510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k092601

B. Purpose for Submission:
New Device

C. Measurand:
Antineutrophil Cytoplasmic Antibodies (ANCA)

D. Type of Test:
ELISA (Qualitative)

E. Applicant:
Immco Diagnostics, Inc.

F. Proprietary and Established Names:
ImmuLisa™ ANCA ELISA for PR3 and MPO Antibodies

G. Regulatory Information:

1. Regulation section:
   21 CFR § 866.5660, Multiple Autoantibodies Immunological Test System
2. Classification:
   Class II
3. Product codes:
   MOB, Test System, Antineutrophil Cytoplasmic Antibodies (ANCA)
4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use(s):
   An enzyme linked immunosorbent assay (ELISA) for the qualitative detection of anti-neutrophil cytoplasmic antibodies (ANCA) with specificity for proteinase 3 (PR3) and myeloperoxidase (MPO) in human serum to aid in the diagnosis of small vessel vasculitis in conjunction with other laboratory and clinical findings.
2. Indication(s) for use:
   Same as intended use
3. Special conditions for use statement(s):
   For prescription use only
4. Special instrument requirements:
   Microplate reader capable of measuring OD at 450 and a reference wavelength of 600-650 nm.

I. Device Description:

Each device contains the following: microwell strips (12x8) coated with MPO and PR3, one calibrators (30 EU/ml), HRP goat anti-human IgG conjugate, TMB enzyme substrate, positive control, negative control, serum diluent, wash buffer and sulfuric acid stop solution. All reagents are ready to use except for the wash buffer which requires reconstitution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Inova Quanta Lite MPO Antibody ELISA

Inova Quanta Lite PR3 Antibody ELISA

2. Predicate K number(s):
   k981330 and k091328
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	ImmuLisa ANCA ELISA for PR3 and MPO	**Inova Quanta Lite MPO and PR3 ELISA**
Intended use	An enzyme linked immunosorbent assay (ELISA) for the qualitative detection of anti-neutrophil cytoplasmic antibodies (ANCA) with specificity for proteinase 3 (PR3) and myeloperoxidase (MPO) in human serum	Same
Methodology	ELISA	Same
Analyte detected	Human IgG antibodies to MPO and PR3	Same
Component set	Includes positive control, negative control, calibrators,conjugate, substrate, diluent, wash buffer, stop solution, microplate	Same
Conjugate antibody	HRP	Same
Specimen type	Serum	Same
Substrate/chromogen	TMB	Same
Positive control	MPO and PR3 IgG antibodies	Same
Stop solution	H2SO4	Same
Assay dilution	1:101	Same
Signal detection	450nm on spectrophotometer	Same
Storage	2-8°C	Same

Differences		
Item	Device	Predicate
	ImmuLisa ANCA ELISA for PR3 and MPO	**Inova Quanta Lite MPO and PR3 ELISA**
Assay type	Qualitative	Semi-Quantitative
Capture antigen	MPO and PR3	MPO or PR3
Cut-off	20 EU/ml	20 EU/ml

Differences		
Item	Device	Predicate
	<20 EU/ml is negative ≥20 EU/ml is positive	
Wash buffer	Powdered or liquid concentrate	Liquid concentrate
Positive control	Acceptance range printed on vial	No value/range assigned
Calibrators	Single; value in units 30 EU/ml	Single; value in units 25 EU/ml
Limit of detection	3.3 EU/ml	Not specified

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

CLSI EP5-A2, EP7-A2, EP9-A2, EP12-A2, and EP17-A

L. Test Principle:

The test is performed as a solid phase immunoassay. Microwells are coated with purified MPO and PR3 antigen followed by a blocking step to reduce non-specific binding during the assay run. Controls, calibrators and patient sera are incubated in the antigen coated wells to allow specific antibodies present in the serum to bind to the MPO and PR3 antigen. Unbound antibodies and other serum proteins are removed by washing the microwells. Bound antibodies are detected by adding an enzyme labeled anti-human IgG conjugate to the microwells. Unbound conjugate is removed by washing. Specific enzyme substrate (TMB) is then added to the wells and the presence of antibodies is detected by a color change produced by the conversion of TMB substrate to a colored reaction product. The reaction is stopped and the intensity of the color change, which is proportional to the concentration of antibody, is ready by a spectrophotometer at 450 nm. Results are expressed in ELISA Units per milliliter (EU/ml) and reported as positive or negative.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Per CLSI EP5-A2, precision of the assay was tested with five specimens ranging in values from 10.43 to 87.21 EU/ml. The percent total imprecision ranged from 5.9-12.2%. Averaged results are shown in table below. Multiple studies were conducted. Assay runs of three replicates of five specimens were conducted using three different lots (n=45). Separately, assay runs of three replicates of five specimens were conducted over five days, twice a day (n=135). Lastly, assay runs of six replicates of five specimens were conducted. Repeatability was determined with 28 replicates of each of the five specimens. Results are also shown in the table below (last column). Percent CVs ranged from 4.1-9.5%. Sponsor's pre-defined acceptance criteria for precision was 20%.

						Within run	
		Total Imprecision		Between days		(Repeatability)	
	Mean	SD		SD		SD	
Sample	(IU/ml)	(IU/ml)	CV%	(IU/ml)	CV%	(IU/ml)	CV%
1	10.43	1.274	12.2%	1.442	13.8%	0.988	9.5%

2	16.03	1.211	7.6%	1.335	8.5%	0.868	5.3%
3	22.58	1.540	6.8%	1.669	7.4%	1.346	6.0%
4	25.29	1.502	5.9%	1.735	6.8%	1.039	4.1%
5	87.21	5.163	5.9%	5.806	6.6%	4.103	4.7%

b. Linearity/assay reportable range:
Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):
International reference materials for anti-MPO or anti-PR3 antibodies are not available. The assay is calibrated in relative arbitrary units (EU/ml).

Stability
Accelerated, real-time and open kit/reagent stability studies were conducted as part of design control to assign expiration dating to components and as part of ongoing quality control/quality assurance analysis. Accelerated and open kit studies were performed on three lots of components/reagents. They included materials incubated at 37°C where one day is considered to equivalent to one month stored at 2-8°C. Materials are removed from the incubator for testing at three day intervals for a minimum of 21 days. For open kit stability studies, materials are opened as required for bench-top usage, then assayed at 15, 45, and 90 day intervals. Based on these studies, the expiration date for this assay is 18 months. Real time stability studies are ongoing.

Positive control and calibrator were derived from the sera of subjects with autoimmune vasculitides obtained from a commercial source. For assignment of values, the samples were tested at various dilutions on at least two different lots of MPO and PR3 antigen coated plates.

d. Detection limit:
Per CLSI EP17-A, the limit of detection (LoD) for this assay was determined to be 3.3 EU/ml based on 60 replicates of the blank and 10 replicates of each of the six low-level samples. The limit of blank is 2.9 EU/ml.

e. Analytical specificity:
Interfering Substances:
Per CLSI EP7-A2, inteference was studied by mixing sera with known ANCA levels with potentially interfering serum samples and analyzing deviation from expected results. Interference was calculated as follows: 1 – (obtained/expected)%. Results are presented in table below. No significant interference was demonstrated for the following substances at the levels indicated: hemoglobin range of interference -5.2-7.3% (2 g/L), bilirubin range of interference -13.9-2% (342 umol/L), rheumatoid factor range of interference 1.5-10.2% (100 EU/ml), and triglycerides range of interference -9.1-7.7% (37 mmol/L). Interference study with triglycerides was conducted separately. The sponsor's acceptance criteria for interference was set at less than 20% for negative samples and less than 15% for positive samples. Testing of grossly hemolyzed or lypemic samples is not recommended as

stated in the package insert.

Sample		Hemoglobin		Bilirubin		RF		Sample		Triglycerides	
	EU/ml	EU/ml	% Int	EU/ml	% Int	EU/ml	% Int		EU/ml	EU/ml	% Int
1	13.7	14.7	7.3	11.8	-13.9	15.1	10.2	1	11	12	-9.1
2	19.4	18.4	-5.2	16.8	-13.4	19.7	1.5	2	18.1	16.8	7.7
3	24.8	25.1	1.2	22.7	-8.5	27.2	9.7	3	21.4	22.8	-6.1
4	62.1	63.8	2.7	58.3	-6.1	67.4	8.5	4	46.2	48.3	-4.3
5	92.1	92.6	0.5	89.4	-2.9	95.6	3.8	5	63.8	64.3	-0.8
6	145.2	151.7	4.5	148.1	2	158.2	9	6	117.7	113.5	3.7

Cross-Reactivity:
A total of 96 potentially cross-reactive specimens from individuals with other autoimmune disorders or positive for other autoantibodies were tested for ANCA using the ImmuLisa ANCA ELISA for PR3 and MPO. ANCA may occur in patients with various connective tissue disorders. Inflammatory bowel disease samples were also included. The results are presented in the table below. Overall cross-reactivity studies are within acceptance criteria (set by sponsor at 10%).

Condition	n	n Positive
Non-ANCA associated vasculitis	24	1
Celiac disease	8	0
Crohn's disease	10	0
Ulcerative colitis	6	0
Hashimoto's thyroiditis	8	0
Rheumatoid arthritis	8	1
ENA* positive		
La Ab	8	1
Ro Ab	8	1
Sm Ab	8	1
RNP Ab	8	0
Total	96	5 (5.2%)

* Antibodies to Extractable Nuclear Antigens

Hook effect:
Not applicable

f. *Assay cut-off*:
A normal range study was conducted using 64 normal human sera and 16 diseased controls on the assay. These samples were obtained from commercial sources. Based on ROC analysis, the mean plus 2.5 standard deviation of these values was established as the cut-off between normal and abnormal results at 0.367 OD. This value was assigned to 20 EU/ml.

2. Comparison studies:

a. *Method comparison with predicate device*:
Per CLSI EP9-A2, the ImmuLisa ANCA ELISA for PR3 and MPO was tested in comparison with Inova Quanta Lite MPO ELISA and Inova Quanta Lite PR3 ELISA separately, using well-characterized sera of ANCA antibody positive subjects (88 ANCA positive), disease controls (56) and healthy individuals (54). Disease controls included Ceiliac Disease, ENA positive collagen vascular autoimmunity, Hashimoto's, and RA. The results are shown in the following 3 tables. 1) Table of IMMCO ANCA ELISA compared to Inova PR3 ELISA alone; 2) IMMCO ANCA ELISA compared to Inova MPO ELISA alone; and 3) Combined data from above two method comparison studies. These are results when the indeterminate range of the predicate assays were considered positive.

Indeterminate as positive				
			Inova PR3 Ab	
		Pos	Neg	Total
IMMCO	Pos	37	41	78
ANCA	Neg	1	119	120
ELISA	Total	38	160	198
Positive. % Agreement		97.4%	(95% CI 84.6% to 99.9%)	
Negative. % Agreement		74.4%	(95% CI 66.8% to 80.8%)	
Overall % Agreement		78.8%	(95% CI 72.3% to 84.1%)	

Indeterminate as positive				
			Inova MPO Ab	
		Pos	Neg	Total
IMMCO	Pos	35	43	78
ANCA	Neg	0	120	120
ELISA	Total	35	163	198
Positive. % Agreement		100.0%	(95% CI 87.7% to 100%)	
Negative. % Agreement		73.6%	(95% CI 66.0% to 80.1%)	
Overall % Agreement		78.3%	(95% CI 71.8% to 83.7%)	

These values are calculated by combining data from above two tables

Indeterminate as positive				
		Inova MPO or	Inova MPO and	
		PR3 Ab Pos	PR3 Ab Neg	Total
IMMCO	Pos	72	6	78
ANCA	Neg	1	119	120
ELISA	Total	73	125	198
Positive. % Agreement		98.6%	(95% CI 91.6% to 99.9%)	
Negative. % Agreement		95.2%	(95% CI 89.4% to 98.5%)	
Overall % Agreement		96.5%	(95% CI 92.6% to 98.4%)	

These are results for the combined predicate assays (MPO and PR3) when the

indeterminate range of the predicate assays were considered negative.

Borderline as negative				
		Inova MPO or	Inova MPO and	
		PR3 Ab Pos	PR3 Ab Neg	Total
IMMCO	Pos	67	1	68
ANCA	Neg	6	124	130
ELISA	Total	73	125	198
Positive. % Agreement		91.8%	(95% CI 82.4% to 96.6%)	
Negative. % Agreement		99.2%	(95% CI 95.0% to 99.9%)	
Overall % Agreement		96.5%	(95% CI 92.6% to 98.4%)	

b. *Matrix comparison*:
Not applicable

3. Clinical studies:
   a. *Clinical sensitivity and specificity*:
   A set of 272 clinical samples from various disease groups (see table below for breakdown of actual disease groups) confirmed IFA+ were tested with the ImmunoLisa ANCA ELISA. Other autoimmune disease samples include Celiac Disease and Hashimoto's Thyroiditis. Results are presented in table below. The calculated clinical sensitivity of the assay is 98.7% (95% CI 92.2-99.9%). The calculated clinical specificity of the assay is 96.4% (95% CI 92.4-98.4%).

ANCA ELISA	Positive > Cutoff 20 EU/ml			
			Disease Status	
		Pos	Neg	Total
	Pos	78	7	85
IMMCO	Neg	1	186	187
	Total	79	193	272
Sensitivity		98.7%	(95% CI 92.2% to 99.9%)	
Specificity		96.4%	(95% CI 92.4% to 98.4%)	
Agreement		97.1%	(95% CI 94.1% to 98.6%)	

Patient Group	**n**	**n Pos**	**% Pos**
Disease Associated			
Glomerulonephritis	32	32	100.0%
Wegener's granulomatosis	47	46	97.9%
Undifferentiated ANCA positive	17	1	5.9%
Disease Control			
Non-ANCA associated vasculitis	24	1	4.2%
Crohn's disease	10	0	0.0%
Ulcerative colitis	6	0	0.0%
Systemic lupus erythematosus	32	3	9.4%
Rheumatoid arthritis	8	1	12.5%

Other autoimmune disease	16	0	0.0%
Healthy normals	80	1	1.3%

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

4. Clinical cut-off:
   Not applicable
5. Expected values/Reference range:
   Expected values in a normal population are negative. However, 2-4% of apparently healthy, asymptomatic individuals may test positive for ANCA antibodies. The following table depicts the frequency of MPO and PR3 specific ANCA in sera from 112 ANCA associated vasculitides patients.

Incidence of anti-PR3 and anti-MPO in ANCA associated vasculitides

Antibody association	Wegener's granulomatosis	Microscopic polyangiitis	Churg-Strauss syndrome
ANCA positive by IFA	78%	59%	67%
anti-PR3 positive	90%	0%	10%
anti-MPO positive	0%	62%	17%

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.