

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k050715

B. Purpose for Submission:

This is a new submission.

C. Measurand:

Antineutrophil Cytoplasmic Antibodies (ANCA)

D. Type of Test:

ELISA (Semi-quantitative)

E. Applicant:

INOVA Diagnostics, Inc.

F. Proprietary and Established Names:

QUANTA Plex™ ANCA Profile

G. Regulatory Information:

1. Regulation section:

21 CFR§ 866.5660 Multiple Antibodies Immunological Test System

2. Classification:

Class II

3. Product code:

MOB, Anti-neutrophil cytoplasmic antibodies (ANCA)

4. Panel:

(82) Immunology

H. Intended Use:

1. Intended use(s):

The QUANTA Plex™ ANCA Profile is a fluorescent immunoassay for the semi-quantitative detection of anti-MPO and anti-PR3 autoantibodies in human serum. The presence of these antibodies can be used in conjunction with clinical findings and other laboratory aids to aid in the diagnosis of the autoimmune vasculitides microscopic polyarteritis, crescentic glomerulonephritis, and Wegener's granulomatosis.

2. Indication(s) for use:

Same as above

3. Special conditions for use statement(s):

The device is for prescription use only.

4. Special instrument requirements:

Luminex^{100™} Flow Cytometer vs.2.2

I. Device Description:

The device consists of the following: a foil package containing 12 (1 X 8) microwell strips with holder (each microwell contains 3 different color beads and each color bead is coated with a different purified antigen (MPO, PR3 and an IgG control), a negative control, a low positive control, a high positive control, HRP sample diluent, PBS concentrate, PE-labeled goat anti-human IgG conjugate (fc-specific) and

conjugate diluent.

J. Substantial Equivalence Information:

1. Predicate device name(s):
 QUANTA Lite™ MPO IgG ELISA
 QUANTA Lite™ PR3 IgG ELISA
2. Predicate 510(k) number(s):
 k981330
 k981328
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	QUANTA Plex™ ANCA	QUANTA Lite™ MPO and QUANTA Lite™ PR3
Intended Use	Detection of anti-MPO and anti-PR3 autoantibodies	Same
Sample type	Serum	Same
Type of test	Semi-quantitative	Same

Differences		
Item	Device	Predicate
Assay type	Flow Cytometer based	ELISA
Assay Format	Multiplexed	Individual analytes
Instrument	Luminometer (Luminex vs. 2.2)	Spectrophotometer
Detection method	Fluorescence	Colorimetric
Conjugate	Phycoerythrin	HR
Solid Phase Capture	Color-coded microspheres	Microwells

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

None referenced.

L. Test Principle:

Native MPO and PR3 are each bound to different, fluorescently “colored” beads. The two different antigen coated beads, as well as an IgG-coated bead, are mixed together, and put into wells of a microwell plate under conditions that preserve the antigens in their native state. Pre-diluted controls and diluted patient sera are added to separate microwells, allowing any MPO and PR3 autoantibodies present to bind to the immobilized antigen. Then an anti-human IgG conjugated to a fluorescent probe is added to each microwell. A second incubation allows the anti-human IgG fluorescent conjugate to bind to any patient autoantibodies that have become attached to the antigen on the beads. The samples are then measured in the Luminex™ 100 flow Cytometer. This flow Cytometer can discriminate the color of each bead from the others as well as measure the fluorescent intensity of the conjugate on each bead. The

conjugate fluorescent intensity is proportional to the amount of labeled anti-human IgG bound to the patient autoantibodies on the bead. Each antigen can be semi-quantitated by comparing the fluorescent intensity of the patient sample with the fluorescence of the corresponding Low Positive. An IgG-coated control bead is included in each microwell to ensure that false negative results due to operational errors are detected.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The automated pipetting method precision testing was not performed on this application. On previous applications for the ENA Profile 4, ENA Profile 5, ENA Profile 6, and SLE Profile 8, it was shown that automated method showed same results as the manual method. Only the manual method was performed on this application.

Intra-assay reproducibility was determined by assaying 10 samples 9 times on one plate. A representative data of the data are shown on the table below.

Antigen	Average LU	Mean %CV
MPO	26	10%
MPO	62	5%
MPO	161	5%
PR3	41	15%
PR3	76	9%
PR3	281	5%

Another form of intra-assay variation was tested by running one sample on an entire plate. The processed data for that sample showed a 7% CV for the MPO bead, 5% CV for the PR3 bead and 3%CV for the IgG control bead.

Inter-assay reproducibility was determined by assaying 12 samples 5 times for 5 days. A representative data of the data are shown on the table below.

Antigen	Average LU	Mean %CV
MPO	24	14%
MPO	63	15%
MPO	183	7%
PR3	41	10%
PR3	85	10%
PR3	423	7%

b. *Linearity/assay reportable range:*

Dynamic range of the assay is 0 MFI (median fluorescence intensity) to 25,000 MFI.

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

Negative, low positive and high positive controls are included.

Accelerated stability studies were performed for the ANCA profile beads and the Low and High controls specific for the ANCA profile. Results were

acceptable. Stability testing was not repeated for components used in other products such as the Quanta Plex negative control, HRP sample diluent, PE conjugate and Conjugate diluent because data for all of these products have been submitted in previous applications.

d. *Detection limit:*

The Luminex Users manual states that the limit of detection is 1000 fluorochromes of phycoerythrin per microsphere. Serial dilutions of positive sera to end point were performed using the QUANTA Plex™ ANCA and detection limit were set at:

- MPO 5.3 LU
- PR3 3.3 LU

e. *Analytical specificity:*

Cross-reactivity

To assess cross-reactivity of other autoantibodies, 90 sera samples were tested. The disease control group consisted of 7 patients with rheumatoid arthritis, 3 with Lyme disease, 3 with hepatitis C, 4 with toxoplasmosis, 3 with cytomegalovirus, and 3 with herpes simplex virus. 67 samples that yielded an atypical ANCA pattern or an X-ANCA pattern by immunofluorescence were also included. This class of samples yields positive ANCA immunofluorescence. However, they are expected to be negative for anti-MPO and anti-PR3 because they do not have the typical pANCA or cANCA pattern associated with the latter autoantibodies.

Comparison of samples from the Other Controls on the MPO portion of the ANCA Profile and the MPO ELISA

Other Controls n=90		Luminex ANCA Profile (MPO)	
MPO ELISA 98.8% the same		Negative	Positive
	Negative	89 (98.9%)	1* (1.1%)
	Positive	0 (0%)	0 (0%)

* This sample was negative for ANCA by IFA.

Comparison of samples from the Other Controls on the PR3 portion of the ANCA Profile and the MPO ELISA

Other Controls n=90		Luminex ANCA Profile (PR3)	
PR3 ELISA 96.7% the same		Negative	Positive
	Negative	81 (90.0%)	3* (3.3%)
	Positive	0 (0%)	6 (6.7%)

*One sample was 19 U on ELISA and cANCA by IFA, so was likely a true positive. 2 samples were 11 U by ELISA and atypical ANCA by IFA.

Interfering substances

Interference studies were performed by mixing each of 6 serum samples: 1 normal, 1 hemolyzed (hemoglobin 1000 mg/dL), 1 icteric (bilirubin 29.7) -2 lipemic (one with cholesterol at 369 and one with triglycerides at 1016) and 1 high IgG, in equal volumes with a sera positive for MPO and PR3. Little or no

interference was observed.

f. *Assay cut-off:*

The cut-off value was determined using 256 normal samples and 20 arbitrary units were chosen for continuity with the predicate devices. The fluorescence unit that was assigned 20 units was based on non-parametric statistical analysis of the data.

2. Comparison studies:

a. *Method comparison with predicate device:*

Clinical samples:

Three hundred twenty seven clinically defined patient samples were tested with the new device and the predicate device. These samples included 237 samples from INOVA Technical Service for evaluation of ANCA, 7 patients with rheumatoid arthritis, 16 with infectious diseases, and 67 with atypical ANCA. Age and sex information were provided for 216 samples. There were 125 males and 91 females. The average age of the males was 39.4 years, while the average age for females was 35.9 years. Discrepant results were re-tested by both devices and the retest result was used as the final result.

Total sample N = 327	Both Pos	Both Neg	E Pos Q Neg	E Neg Q Pos	% Positive Agreement	% Negative Agreement	%Total Agreement
MPO	247	71	3	6	95.9%	97.6%	97.2%
PR3	212	106	1	8	99.1%	96.4%	97.2%

All Samples:

Five hundred eighty three serum samples were tested with the new device and the predicate device. The samples included 256 normal, and the 327 clinical samples.

All Samples N=583	Both Neg	Both Pos	ELISA Neg Q.Plex Pos	ELISA Pos Q.Plex Neg	%Positive Agreement (95% CII)	%Negative Agreement (95% CI)	Percent Agreement
MPO	502	71	7	3	95.9% (88.6-99.2%)	98.6% (97.2-99.4%)	98.3%
PR3	465	107	9	2	98.2% (93.5-99.8%)	98.1% (96.2-99.0%)	98.1%

b. *Matrix comparison:*

Serum is the only recommended matrix.

3. Clinical studies:

a. *Clinical Sensitivity:*

Not applicable.

b. *Clinical specificity:*

Not applicable.

c. *Other clinical supportive data (when a. and b. are not applicable):*

Not applicable.

4. Clinical cut-off:

See assay cut-off.

5. Expected values/Reference range:

Two hundred fifty six from normal blood donors fro INOVA Technical Service

Library was run on the QUANTA Plex™ ANCA Profile test. The normal range results were shown below.

Analyte	% Negative	Range LU*	Mean ±SD (LU)
MPO	99.6	0-41	2.1±2.8
PR3	99.2	0-11.5	1.4±1.6

N. Proposed Labeling:

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

O. Conclusion:

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