99.7%)	59/60 - 98.3% (91.1 - 99.7%)
MPO (p-ANCA) Low Positive	60/60 - 100% (94 - 100%)	58/60 - 96.7% (88.6 - 99.1%)	58/60 - 96.7% (88.6 - 99.1%)
PR3 (c-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
MPO (p-ANCA) Medium Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)
PR3 (c-ANCA) High Positive	60/60 - 100% (94 - 100%)	58/60 - 96.7% (88.6 - 99.1%)	58/60 - 96.7% (88.6 - 99.1%)
MPO (p-ANCA) High Positive	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)	60/60 - 100% (94 - 100%)

Between-Method Pattern Result Agreement (Technician 1 &2 Combined)

Sample	Method A vs Method B Agreement (95% CI)	Method A vs Method C Agreement (95% CI)	Method B vs Method C Agreement (95% CI)
PR3 (c-ANCA) Low Positive	120/120 - 100% (96.9 - 100%)	118/120 - 98.3% (94.1 - 99.5%)	118/120 - 98.3% (94.1 - 99.5%)
MPO (p-ANCA) Low Positive	120/120 - 100% (96.9 - 100%)	116/120 - 96.7% (91.7 - 98.7%)	116/120 - 96.7% (91.7 - 98.7%)
PR3 (c-ANCA) Medium Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
MPO (p-ANCA) Medium Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)
PR3 (c-ANCA) High Positive	120/120 - 100% (96.9 - 100%)	116/120 - 96.7% (91.7 - 98.7%)	116/120 - 96.7% (91.7 - 98.7%)
MPO (p-ANCA) High Positive	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)	120/120 - 100% (96.9 - 100%)

e. Five-day, Multi-Site Reproducibility Study:

The following eight serum samples were assembled: Two low positive samples (~1:20-1:40 endpoint); One anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA). Two mid positive samples (~1:80-1:160 endpoint); One anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA). Two high positive samples (> 1:160 endpoint); One anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), as well as two negative samples. All eight samples were assayed in triplicate, at a 1:20 screening dilution, twice per day, on five different days, by three different technicians, using the ANCA Ethanol-fixed IFA test system. At three different laboratories, 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). Sample identities were blinded and randomized independently prior to each day of testing.

Results:

i. **Qualitative Result Agreement**

a. **Within Method**

Site 1 - Within-Method Qualitative Result Agreement

Within-Method Qualitative Result Agreement (Technician 1)

Sample	Method A (95% CI)	Method B (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-2	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)

Within-Method Qualitative Result Agreement (Technician 2)

Sample	Method A (95% CI)	Method B (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-2	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)

Sample	Method C (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)
Negative-1	30/30 - 100% (88.7 - 100.0%)
Negative-2	30/30 - 100% (88.7 - 100.0%)

Site 2 - Within-Method Qualitative Result Agreement

Within-Method Qualitative Result Agreement (Technician 1)

Sample	Method A (95% CI)	Method B (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-2	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)

Within-Method Qualitative Result Agreement (Technician 2)

Sample	Method A (95% CI)	Method B (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-2	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)

Sample	Method C (95% CI)
MPO (p-ANCA) Low Pos	29/30 - 96.7% (83.3 - 99.4%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)
Negative-1	30/30 - 100% (88.7 - 100.0%)
Negative-2	30/30 - 100% (88.7 - 100.0%)

Site 3 - Within-Method Qualitative Result Agreement

Within-Method Qualitative Result Agreement (Technician 1)

Sample	Method A (95% CI)	Method B (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-2	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)

Within-Method Qualitative Result Agreement (Technician 2)

Sample	Method A (95% CI)	Method B (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-2	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)

Sample	Method C (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)
Negative-1	29/30 - 96.7% (83.3 - 99.4%)
Negative-2	30/30 - 100% (88.7 - 100.0%)

b. **Between Method:**

Site 1 – Between-Method Qualitative Result Agreement

Between-Method Qualitative Result Agreement (Technician 1)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-2	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)

Between-Method Qualitative Result Agreement (Technician 2)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-2	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)

Site 2 – Between-Method Qualitative Result Agreement

Between-Method Qualitative Result Agreement (Technician 1)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	29/30 - 96.7% (83.3 - 99.4%)	29/30 - 96.7% (83.3 - 99.4%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-2	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)

Between-Method Qualitative Result Agreement (Technician 2)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	29/30 - 96.7% (83.3 - 99.4%)	29/30 - 96.7% (83.3 - 99.4%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-2	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)

Site 3 - Between-Method Qualitative Result Agreement

Between-Method Qualitative Result Agreement (Technician 1)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	30/30 - 100% (88.7 - 100.0%)	29/30 - 96.7% (83.3 - 99.4%)	29/30 - 96.7% (83.3 - 99.4%)
Negative-2	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)

Between-Method Qualitative Result Agreement (Technician 2)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	30/30 - 100% (88.7 - 100.0%)	29/30 - 96.7% (83.3 - 99.4%)	29/30 - 96.7% (83.3 - 99.4%)
Negative-2	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)

After combining results from all eight samples, the qualitative results can also be summarized as follows:

Method A Multisite Reproducibility: Qualitative results from three sites and two technicians per site comparing site to site and tech to tech.

			Site 1		Site 2		Site 3	
			Technician 1	Technician 2	Technician 1	Technician 2	Technician 1	Technician 2
			Method A		Method A		Method A	
Site 1	Technician 1	Method A		240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)
	Technician 2				240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)
Site 2	Technician 1	Method A				240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)
	Technician 2						240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)
Site 3	Technician 1	Method A						240/240 - 100% (98.42 - 100.00)
	Technician 2							

Method B Multisite Reproducibility: Qualitative results from three sites and two technicians per site comparing site to site and tech to tech.

			Site 1		Site 2		Site 3	
			Technician 1	Technician 2	Technician 1	Technician 2	Technician 1	Technician 2
			Method B		Method B		Method B	
Site 1	Technician 1	Method B		240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)
	Technician 2				240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)
Site 2	Technician 1	Method B				240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)
	Technician 2						240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)
Site 3	Technician 1	Method B						240/240 - 100% (98.42 - 100.00)
	Technician 2							

Method C Multisite Qualitative Reproducibility Comparing Site to Site

		Site 1	Site 2	Site 3
		Method C		
Site 1	Method C		239/240 - 99.58% (97.68 - 99.93)	239/240 - 99.58% (97.68 - 99.93)
Site 2				238/240 - 99.17% (97.01 - 99.77)
Site 3				

ii. **Pattern Result Agreement**

a. **Within Method**

Site 1 - Within-Method Pattern Result Agreement

Within-Method Pattern Agreement (Technician 1)

Sample	Method A (95% CI)	Method B (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	N/A	N/A
Negative-2	N/A	N/A

Within-Method Pattern Agreement (Technician 2)

Sample	Method A (95% CI)	Method B (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	N/A	N/A
Negative-2	N/A	N/A

Sample	Method C (95% CI)
MPO (p-ANCA) Low Pos	28/30 - 93.3% (78.7 - 98.2%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	27/30 - 90.0% (74.4 - 96.5%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)
Negative-1	N/A
Negative-2	N/A

Site 2 - Within-Method Pattern Result Agreement

Within-Method Pattern Agreement (Technician 1)

Sample	Method A (95% CI)	Method B (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	N/A	N/A
Negative-2	N/A	N/A

Within-Method Pattern Agreement (Technician 2)

Sample	Method A (95% CI)	Method B (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	N/A	N/A
Negative-2	N/A	N/A

Sample	Method C (95% CI)
MPO (p-ANCA) Low Pos	25/30 - 83.3% (66.4 - 92.7%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	28/30 - 93.3% (78.7 - 98.2%)
PR3 (c-ANCA) Mid Pos	28/30 - 93.3% (78.7 - 98.2%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)
Negative-1	N/A
Negative-2	N/A

Site 3 - Within-Method Pattern Result Agreement

Within-Method Pattern Agreement (Technician 1)

Sample	Method A (95% CI)	Method B (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	N/A	N/A
Negative-2	N/A	N/A

Within-Method Pattern Agreement (Technician 2)

Sample	Method A (95% CI)	Method B (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	N/A	N/A
Negative-2	N/A	N/A

Sample	Method C (95% CI)
MPO (p-ANCA) Low Pos	25/30 - 83.3% (66.4 - 92.7%)
MPO (p-ANCA) Mid Pos	28/30 - 93.3% (78.7 - 98.2%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)
Negative-1	N/A
Negative-2	N/A

b. **Between Method:**

Site 1 – Between-Method Pattern Result Agreement

Between-Method Pattern Agreement (Technician 1)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	28/30 - 93.3% (78.7 - 98.2%)	28/30 - 93.3% (78.7 - 98.2%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	27/30 - 90.0% (74.4 - 96.5%)	27/30 - 90.0% (74.4 - 96.5%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	N/A	N/A	N/A
Negative-2	N/A	N/A	N/A

Between-Method Pattern Agreement (Technician 2)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	28/30 - 93.3% (78.7 - 98.2%)	28/30 - 93.3% (78.7 - 98.2%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	27/30 - 90.0% (74.4 - 96.5%)	27/30 - 90.0% (74.4 - 96.5%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	N/A	N/A	N/A
Negative-2	N/A	N/A	N/A

Site 2 - Between-Method Pattern Result Agreement

Between-Method Pattern Agreement (Technician 1)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	25/30 - 83.3% (66.4 - 92.7%)	25/30 - 83.3% (66.4 - 92.7%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	28/30 - 93.3% (78.7 - 98.2%)	28/30 - 93.3% (78.7 - 98.2%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	28/30 - 93.3% (78.7 - 98.2%)	28/30 - 93.3% (78.7 - 98.2%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	N/A	N/A	N/A
Negative-2	N/A	N/A	N/A

Between-Method Pattern Agreement (Technician 2)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	25/30 - 83.3% (66.4 - 92.7%)	25/30 - 83.3% (66.4 - 92.7%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	28/30 - 93.3% (78.7 - 98.2%)	28/30 - 93.3% (78.7 - 98.2%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	28/30 - 93.3% (78.7 - 98.2%)	28/30 - 93.3% (78.7 - 98.2%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	N/A	N/A	N/A
Negative-2	N/A	N/A	N/A

Site 3 - Between-Method Pattern Result Agreement

Between-Method Pattern Agreement (Technician 1)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	25/30 - 83.3% (66.4 - 92.7%)	25/30 - 83.3% (66.4 - 92.7%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	28/30 - 93.3% (78.7 - 98.2%)	28/30 - 93.3% (78.7 - 98.2%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	N/A	N/A	N/A
Negative-2	N/A	N/A	N/A

Between-Method Pattern Agreement (Technician 2)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
MPO (p-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	25/30 - 83.3% (66.4 - 92.7%)	25/30 - 83.3% (66.4 - 92.7%)
MPO (p-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	28/30 - 93.3% (78.7 - 98.2%)	28/30 - 93.3% (78.7 - 98.2%)
MPO (p-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Low Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) Mid Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
PR3 (c-ANCA) High Pos	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)	30/30 - 100% (88.7 - 100.0%)
Negative-1	N/A	N/A	N/A
Negative-2	N/A	N/A	N/A

After combining results that were in positive agreement from all six samples, the pattern results can also be summarized as follows:

Method A Multisite Reproducibility: Pattern results from three sites and two technicians per site comparing site to site and tech to tech.

			Site 1		Site 2		Site 3	
			Technician 1	Technician 2	Technician 1	Technician 2	Technician 1	Technician 2
			Method A		Method A		Method A	
Site 1	Technician 1	Method A		180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)
	Technician 2				180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)
Site 2	Technician 1	Method A				180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)
	Technician 2						180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)
Site 3	Technician 1	Method A						180/180 - 100% (97.91 - 100.00)
	Technician 2							

Method B Multisite Reproducibility: Pattern results from three sites and two technicians per site comparing site to site and tech to tech.

			Site 1		Site 2		Site 3	
			Technician 1	Technician 2	Technician 1	Technician 2	Technician 1	Technician 2
			Method B		Method B		Method B	
Site 1	Technician 1	Method B		180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)
	Technician 2				180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)
Site 2	Technician 1	Method B				180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)
	Technician 2						180/180 - 100% (97.91 - 100.00)	180/180 - 100% (97.91 - 100.00)
Site 3	Technician 1	Method B						180/180 - 100% (97.91 - 100.00)
	Technician 2							

Method C Multisite Pattern Reproducibility Comparing Site to Site

		Site 1	Site 2	Site 3
		Method C		
Site 1	Method C		166/179 - 92.74% (87.97 - 95.71)	170/180 - 94.44% (90.08 - 96.95)
Site 2				164/179 - 91.62% (86.64 - 94.86)
Site 3				

f. Interference Study:

The following eight serum samples were assembled: Two low positive samples (~1:20-1:40 endpoint); One anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA). Two mid positive samples (~1:80-1:160 endpoint); One anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA). Two high positive samples (> 1:160 endpoint); One anti-PR3 (c-ANCA) and one anti-MPO (p-ANCA), as well as two negative samples. These eight specimens were spiked with two different concentrations (low and high) of nineteen different interferents as outlined in the table below. All specimens were assayed in triplicate at a 1:20 dilution and interpreted by all three methods noted above. Qualitative 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 mg/mL	2.5 mg/mL
Albumin	35 mg/mL	52 mg/mL
Hemoglobin	100 mg/mL	200 mg/mL
RF	200 U/mL	400 U/mL
Exogenous Substances		
Substance	**Low Concentration**	**High Concentration**
Intralipids	2.0 mg/mL	20 mg/mL
Cyclophosphamide	0.183 mg/mL	0.549 mg/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 mg/mL
Belimumab	2 mg/mL	8 mg/mL
Methotrexate	0.454 mg/mL	1.36 mg/mL
Naproxen	0.12 mg/mL	0.36 mg/mL

None of the interferents affected the expected results for any test replicates when read by Methods A and B. Method C yielded uncertain and/or negative results for a few test replicates of positive samples, as well as incorrect pattern results, for some positive test replicates. However, the relatively low frequency of these incorrect results for Method C do not suggest they are a consequence of assay interference, since Methods A and B results were 100% acceptable. Overall, it can be concluded that the ANCA Ethanol-fixed IFA test system is not at risk of generating erroneous results due to the presence of the interferents tested.

2. Clinical Performance Studies:

a. Cohort Description

The 583 clinically characterized specimens that were utilized are outlined in the table below. These specimens were aliquoted, blinded, and randomized prior to testing. All specimens were assayed by three different technicians at a 1:20 screening dilution via the Sebia ANCA Ethanol-fixed IFA test system, and by a single technician via the predicate ANCA Ethanol-fixed IFA test system. Qualitative and pattern results for the Sebia ANCA Ethanol-fixed IFA test system were interpreted by two technicians at three different laboratories 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). Qualitative and pattern results for the predicate ANCA Ethanol-fixed IFA test system were interpreted at a single laboratory via Method A. The results were used to assess clinical specificity, clinical sensitivity, qualitative agreement, and pattern agreement between interpretation methods.

Diagnosis		n
ANCA Associated Vasculitis (AAV)	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**

b. Qualitative Results Summary

i. Sebia ANCA Ethanol-fixed IFA test system qualitative results summary - Site 1

Site 1														
Diagnosis		n	Sebia ANCA IFA - Ethanol											
			Method A				Method B				Method C (dIFine)			
			Technician A		Technician B		Technician A		Technician B		UNC - NEG		UNC - POS	
			n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos
ANCA Associated Vasculitis	MPA	123	120	97.56	121	98.37	121	98.37	121	98.37	121	98.37	122	99.19
	GPA	92	85	92.39	85	92.39	85	92.39	85	92.39	82	89.13	85	92.39
	eGPA	8	7	87.50	8	100.00	7	87.50	7	87.50	7	87.50	8	100.00
Control Diseases	Non-ANCA Associated Vasculitis	30	12	40.00	12	40.00	12	40.00	12	40.00	13	43.33	15	50.00
	Autoimmune Thyroid	30	11	36.67	11	36.67	12	40.00	11	36.67	12	40.00	14	46.67
	Rheumatoid Arthritis	30	16	53.33	16	53.33	16	53.33	16	53.33	15	50.00	17	56.67
	Chronic Kidney Disease	30	14	46.67	13	43.33	14	46.67	13	43.33	11	36.67	15	50.00
	Systemic Sclerosis	30	17	56.67	17	56.67	17	56.67	17	56.67	17	56.67	17	56.67
	Systemic Lupus Erythematosus	30	25	83.33	25	83.33	25	83.33	25	83.33	26	86.67	27	90.00
	Dermatomyositis/Polymyositis	30	8	26.67	8	26.67	8	26.67	8	26.67	7	23.33	9	30.00
	Inflammatory Bowel Disease	30	12	40.00	12	40.00	12	40.00	12	40.00	12	40.00	12	40.00
	Sinusitis/Allergic Rhinitis	30	4	13.33	4	13.33	4	13.33	4	13.33	6	20.00	6	20.00
	Infectious Disease	30	8	26.67	9	30.00	8	26.67	8	26.67	8	26.67	14	46.67
	Asthma/COPD	30	4	13.33	4	13.33	4	13.33	4	13.33	4	13.33	4	13.33
	Cancer	30	5	16.67	4	13.33	5	16.67	5	16.67	4	13.33	7	23.33
Total		**583**												

ii. Sebia ANCA Ethanol-fixed IFA test system qualitative results summary - Site 2

Site 2														
Diagnosis		n	Sebia ANCA IFA - Ethanol											
			Method A				Method B				Method C (dIFine)			
			Technician A		Technician B		Technician A		Technician B		UNC - NEG		UNC - POS	
			n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos
ANCA Associated Vasculitis	MPA	123	121	98.37	121	98.37	121	98.37	121	98.37	121	98.37	122	99.19
	GPA	92	83	90.22	82	89.13	83	90.22	82	89.13	82	89.13	83	90.22
	eGPA	8	8	100.00	8	100.00	8	100.00	8	100.00	8	100.00	8	100.00
Control Diseases	Non-ANCA Associated Vasculitis	30	12	40.00	12	40.00	12	40.00	13	43.33	11	36.67	13	43.33
	Autoimmune Thyroid	30	8	26.67	9	30.00	10	33.33	8	26.67	8	26.67	11	36.67
	Rheumatoid Arthritis	30	15	50.00	15	50.00	15	50.00	15	50.00	14	46.67	15	50.00
	Chronic Kidney Disease	30	11	36.67	12	40.00	12	40.00	12	40.00	11	36.67	13	43.33
	Systemic Sclerosis	30	16	53.33	17	56.67	17	56.67	16	53.33	16	53.33	18	60.00
	Systemic Lupus Erythematosus	30	24	80.00	24	80.00	24	80.00	24	80.00	23	76.67	25	83.33
	Dermatomyositis/Polymyositis	30	4	13.33	4	13.33	5	16.67	4	13.33	5	16.67	6	20.00
	Inflammatory Bowel Disease	30	11	36.67	11	36.67	11	36.67	11	36.67	12	40.00	13	43.33
	Sinusitis/Allergic Rhinitis	30	2	6.67	2	6.67	2	6.67	2	6.67	2	6.67	2	6.67
	Infectious Disease	30	6	20.00	6	20.00	6	20.00	6	20.00	6	20.00	8	26.67
	Asthma/COPD	30	3	10.00	3	10.00	3	10.00	3	10.00	3	10.00	3	10.00
	Cancer	30	5	16.67	5	16.67	5	16.67	5	16.67	4	13.33	5	16.67
Total		**583**												

iii. Sebia ANCA Ethanol-fixed IFA test system qualitative results summary - Site 3

Site 3														
Diagnosis		n	Sebia ANCA IFA - Ethanol											
			Method A				Method B				Method C (dIFine)			
			Technician A		Technician B		Technician A		Technician B		UNC - NEG		UNC - POS	
			n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos	n Pos	% Pos
ANCA Associated Vasculitis	MPA	123	122	99.19	122	99.19	122	99.19	122	99.19	122	99.19	122	99.19
	GPA	92	88	95.65	88	95.65	88	95.65	87	94.57	84	91.30	86	93.48
	eGPA	8	8	100.00	8	100.00	8	100.00	8	100.00	8	100.00	8	100.00
Control Diseases	Non-ANCA Associated Vasculitis	30	13	43.33	13	43.33	14	46.67	14	46.67	14	46.67	15	50.00
	Autoimmune Thyroid	30	14	46.67	12	40.00	15	50.00	14	46.67	13	43.33	16	53.33
	Rheumatoid Arthritis	30	17	56.67	17	56.67	17	56.67	17	56.67	15	50.00	17	56.67
	Chronic Kidney Disease	30	15	50.00	14	46.67	15	50.00	15	50.00	14	46.67	17	56.67
	Systemic Sclerosis	30	17	56.67	18	60.00	20	66.67	20	66.67	19	63.33	20	66.67
	Systemic Lupus Erythematosus	30	24	80.00	25	83.33	25	83.33	25	83.33	25	83.33	26	86.67
	Dermatomyositis/Polymyositis	30	8	26.67	8	26.67	9	30.00	9	30.00	7	23.33	11	36.67
	Inflammatory Bowel Disease	30	14	46.67	13	43.33	14	46.67	15	50.00	12	40.00	14	46.67
	Sinusitis/Allergic Rhinitis	30	3	10.00	5	16.67	4	13.33	4	13.33	4	13.33	5	16.67
	Infectious Disease	30	8	26.67	7	23.33	10	33.33	10	33.33	7	23.33	11	36.67
	Asthma/COPD	30	3	10.00	3	10.00	4	13.33	4	13.33	3	10.00	4	13.33
	Cancer	30	8	26.67	8	26.67	7	23.33	8	26.67	6	20.00	9	30.00
Total		**583**												

iv. Predicate ANCA Ethanol-fixed IFA test system qualitative results summary

Diagnosis		n	Predicate ANCA IFA - Ethanol	
			Method A	
			n Pos	% Pos
ANCA Associated Vasculitis	MPA	123	119	96.75
	GPA	92	82	89.13
	eGPA	8	6	75.00
Control Diseases	Non-ANCA Associated Vasculitis	30	16	53.33
	Autoimmune Thyroid	30	14	46.67
	Rheumatoid Arthritis	30	15	50.00
	Chronic Kidney Disease	30	11	36.67
	Systemic Sclerosis	30	20	66.67
	Systemic Lupus Erythematosus	30	23	76.67
	Dermatomyositis/Polymyositis	30	8	26.67
	Inflammatory Bowel Disease	30	12	40.00
	Sinusitis/Allergic Rhinitis	30	2	6.67
	Infectious Disease	30	8	26.67
	Asthma/COPD	30	4	13.33
	Cancer	30	6	20.00

c. Clinical Sensitivity and Clinical Specificity:

The clinical sensitivity was calculated at each site using the qualitative results derived from the ANCA Associated Vasculitis samples (n = 223). Specificity was calculated using the combined set of qualitative results derived from the control disease samples (n = 360).

i. Sebia ANCA Ethanol-fixed IFA test system

Site	Technician	Interpretation	AAV (n = 223)		CD (n = 360)	
			% Sensitivity	95% CI	% Specificity	95% CI
1	A	Method A	95.07	91.38 - 97.22	62.22	57.11 - 67.08
		Method B	95.52	91.94 - 97.55	61.94	56.83 - 66.81
	B	Method A	95.96	92.51 - 97.86	62.50	57.39 - 67.34
		Method B	95.52	91.94 - 97.55	62.50	57.39 - 67.34
	dIFine	Method C (UNC = NEG)	94.17	90.28 - 96.56	62.50	57.39 - 67.34
		Method C (UNC = POS)	96.41	93.08 - 98.17	56.67	51.50 - 61.69
2	A	Method A	95.07	91.38 - 97.22	67.50	62.50 - 72.13
		Method B	95.07	91.38 - 97.22	66.11	61.07 - 70.81
	B	Method A	94.62	90.83 - 96.90	66.67	61.64 - 71.34
		Method B	94.62	90.83 - 96.90	66.94	61.93 - 71.60
	dIFine	Method C (UNC = NEG)	94.62	90.83 - 96.90	68.06	63.07 - 72.66
		Method C (UNC = POS)	95.52	91.94 - 97.55	63.33	58.24 - 68.15
3	A	Method A	97.76	94.86 - 99.04	60.00	54.86 - 64.93
		Method B	97.76	94.86 - 99.04	57.22	52.06 - 62.23
	B	Method A	97.76	94.86 - 99.04	60.28	55.14 - 65.20
		Method B	97.31	94.26 - 98.76	56.94	51.78 - 61.96
	dIFine	Method C (UNC = NEG)	95.96	92.51 - 97.86	61.39	56.26 - 66.27
		Method C (UNC = POS)	96.86	93.66 - 98.47	54.17	49.00 - 59.24

ii. Predicate ANCA Ethanol-fixed IFA test system

Interpretation	AAV (n = 223)		CD (n = 360)	
	% Sensitivity	95% CI	% Specificity	95% CI
Method A	92.83	88.66 - 95.54	61.39	56.26 - 66.27

iii. **Sensitivity and Specificity Conclusion**:

Sensitivity values for the AAV cohort ranged from 94.17% to 97.76% across all three methods and all three sites. Clinical specificity among the Control Diseases cohort ranged from 54.17% to 68.06% across all three methods, across all three sites. If one averages all methods of interpretation across all three sites, the clinical sensitivity in the AAV group averaged 95.86% and the clinical specificity in the Control Disease group averaged 62.05%. Considering that the AAV serum samples utilized in this study were collected at the time of diagnosis, the high level of sensitivity obtained is consistent with published literature,[15,16] as well as the predicate device that was tested

in parallel using the same samples. The specificity values obtained herein are also consistent with 510k summaries from similar devices, as well as the predicate device that was tested in parallel using the same samples.

d. Interpretation Method Agreement:

A summary of the Method A versus Method B, Method A versus Method C, and Method B versus Method C qualitative and pattern result agreement comparisons appears in the tables below:

i. **Method A vs Method B Qualitative Comparison**

Method A vs Method B		Positive Agreement (95% CI)	Negative Agreement (95% CI)	Total Agreement (95% CI)
Site 1	Technician A	347/348, 99.71%	232/235, 98.72%	579/583, 99.31%
		(98.39 - 99.95)	(96.31 - 99.56)	(98.25 - 99.73)
	Technician B	347/349, 99.43%	233/234, 99.57%	580/583, 99.49%
		(97.93 - 99.84)	(97.62 - 99.92)	(98.50 - 99.82)
Site 2	Technician A	329/329, 100.00%	249/254, 98.03%	578/583, 99.14%
		(98.85 - 100.00)	(95.48 - 99.16)	(98.01 - 99.63)
	Technician B	329/331, 99.40%	251/252, 99.60%	580/583, 99.49%
		(97.82 - 99.83)	(97.79 - 99.93)	(98.50 - 99.82)
Site 3	Technician A	359/362, 99.17%	208/221, 94.12%	567/583, 97.26%
		(97.59 - 99.72)	(90.20 - 96.53)	(95.59 - 98.30)
	Technician B	356/361, 98.61%	206/222, 92.79%	562/583, 96.40%
		(96.80 - 99.41)	(88.61 - 95.52)	(94.56 - 97.63)

ii. **Combined Qualitative Agreement for Method A vs Method B All Sites/All Technicians**

Positive Agreement (95% CI)	Negative Agreement (95% CI)	Total Agreement (95% CI)
2067/2080, 99.38%	1379/1418, 97.25%	3446/3498, 98.51%
(98.93 - 99.63)	(96.26 - 97.98)	(98.06 - 98.86)

iii. **Method A vs Method C Qualitative Comparison**

Comparisons between Method A and Method C were calculated twice; once assuming that all Method C results that were UNC were considered as negative and once assuming that all Method C results that were UNC were considered as positive.

Method A vs Method C (UNC = Neg)		Positive Agreement (95% CI)	Negative Agreement (95% CI)	Total Agreement (95% CI)
Site 1	Technician A	337/348, 96.84%	227/235, 96.60%	564/583, 96.74%
		(94.43 - 98.23)	(93.43 - 98.27)	(94.97 - 97.90)
	Technician B	337/349, 96.56%	226/234, 96.58%	563/583, 96.57%
		(94.09 - 98.02)	(93.40 - 98.26)	(94.76 - 97.77)
Site 2	Technician A	322/329, 97.87%	250/254, 98.43%	572/583, 98.11%
		(95.67 - 98.97)	(96.02 - 99.39)	(96.65 - 98.94)
	Technician B	322/331, 97.28%	248/252, 98.41%	570/583, 97.77%
		(94.91 - 98.56)	(95.99 - 99.38)	(96.22 - 98.69)
Site 3	Technician A	347/362, 95.86%	215/221, 97.29%	562/583, 96.40%
		(93.28 - 97.47)	(94.20 - 98.75)	(94.56 - 97.63)
	Technician B	346/361, 95.84%	215/222, 96.85%	561/583, 96.23%
		(93.26 - 97.47)	(93.64 - 98.46)	(94.35 - 97.49)

Method A vs Method C (UNC = POS)		Positive Agreement (95% CI)	Negative Agreement (95% CI)	Total Agreement (95% CI)
Site 1	Technician A	348/348, 100.00%	212/235, 90.21%	560/583, 96.05%
		(98.91 - 100.00)	(85.74 - 93.39)	(94.15 - 97.36)
	Technician B	349/349, 100.00%	212/234, 90.60%	561/583, 96.23%
		(98.91 - 100.00)	(86.18 - 93.71)	(94.35 - 97.49)
Site 2	Technician A	327/329, 99.39%	236/254, 92.91%	563/583, 96.57%
		(97.81 - 99.83)	(89.08 - 95.47)	(94.76 - 97.77)
	Technician B	329/331, 99.40%	236/252, 93.65%	565/583, 96.91%
		(97.82 - 99.83)	(89.94 - 96.05)	(95.17 - 98.04)
Site 3	Technician A	358/362, 98.90%	198/221, 89.59%	556/583, 95.37%
		(97.19 - 99.57)	(84.87 - 92.96)	(93.35 - 96.80)
	Technician B	355/361, 98.34%	196/222, 88.29%	551/583, 94.51%
		(96.42 - 99.24)	(83.39 - 91.88)	(92.35 - 96.09)

iv. **Combined Qualitative Agreement for Method A vs Method C All Sites/All Technicians**

Method C (UNC = Neg)		
Positive Agreement (95% CI)	**Negative Agreement (95% CI)**	**Total Agreement (95% CI)**
2011/2080, 96.68%	1381/1418, 97.39%	3392/3498, 96.97%
(95.82 - 97.37)	(96.42 - 98.10)	(96.35 - 97.49)

Method C (UNC = [Pos)		
Positive Agreement (95% CI)	**Negative Agreement (95% CI)**	**Total Agreement (95% CI)**
2066/2080, 99.33%	1290/1418, 90.97%	3356/3498, 95.94%
(98.87 - 99.60)	(89.37 - 92.36)	(95.23 - 96.55)

v. **Method B vs Method C Qualitative Comparison**

Comparisons between Method B and Method C were calculated twice; once assuming that all Method C results that were UNC were considered as negative and once assuming that all Method C results that were UNC were considered as positive.

Method B vs Method C (UNC = Neg)		Positive Agreement (95% CI)	Negative Agreement (95% CI)	Total Agreement (95% CI)
Site 1	Technician A	338/350, 96.57%	226/233, 97.00%	564/583, 96.74%
		(94.10 - 98.03)	(93.93 - 98.54)	(94.97 - 97.90)
	Technician B	337/348, 96.84%	227/235, 96.60%	564/583, 96.74%
		(94.43 - 98.23)	(93.43 - 98.27)	(94.97 - 97.90)
Site 2	Technician A	322/334, 96.41%	245/249, 98.39%	567/583, 97.26%
		(93.83 - 97.93)	(95.94 - 99.37)	(95.59 - 98.30)
	Technician B	322/330, 97.58%	249/253, 98.42%	571/583, 97.94%
		(95.29 - 98.77)	(96.01 - 99.38)	(96.44 - 98.82)
Site 3	Technician A	352/372, 94.62%	210/211, 99.53%	562/583, 96.40%
		(91.84 - 96.49)	(97.36 - 99.92)	(94.56 - 97.63)
	Technician B	352/372, 94.62%	210/211, 99.53%	562/583, 96.40%
		(91.84 - 96.49)	(97.36 - 99.92)	(94.56 - 97.63)

Method B vs Method C (UNC = POS)		Positive Agreement (95% CI)	Negative Agreement (95% CI)	Total Agreement (95% CI)
Site 1	Technician A	350/350, 100.00%	212/233, 90.99%	562/583, 96.40%
		(98.91 - 100.00)	(86.62 - 94.03)	(94.56 - 97.63)
	Technician B	348/348, 100.00%	212/235, 90.21%	560/583, 96.05%
		(98.91 - 100.00)	(85.74 - 93.39)	(94.15 - 97.36)
Site 2	Technician A	332/334, 99.40%	236/249, 94.78%	568/583, 97.43%
		(97.84 - 99.84)	(91.27 - 96.92)	(95.80 - 98.43)
	Technician B	328/330, 99.39%	236/253, 93.28%	564/583, 96.74%
		(97.82 - 99.83)	(89.50 - 95.76)	(94.97 - 97.90)
Site 3	Technician A	369/372, 99.19%	199/211, 94.31%	568/583, 97.43%
		(97.66 - 99.73)	(90.32 - 96.72)	(95.80 - 98.43)
	Technician B	368/372, 98.92%	198/211, 93.84%	566/583, 97.08%
		(97.27 - 99.58)	(89.75 - 96.36)	(95.38 - 98.17)

vi. **Combined Qualitative Agreement for Method B vs Method C All Sites/All Technicians**

Method C (UNC = Neg)		
Positive Agreement (95% CI)	Negative Agreement (95% CI)	Total Agreement (95% CI)
2023/2106, 96.06%	1367/1392, 98.20%	3390/3498, 96.91%
(95.14 - 96.81)	(97.36 - 98.78)	(96.29 - 97.44)

Method C (UNC = Pos)		
Positive Agreement (95% CI)	Negative Agreement (95% CI)	Total Agreement (95% CI)
2095/2106, 99.48%	1293/1392, 92.89%	3388/3498, 96.86%
(99.07 - 99.71)	(91.42 - 94.12)	(96.22 - 97.38)

vii. **Pattern Agreement – Individual Sites**

Pattern Agreement		Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
Site 1	Technician A	336/347, 96.83%	290/337, 86.05%	290/338, 85.80%
		(94.41 - 98.22)	(81.95 - 89.35)	(81.67 - 89.12)
	Technician B	327/347, 94.24%	296/337, 87.83%	289/337, 85.76%
		(91.27 - 96.24)	(83.91 - 90.90)	(81.62 - 89.09)
Site 2	Technician A	329/329, 100.00%	281/322, 87.27%	281/322, 87.27%
		(98.85 - 100.00)	(83.18 - 90.47)	(83.18 - 90.47)
	Technician B	329/329, 100.00%	280/322, 86.96%	280/322, 86.96%
		(98.85 - 100.00)	(82.84 - 90.20)	(82.84 - 90.20)
Site 3	Technician A	328/359, 91.36%	295/347, 85.01%	323/352, 91.76%
		(88.00 - 93.85)	(80.88 - 88.39)	(88.42 - 94.20)
	Technician B	324/356, 91.01%	277/346, 80.06%	287/352, 81.53%
		(87.59 - 93.56)	(75.53 - 83.93)	(77.15 - 85.24)

viii. **Combined Pattern Agreement - All Sites/All Technicians**

Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
1973/2067, 95.45%	1719/2011, 85.48%	1750/2023, 86.51%
(94.47 - 96.27)	(83.87 - 86.95)	(84.95 - 87.93)

ix. **Agreement Studies Conclusion:**

In all cases, the qualitative and pattern agreement between interpretation methods is quite high indicating that all three methods (manual microscope, digital read of the Sebia dIFine® scanner and automated call from the Sebia dIFine® scanner) correlate well with each other and exhibited few discrepancies.

Taken altogether, these data demonstrate that the auto-call identified by Sebia dIFine® (Method C) agrees with Method A and/or Method B (non-automated identification methods) for the vast majority of the samples. However, it is still the responsibility of the trained operator to make the final decision.

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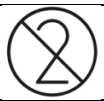
GLOSSARY OF SYMBOLS

The following symbols **may** have been used in the labelling of this product.

Symbol	Description	Symbol	Description
	Manufacturer		Keep away from sunlight
IVD	*In vitro* diagnostic medical device		Single use assay wells
REF	Catalogue number	COVGLS	Cover Glass
Σ n	Sufficient for *n* tests	SLD	ANCA Substrate Slide
LOT	Batch code	BUF PBS	PBS Buffer
	Use by	MNTMED	Mounting Media
	Storage Temperature limitations	CONJ	Conjugate
RX Only	For Prescription Use Only	CTRL +	c-ANCA Positive Control
	Consult electronic instructions for use	CTRL +	p-ANCA Positive Control
	Store in the upright position	CTRL -	Negative Control
		Made in the USA	Made in the USA

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