

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

A. 510(k) Number:

k092600

B. Purpose for Submission:

New Device

C. Measurand:

Anti-PR3 (proteinase 3)

D. Type of Test:

ELISA (Semi-quantitative)

E. Applicant:

Immco Diagnostics, Inc.

F. Proprietary and Established Names:

ImmuLisa™ PR3 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 proteinase 3 (PR3) in human serum to aid in the diagnosis of Wegener's granulomatosis 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 PR3, 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 PR3 Antibody ELISA

2. Predicate K number(s):
k981328
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	ImmuLisa PR3 Antibody ELISA	Inova Quanta Lite PR3 ELISA
Intended use	An enzyme linked immunosorbent assay (ELISA) for the detection and semi-quantitation of antibodies to proteinase 3 (PR3) in human serum, as an aid in the diagnosis of Wegener's granulomatosis in conjunction with other laboratory and clinical findings.	Same
Methodology	ELISA	Same
Capture antigen	PR3	Same
Analyte detected	Human IgG antibodies to PR3	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	PR3 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 PR3 Antibody ELISA	Inova Quanta Lite PR3 ELISA
Cut-off	10 U/ml	20 EU/ml
Wash buffer	Powdered or liquid	Liquid concentrate

Differences		
Item	Device	Predicate
	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	2.2-156 U/ml	Not specified
Indeterminate range	10-12.5 U/ml	None
Limit of detection	2.2 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 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 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 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 2.2-156 U/ml. The seven samples spanned concentrations 6.34 to 141.59. The percent total imprecision ranged from 5.0-12.8%. 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 3.4-8.1%. Sponsor's pre-defined acceptance criteria for precision was 20%.

Sample	Total Imprecision		Between days		Within run (Repeatability)		
	Mean	SD	SD	SD	SD	SD	
	(IU/ml)	(IU/ml)	(IU/ml)	(IU/ml)	(IU/ml)	(IU/ml)	
		CV%	CV%	CV%	CV%	CV%	
1	6.34	0.808	12.8%	0.964	15.2%	0.517	8.1%
2	8.50	0.750	8.8%	0.818	9.7%	0.643	7.5%
3	12.26	1.215	9.9%	1.220	9.8%	1.167	9.8%
4	27.27	2.627	9.6%	3.112	11.4%	1.711	6.3%
5	56.94	3.088	5.4%	3.485	6.1%	2.390	4.2%
6	111.06	5.518	5.0%	6.406	5.8%	3.789	3.4%
7	141.59	7.763	5.5%	8.205	5.8%	6.850	4.8%

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 2.2-156 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	Slope (95% CI)	Y-Intercept (95% CI)	R ²	% Recovery
1	3.7 to 77.2	1.07 (0.94 to 1.20)	-0.031 (-0.134 to 0.071)	0.986	89.8 to 111.9
2	3.8 to 158.2	1.012 (0.934 to 1.090)	-0.053 (-0.153 to 0.047)	0.994	88.9 to 102.2
3	3.4 to 43.4	1.023 (0.929 to 1.118)	0.009 (-0.045 to 0.063)	0.991	100 to 112.3

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

An international reference material for anti-PR3 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 PR3 antigen coated plates.

d. *Detection limit:*

Per CLSI EP17-A, the limit of detection (LoD) for PR3 antibody using this assay was determined to be 2.2 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 2.1 U/ml.

e. *Analytical specificity:*

Interfering Substances:

Per CLSI EP7-A2, interference was studied by mixing sera with known PR3 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 -9.6-10.6% (2 g/L), bilirubin range of interference -5.7-1.7% (342 umol/L), rheumatoid factor range of interference -13.4-4.2% (100 EU/ml), and triglycerides range of interference -4.4-8.5% (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	% Int	EU/ml	% Int	EU/ml	% Int	EU/ml	% Int	EU/ml	% Int	EU/ml	% Int	EU/ml	% Int	EU/ml	% Int	
1	4	4.4	-9.6	4.1	-1.7	4.5	-13.4	1	3.8	3.7	2.7							
2	9.8	9.6	1.4	9.7	0.8	10	-2	2	8.9	8.2	8.5							
3	9.8	8.7	10.6	9.8	0	9.6	2	3	7.9	8.3	-4.4							
4	36	35.4	1.7	38	-5.7	37	-3	4	18.8	17.8	5.6							
5	72	75.1	-4.3	75.9	-5.5	73.9	-2.6	5	42.1	41.4	1.7							
6	192.9	176.9	8.3	189.7	1.7	184.7	4.2	6	134.4	138.1	-2.7							

Cross-Reactivity:

A total of 136 potentially cross-reactive specimens from individuals with other autoimmune disorders or positive for other autoantibodies were tested for PR3 antibodies using the Immulisa PR3 Antibody ELISA. MPO positive samples were tested MPO positive by ELISA and confirmed for a pANCA 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
Glomerulonephritis	29	2
Undifferentiated pANCA positive	11	0
Non-ANCA associated vasculitis	24	1

Crohn's disease	10	0
Ulcerative Colitis	6	0
Systemic lupus erythematosus	32	0
Rheumatoid Arthritis	8	0
Other autoimmune disease	16	0
Total	136	3 (2.2%)

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 0.250 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 PR3 Antibody ELISA was tested in comparison with Inova Quanta Lite PR3 ELISA using well-characterized sera of ANCA antibody positive subjects (53 cANCA positive), disease controls (28) and healthy individuals (24). Included were 12 samples near the cut-off of 10 U/ml. Only samples within the measuring range of the assay were included for method comparison analysis. The disease controls included Celiac Disease, ENA positive collagen vascular autoimmunity, Hashimoto's, and RA. Of the four samples that tested negative on the Inova assay and positive on the IMMCO assay, three were IFA positive Wegener's cases and one was a disease control. The two samples that tested negative on the IMMCO assay and positive on the Inova assay were IFA positive Wegener's 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	41	4	45
IMMCO	Neg	2	58	60
	Total	43	62	105
Positive % Agreement		95.3%	(95% CI 82.9% to 99.2%)	
Negative % Agreement		93.5%	(95% CI 83.5% to 99.2%)	
Overall % Agreement		94.3%	(95% CI 87.5% to 97.7%)	

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
IMMCO	Pos	38	0	38
	Neg	5	62	67
Total		43	62	105
Positive % Agreement		88.4%	(95% CI 74.1% to 95.6%)	
Negative % Agreement		100.0%	(95% CI 92.7% to 100%)	
Overall % Agreement		95.2%	(95% CI 88.7% to 98.2%)	

b. *Matrix comparison:*
Not applicable

3. Clinical studies:

a. *Clinical sensitivity and specificity:*

A set of 75 clinical samples from various disease groups (see table below for breakdown of actual disease groups) confirmed IFA+ were tested with the ImmunoLisa PR3 Antibody ELISA and another commercially available PR3 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 96.6% (95% CI 87.3-99.4%). The calculated clinical specificity of the assay is 98.1% (95% CI 95-99.4%).

PR3	Positive > Cutoff 10 IU/ml, 5 point Calibrator			
		Disease Status		
		Pos	Neg	Total
IMMCO	Pos	57	4	61
	Neg	2	212	214
Total		59	216	275
Sensitivity		96.6%	(95% CI 87.3% to 99.4%)	
Specificity		98.1%	(95% CI 95.0% to 99.4%)	
Agreement		97.8%	(95% CI 95.1% to 99.1%)	

Patient Group	IMMCO			Inova PR3 Ab ELISA		
	n	n Pos	% Pos	n	n Pos	% Pos
Disease Associated						
Wegener's granulomatosis	59	57	96.6%	59	56	94.9%
Glomerulonephritis	29	2	6.9%	29	2	6.9%
Undifferentiated ANCA positive	11	0	0.0%	11	0	0.0%
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	1	3.1%	32	0	0.0%
Rheumatoid arthritis	8	0	0.0%	8	0	0.0%
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, 2-4% of apparently healthy, asymptomatic individuals may test positive for anti-PR3 antibodies. The following table depicts the frequency of PR3 and MPO 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.