

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

A. 510(k) Number:

k092599

B. Purpose for Submission:

New Device

C. Measurand:

Anti-MPO (myeloperoxidase)

D. Type of Test:

ELISA (Semi-quantitative)

E. Applicant:

Immco Diagnostics, Inc.

F. Proprietary and Established Names:

ImmuLisa™ MPO Antibody ELISA

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 detection and semi quantitation of antibodies to myeloperoxidase (MPO) in human serum as an aid in the diagnosis of glomerulonephritis 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, Calibrators A-E (156, 62.5, 25, 10, 1 U/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

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

Similarities		
Item	Device	Predicate
	ImmuLisa MPO Antibody ELISA	Inova Quanta Lite MPO ELISA
Intended use	An enzyme linked immunosorbent assay (ELISA) for the detection and semi quantitation of antibodies to myeloperoxidase (MPO) in human serum as an aid in the diagnosis of glomerulonephritis in conjunction with other laboratory and clinical findings	Same
Methodology	ELISA	Same
Capture antigen	MPO	Same
Analyte detected	Human IgG antibodies to MPO	Same
Assay type	Semi-quantitative	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 IgG antibody	Same
Stop solution	H2SO4	Same
Screening dilution	1:101	Same
Signal detection	450nm on spectrophotometer	Same
Storage	2-8°C	Same

Differences		
Item	Device	Predicate
	ImmuLisa MPO Antibody ELISA	Inova Quanta Lite MPO ELISA
Cut-off	10 U/ml	20 EU/ml

Differences		
Item	Device	Predicate
Wash buffer	Powdered or liquid concentrate	Liquid concentrate
Positive control	Acceptance range printed on vial	No value/range assigned
Calibrators	Set of 5; values in U/ml E: 1 D: 10 C: 25 B: 62.5 A: 156	Single; value in units 25 EU/ml
Linear range	2.7-156 U/ml	Not specified
Indeterminate range	10-12.5 U/ml	None
Limit of detection	2.7 U/ml	Not specified

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

CLSI EP5-A2, EP6-A, 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 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 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 arbitrary Units per milliliter (U/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 seven specimens with values spanning the range of the assay. The linear range of the assay is 2.7-156 U/ml. The seven samples spanned concentrations 2.61 to 137.08. The percent total imprecision ranged from 3.4-13.5%. Averaged results are shown in table below. Multiple studies were conducted. Assay runs of three replicates of seven specimens were conducted using three different lots (n=63). Separately, assay runs of three replicates of seven specimens were conducted over five days, twice a day (n=189). Lastly, assay runs of six replicates of seven specimens were conducted. Repeatability was determined with 28 replicates of each of the seven specimens. Results are also shown in the table below (last column). Percent CVs ranged from 2.6-11.3%.

Sponsor's pre-defined acceptance criteria for precision was 20%.

Sample	Within run (Repeatability)						
	Total Imprecision			Between days			
	Mean (U/ml)	SD (U/ml)	CV%	SD (U/ml)	CV%	SD (U/ml)	
1	2.61	0.352	13.5%	0.382	14.7%	0.286	11.3%
2	8.41	0.582	6.9%	0.632	7.5%	0.496	6.0%
3	11.54	0.743	6.4%	0.825	7.2%	0.604	5.2%
4	48.22	3.028	6.3%	3.274	6.8%	2.660	5.5%
5	55.93	3.166	5.7%	3.058	5.5%	3.349	6.0%
6	126.27	4.334	3.4%	4.712	3.7%	3.772	3.0%
7	137.08	4.746	3.5%	5.439	4.0%	3.542	2.6%

b. Linearity/assay reportable range:

To determine the linear range of the assay, studies were performed per CLSI EP6-A using equidistant dilution series of positive samples with values throughout the calibrator range. Up to nine serial dilutions in sample diluent (low positive or negative samples) were made and run in duplicate. The linear range of the assay is 2.7-156 U/ml. Linear regressions are summarized in the table below. Samples producing results greater than the top calibrator are recommended to be diluted and retested.

Sample	Test Range (U/ml)	Slope (95% CI)	Y-Intercept (95% CI)	R ²	% Recovery
1	5.2 to 182.3	1.009 (0.943 to 1.075)	-0.063 (-0.160 to 0.033)	0.996	99.4 to 113.3
2	4.6 to 74.5	0.947 (0.876 to 1.018)	0.067 (-0.004 to 0.137)	0.994	84.1 to 104.2
3	4.7 to 47.8	1.028 (0.962 to 1.094)	-0.005 (-0.056 to 0.046)	0.996	94.8 to 101.6

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

An international reference material for anti-MPO antibodies is not available. The assay is calibrated in relative arbitrary units (U/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 calibrators 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 antigen coated plates.

d. *Detection limit:*

Per CLSI EP17-A, the limit of detection (LoD) for MPO antibody using this assay was determined to be 2.7 U/ml based on 60 replicates of the blank and 10 replicates of each of the six low-level samples. The limit of blank is 2.0 U/ml.

e. *Analytical specificity:*

Interfering Substances:

Per CLSI EP7-A2, interference was studied by mixing sera with known MPO antibody levels with potentially interfering serum samples and analyzing deviation from expected results. Interference was calculated as follows: $1 - (\text{obtained}/\text{expected})\%$. Results are presented in table below. No significant interference was demonstrated for the following substances at the levels indicated: hemoglobin range of interference -7.7-5.4% (2 g/L), bilirubin range of interference -11.5-10.8% (342 umol/L), rheumatoid factor range of interference -5.9-12.3% (100 EU/ml), and triglycerides range of interference -9.9-5.6% (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	
		IU/ml	%Interference	IU/ml	%Interference	IU/ml	%Interference		IU/ml	IU/ml	%Interference
1	5.2	5.6	-7.7	5.8	-11.5	4.8	7.7	1	5.1	5.7	-9.9
2	10.2	9.7	4.9	9.1	10.8	10.8	-5.9	2	9.4	8.9	5.6
3	12.1	11.8	2.5	11.9	1.7	11.4	5.8	3	11.4	12.4	-8.1
4	45.2	47.8	-5.8	42.5	6	41.7	7.7	4	32.2	35.6	-9.3
5	82.6	78.1	5.4	75.4	8.7	72.4	12.3	5	65.3	68.6	-4.8
6	126.4	132.4	-4.7	138.1	-9.3	115.7	8.5	6	147.3	153.4	-4

Cross-Reactivity:

A total of 149 potentially cross-reactive specimens from individuals with other autoimmune disorders or positive for other autoantibodies were tested for MPO antibodies using the ImmuLisa MPO Antibody ELISA. PR3 is the most common antigen target of ANCA in patients with Wegener's granulomatosis, therefore PR3 positive samples are included (confirmed for a cANCA positive reaction using FDA cleared IFA). 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	Positive n
Wegener's granulomatosis	47	0
Undifferentiated cANCA positive	6	0
Non-ANCA associated vasculitis	24	0

Crohn's disease	10	0
Ulcerative colitis	6	0
Systemic lupus erythematosus	32	0
Rheumatoid Arthritis	8	1
Other autoimmune disease	16	0
Total	149	1 (0.7%)

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 0.294 OD. This value was assigned to 10 U/ml. An indeterminate range has been established 20% above the cut-off at 12.5 U/ml. Samples in this indeterminate range should be retested or confirmed using a secondary method such as IFA.

2. Comparison studies:

a. Method comparison with predicate device:

Per CLSI EP9-A2, the Immulisa MPO Antibody ELISA was tested in comparison with Inova Quanta Lite MPO ELISA using well-characterized sera of ANCA antibody positive subjects (45 pANCA positive), disease controls (35) and healthy individuals (30). Included were 21 samples near the cut-off of 10 U/ml. Only samples within the measuring range of the assay were included for method comparison analysis. Disease controls included Celiac Disease, ENA positive collagen vascular autoimmunity, Hashimoto's, and RA. The two samples testing negative on the Inova assay and positive on the IMMCO assay are IFA positive glomerulonephritis cases. The results are as follows for a cut-off of 10 U/ml using a 5 point calibrator (indeterminate results are considered positive):

5 point calibrator, indeterminate as positive				
		Inova		
		Pos	Neg	Total
	Pos	43	2	45
IMMCO	Neg	0	65	65
	Total	43	67	110
Positive % Agreement		100.0%	(95% CI 89.8% to 100%)	
Negative % Agreement		97.0%	(95% CI 88.7% to 99.5%)	
Overall % Agreement		98.2%	(95% CI 92.9% to 99.7%)	

The following results are for a cut-off of 12.5 U/ml using a 5 point calibrator (indeterminate results are considered negative):

5 point calibrator, indeterminate as negative				
			Inova	
		Pos	Neg	Total
	Pos	34	2	36
IMMCO	Neg	4	70	74
	Total	38	72	110
Positive % Agreement		89.5%	(95% CI 74.3% to 96.6%)	
Negative % Agreement		97.2%	(95% CI 89.4% to 99.5%)	
Overall % Agreement		94.5%	(95% CI 88.0% to 97.8%)	

b. *Matrix comparison:*
Not applicable

3. Clinical studies:

a. *Clinical sensitivity and specificity:*

A set of 286 clinical samples from various disease groups (see table below for breakdown of actual disease groups) confirmed IFA+ were tested with the ImmunoLisa MPO Antibody ELISA and another commercially available MPO antibody ELISA. Other autoimmune disease samples include Celiac Disease and Hashimoto's Thyroiditis. Results are presented in table below. When indeterminate results are considered positive, the calculated clinical sensitivity of the assay is 100% (95% CI 92.1-100%). The calculated clinical specificity of the assay is 99.6% (95% CI 97.2-100%).

MPO	Positive > Cutoff 10 U/ml, 5 point Calibrator			
		Disease Status		
		Pos	Neg	Total
	Pos	57	1	58
IMMCO	Neg	0	228	228
	Total	57	229	286
Sensitivity		100.0%	(95% CI 92.1% to 100%)	
Specificity		99.6%	(95% CI 97.2% to 100%)	
Agreement		99.7%	(95% CI 97.8% to 100%)	

Patient Group	IMMCO			Other MPO Ab ELISA		
	n	n Pos	% Pos	n	n Pos	% Pos
Disease Associated						
Glomerulonephritis	57	57	100%	54	53	98.1%
Wegener's granulomatosis	47	0	0.0%	47	0	0.0%
Undifferentiated ANCA positive	6	0	0.0%	6	0	0.0%
Disease Control						
Non-ANCA associated vasculitis	24	0	0.0%			
Crohn's disease	10	0	0.0%			
Ulcerative colitis	6	0	0.0%			
Systemic lupus erythematosus	32	0	0.0%	32	0	0.0%

Rheumatoid arthritis	8	1	12.5%	8	1	12.5%
Other autoimmune disease	16	0	0.0%	16	0	0.0%
Healthy normals	80	0	0.0%	80	0	0.0%

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, 4% of apparently healthy, asymptomatic individuals may test positive for anti-MPO 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%
unknown specificity positive	40%	31%	73%

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.