

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

A. 510(k) Number:

k051458

B. Purpose for Submission:

New Device

C. Measurand:

Anti- PR3 antibodies

D. Type of Test:

Qualitative and Semi-quantitative ELISA

E. Applicant:

Eurodiagnostica

F. Proprietary and Established Names:

Wieslab™ Cap PR-3 ANCA

G. Regulatory Information:

1. Regulation section:
21 CFR 866.5660, Multiple autoantibodies immunological test system
2. Classification:
II
3. Product codes:
MOB, Test system, antineutrophil cytoplasmic antibodies (ANCA)
4. Panel:
Immunology 82

H. Intended Use:

1. Intended use(s):
The Wieslab™ Cap PR-3 ANCA test kit is an enzyme-linked immunosorbent assay (ELISA) for detection and semi-quantitation of IgG antibodies to proteinase 3 (PR3) in human serum. The assay is used to detect antibodies in a single serum specimen. The results of the assay are to be used as an aid to the diagnosis of Wegener's granulomatosis. The analysis should be performed by trained laboratory professionals.
For In Vitro Diagnostic Use.
2. Indication(s) for use:
The Wieslab™ Cap PR-3 ANCA test kit is an enzyme-linked immunosorbent assay (ELISA) for detection and semi-quantitation of IgG antibodies to proteinase 3 (PR3) in human serum. The assay is used to detect antibodies in a single serum specimen. The results of the assay are to be used as an aid to the diagnosis of Wegener's granulomatosis. The assay is intended for use in patients with signs and symptoms consistent with WG. It is not intended for screening a healthy population. The analysis should be performed by trained laboratory professionals.
3. Special conditions for use statement(s):
The device is for prescription use only.
4. Special instrument requirements:
Microplate reader capable of measuring OD at 405 nm.
Microplate washer (300µL volume).

I. Device Description:

Each device contains the following: microplate strips (red colored) coated with monoclonal anti-proteinase 3/proteinase 3 antigen; five levels calibrators (10, 40, 80, 160, 320 U/mL); positive and negative controls (human serum in diluent); wash solution concentrate; sample diluent; goat anti-human IgG alkaline phosphatase conjugate; *p*-Nitro phenyl Phosphate (*p*NPP) substrate.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Wieslisa® PR-3 ANCA Kit
2. Predicate 510(k) number(s):
k974167
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Device
Intended use	To aid in the diagnosis of Wegener's granulomatosis (WG)	Same
Assay Format	Qualitative and semi-quantitative	Same
Enzyme-Conjugate	Alkaline Phosphatase	Same
Positive and Negative controls	Ready to use	Same
Calibrators: Five Levels	Ready to use	Same
Sample volume required	5 µL	Same
Sample type and dilution	Serum at 1:80	Same
Sample diluent	PBS	Same
Wash solution	30x Concentrate	Same
Substrate	<i>p</i> NPP	Same
Incubation times	30-30-60 minutes	Same
OD reading	405 nm	Same
Platform	96 well microtiter plates	Same

Differences		
Item	Device	Predicate
Technology	Capture ELISA	Direct ELISA
Antigen	Purified anti-PR3 monoclonal antibody and PR3 capture complex	Purified PR3
OD measurement	Within ± 5 minutes	None specified
Anti-PR-3 Antibody Results Interpretation	Negative: < 21 U/mL Equivocal: 21-30 U/mL Positive: > 30 U/mL	Negative: < 10 Units Equivocal: 10-20 Units Positive: > 20 Units

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

None provided.

L. Test Principle:

The Wieslab™ Cap PR-3 ANCA test kit is an enzyme-linked immunosorbent assay (ELISA) for detection and semi-quantitation of IgG antibodies to proteinase 3 (PR3) in human serum. The wells

of the microtiter plate strips are coated with a capture complex of monoclonal antibody to proteinase 3 and purified PR3 antigen. Antibodies specific to PR3 in diluted serum bind to the capture antigen during the first incubation. The wells are then washed to remove unbound antibodies. An enzyme labeled anti-human IgG conjugate is added to bind the capture antigen-antibody complex in the well during the second incubation step. After a further washing step, detection of the specific antibodies is obtained by incubation with substrate solution. The amount of bound antibodies correlated to the color intensity and is measured in terms of absorbance (optical density, OD). The absorbance is then calculated against the calibrator curve and the results are given in arbitrary Units/mL.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The intra-assay reproducibility was determined by testing eight samples 24 times. Five samples with high anti-PR3 concentrations (102-141 U/mL) had a CV of 6-9% and three samples close to the assay cut-off (24-42 U/mL) had a CV of 3-13%. The inter-assay reproducibility was determined by testing seven samples in duplicate for four times. Three samples with high anti-PR3 concentrations (77-138 U/mL) had a CV of 6-7%. Four samples close to the assay cut-off (23-49 U/mL) had a CV of 4-14%.

b. *Linearity/assay reportable range:*

Three positive sera were diluted serially from neat, 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions. The values were compared to log 2 of dilution by standard regression. The values indicate that the assay has a linear relationship with serum dilutions. The assay reportable range is from 10-320 U/mL.

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

There is no reference standard for anti-PR3. The calibrators and controls (positive and negative) are prepared in-house and arbitrary units are assigned during the development process.

d. *Detection limit:*

Not applicable.

e. *Analytical specificity:*

Interference by endogenous substances: No data provided. The package insert states to avoid sera which are icteric, lipemic, and hemolyzed; and heat-inactivated sera should not be used.

Crossreactivity with heterophile antibodies: Since one of the capture antigen component is a monoclonal antibody, some in-house interference studies (~12000 samples) were performed. The effect was determined to be very low (0.025%). The package insert states that ‘individuals receiving mouse anti-human antibodies for treatment or diagnosis, or those patients who have otherwise exposed to mouse immunoglobulin, may produce Human Anti-Mouse Antibodies (HAMA). These antibodies can interfere with assays using mouse monoclonal antibodies and may cause falsely elevated levels’.

f. *Assay cut-off:*

The cut-off value of >30 U/mL was based on testing 120 normal blood donor sera, 17 rheumatoid arthritis (RA) samples, 46 systemic lupus erythematosus (SLE). All of the

normal donors were negative. One of the RA and one of the SLE samples were equivocal (23 and 27 U/mL respectively).

2. Comparison studies:

a. *Method comparison with predicate device:*

Testing was performed on 180 samples. The positive percent agreement was 97.6% (40/41); the negative percent agreement was 98.5% (133/135) and the Overall Agreement was 98.3% (173/176).

		Wieslisa PR3-ANCA ELISA Kit			
		Positive	Equivocal*	Negative	Total
WiesLab™ Cap PR3- ANCA	Positive	40	(2)*	2	42
	Equivocal	0	0	(2)*	(2)*
	Negative	1	0	133	134
	Total	41	(2)*	135	176

*Equivocal results were excluded in the calculation.

b. *Matrix comparison:*

Not applicable.

3. Clinical studies:

a. *Clinical sensitivity and specificity:*

The clinical sensitivity and specificity study were evaluated on 363 clinically characterized sera from patients with the following diagnosis: 50 WG; 50 microscopic polyangiitis (MP); 45 SLE; 18 RA; 80 glomerular basement membrane antibody (GBM); and 120 healthy blood donors. Sensitivity for WG was 97%. Sensitivity for MP patients was 31.2% because PR3-ANCA antibodies are only found in approximately one third of the MP patients. The overall specificity of the new device (healthy and disease controls) was 97.3%.

N= 363		ELISA		
Patient Group	n=	positive	Equivocal*	negative
WG	50	48	0	2
MP	50	15	(2)*	33
Healthy controls	120	0	0	120
Disease controls				
SLE	45	0	1	44
RA	18	0	1	17
GBM	80	4	0	76

*Equivocal results were excluded from the calculation.

Sensitivity:

WG: 96.0% (48/50) 95% CI: 83.3% to 99.5%
 MP: 31.2% (15/48) 95% CI: 18.7% to 46.2%

Specificity:

Healthy controls: 100% (120/120) 95% CI: 97.0% to 100%
 SLE: 100% (44/44) 95% CI: 92.0% to 100%

RA:	100% (17/17)	95% CI: 80.5% to 100%)
GBM:	95% (76/80)	95% CI: 97.0% to 100%)

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

Not Applicable.

4. Clinical cut-off:

Same as assay cut-off.

5. Expected values/Reference range:

Expected values in the normal population should be negative.

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.