510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k091859

B. Purpose for Submission:
New assay

C. Measurand:
Anti-neutrophil cytoplasmic antibody (ANCA)

D. Type of Test:
Semi-quantitative and qualitative ELISA

E. Applicant:
Aesku.Diagnostics

F. Proprietary and Established Names:
AESKULISA® PR3 test kit

G. Regulatory Information:

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

H. Intended Use:

1. Intended use(s):
   AESKULISA PR3 is a solid phase enzyme immunoassay employing purified native human proteinase 3 (PR3) from human neutrophil granulocytes for the semi-quantitative and qualitative detection of antibodies against proteinase 3 in human serum. The assay is an aid in the diagnosis of Wegener's granulomatosis and should be used in conjunction with other serological tests and clinical findings.
2. Indication(s) for use:
   See intended use above.
3. Special conditions for use statement(s):
   For prescription use only
4. Special instrument requirements:
   Microplate reader capable of reading absorbance values at 450 nm

I. Device Description:
AESKULISA PR3 is a solid phase enzyme immunoassay. The test kit includes the following components: 96-well microtiter plate, coated with purified PR3 isolated from human neutrophil granular cells, 6 calibrators (0, 3, 10, 30, 100, and 300 U/mL), cut-off control for qualitative analysis, positive control containing human serum, negative control containing human serum, sample buffer concentrate (5x), wash buffer concentrate (50x), polyclonal goat anti-human IgG HRP (horseradish peroxide) conjugate, TMB chromogenic substrate, stop solution containing 1M HCl.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   VARELISA PR3 ANCA EIA kit
2. Predicate K number(s):
   k041043
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Name	AESKULISA PR3	VARELISA PR3
Intended Use	The assay is an aid in the diagnosis of Wegener's granulomatosis and should be used in conjunction with other serological tests and clinical findings.	Same.
Measurement	Semi-quantitative and qualitative	Same
Conjugate	HRP conjugated anti-human IgG antibody	Same
Assay principle	Indirect noncompetitive enzyme immunoassay	Same

Differences		
Item	Device	Predicate
Name	AESKULISA PR3	VARELISA PR3
Specimen requirements	Serum	Serum and plasma
Calibrators	0, 3, 10, 30, 100, 300 U/mL	0, 3, 7, 16, 40, 100 U/mL
Cutoff	15 U/mL	9 U/mL
Equivocal Zone	None	6-9 U/mL

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

None utilized.

L. Test Principle:

The assay is an indirect noncompetitive enzyme immunoassay. The wells of a microtiter plate are coated with purified human PR3 antigen. Antibodies specific for PR3 present in the patient samples bind to the antigen. In a second step, the enzyme labeled second antibody (conjugate) binds to the antigen-antibody complex leading to the formation of an enzyme labeled conjugate-antibody-antigen complex. The enzyme labeled antigen-antibody complex converts the added substrate to form a colored solution. The rate of color formation from the chromogen is a function of the amount of conjugate complexed with the bound antibody and thus is proportional to the initial concentration of antibodies in the patient sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:
   Precision of the assay was assessed using a total of eight serum samples with mean values ranging from 6.8 U/mL to 260.7 U/mL. Experiment 1 was performed using three separate runs with eight replicates each. Experiment 2 was performed using five separate runs with eight replicates each:

Experiment	Sample	Mean U/mL	Intra-assay % CV	Inter-assay % CV
1	1	22.9	2.2	2.2
	2	48.9	3.7	4.0
	3	88.2	5.2	5.7
2	4	6.8	7.1	15.8
	5	11.1	2.0	4.7
	6	16.3	3.4	6.4
	7	75.4	3.4	6.0
	8	260.7	4.4	6.0

A lot-to-lot reproducibility was study was performed using the eight samples listed above. The same lot of antigen was used for all three lots, but the plates were coated in separate runs. A different lot of conjugate was used for each of the three lots:

Sample	Result (U/mL)			Mean	%CV
	Lot 1	Lot 2	Lot 3		
1	22.6	22.1	22.2	22.3	1.7
2	50.4	51.6	51.4	51.2	2.8
3	99.6	95.3	105.2	100.0	8.7
4	6.7	5.7	5.9	5.8	12.8
5	10.3	10.2	11.1	11.0	6.5
6	15.1	15.1	16.1	16.2	6.5
7	72.7	74.7	76.2	74.9	3.1
8	262.6	272.7	256.0	263.8	5.7

Because the AESKULISA PR3 assay can be used in either a semi-quantitative or qualitative mode, a separate qualitative analysis was performed on intra- and inter-assay data from experiment 2. Following the package insert directions for determining qualitative results, the absorbance values of each sample replicate were divided by the absorbance the cut-off calibrator on the same plate and the resulting ratio was used to determine if the sample was positive or negative. The data are summarized in the table below:

Sample	Quantitative Value (U/mL)	% Negative	% Positive
4	5.8	100	0
5	11.0	100	0
6	16.2	12.5	87.5
7	74.9	0	100
8	256.0	0	100

b. Linearity/assay reportable range:

Four serum samples were diluted, beginning with a standard dilution of 1:100

and further diluted to a total dilution of 1:200, 1:400, and 1:800. Percent recovery (percent observed/expected ratios) of each sample was in the range 92 - 107%. The linearity experiment encompassed samples in the range 4.2 – 278 U/mL. A linear regression of the measured vs. expected data in this table produced slope = 0.94, intercept = 2.48, r^2 = 0.998.

	Dilution	**Expected (U/mL)**	**Measured (U/mL)**	**Recovery**
Sample 1	1/100	102.0	105.0	97 %
	1/200	49.8	52.5	95 %
	1/400	25.8	26.3	98 %
	1/800	12.1	13.1	92 %
Sample 2	1/100	52.6	54.0	96 %
	1/200	25.8	27.0	96 %
	1/400	14.1	13.5	104 %
	1/800	7.3	6.8	107 %
Sample 3	1/100	278.4	261.8	106 %
	1/200	139.5	130.9	107 %
	1/400	64.2	65.4	98 %
	1/800	32.3	32.7	99 %
Sample 4	1/100	33.6	34.2	98%
	1/200	16.0	17.1	94 %
	1/400	7.9	8.6	92 %
	1/800	4.2	4.3	98 %

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 testing data was provided demonstrating that the AESKULISA PR3 kit is stable for at least 24 months when stored at 4°C.

Opened and reconstituted kits are claimed to be stable for one month when stored at 4°C. Serum sample stability claims and storage recommendations in the package insert are based on CLSI H18-A3 “Procedures for the Handling and Processing of Blood Specimens; Approved Guideline — Third Edition”.

d. *Detection limit:*
Thirty determinations of sample buffer were used to determine the limit of blank (LoB). LoB of the AESKULISA PR3 assay is 0.28 U/mL.
To determine the Limit of Detection (LoD) eight low samples were tested eight times each (a total of 64 determinations). These samples were used to calculate LoD according to CLSI EP17-A. The LoD of the assay is 1.38 U/ml.

e. *Analytical specificity:*
The following substances were spiked into serum in order to test for interference: hemoglobin (800 mg/dL), bilirubin (20 mg/dL), and triglyceride

(3000 mg/dL). Three samples were evaluated for interference—a high negative, a low positive, and a strongly positive sample. Recoveries were all in the range 89-110%.

The analytical specificity of the AESKULISA PR3 assay was partially addressed in the clinical study by testing 69 samples from patients that were positive for anti-MPO antibodies. Two of these samples were positive for both anti-MPO and anti-PR3 antibodies. These two samples were from a patient diagnosed with SLE and a patient diagnosed with acute hearing loss.

The analytical specificity of the AESKULISA PR3 assay was also addressed by testing 21 samples from patients with reactive arthritis that were positive for rheumatoid factor, antibodies to cyclic citrullinated peptides (CCP) and/or antinuclear antibodies (ANA). All were negative in the AESKULISA PR3 assay.

f. Assay cut-off:

A study was performed to confirm the defined cut-off by measuring serum samples from 80 apparently health donors as well as samples from 27 patients with Crohn's disease, reactive arthritis, or ulcerative colitis that should all be expected to be negative for anti-PR3 antibodies. The cut-off of 15 U/mL was determined from the mean plus 3 standard deviations of this population.

2. Comparison studies:

a. Method comparison with predicate device:

139 sera of patients suffering from Wegener's granulomatosis, microscopic polyangiitis and other autoimmune diseases have been tested on the AESKULISA PR3 and a predicate device. Of these, 69 sera lay in range of the assay and were used for a comparison study versus a predicate device:

	Predicate PR3			
AESKULISA PR3	**Pos**	**Equiv**	**Neg**	**Total**
Pos	15	6	5	26
Neg	0	4	39	43
Total	15	10	44	69

Regarding Equivocal as Negative:
Positive Agreement: 100.0% 95% CI: 79.6 – 100.0%
Negative Agreement: 79.6% 95% CI: 67.1 – 88.2.0%
Overall Agreement: 84.1% 95% CI: 76.7 – 90.9%

Regarding Equivocal as Positive:
Positive Agreement: 84.0% 95% CI: 65.4 – 93.6%
Negative Agreement: 88.6% 95% CI: 76.0 – 95.0%
Overall Agreement: 87.0% 95% CI: 77.0 – 93.0%

b. Matrix comparison:

Not applicable; this assay is for serum only.

3. Clinical studies:
   a. *Clinical Sensitivity and Specificity:*
   AESKULISA PR3 assay results from 139 patients suffering from Wegener's granulomatosis, microscopic polyangiitis, other autoimmune diseases, and miscellaneous other conditions (see below) were compared to the clinical diagnosis:

	AESKULISA PR3 Results:		
Number of Samples with	**Positive**	**Negative**	**Total**
acute hearing loss	1		1
chronic renal disease	1		1
Churg-Strauss	1	1	2
COPD		3	3
Crohns disease		6	6
endocarditis	1		1
glomerulonephritis		2	2
glomerulonephritis (c-ANCA positive)	1		1
Goodpasture-Syndrome	1		1
healthy		1	1
HIV	1		1
mPAN		30	30
palsy	1		1
Polymyalgia rheumatica (vasculitis)	1		1
Reactive Arthritis		21	21
rheumatoid arthritis	1		1
SLE	1		1
ulcerative colitis		6	6
ulceritive colitis, septic fungal infection	1		1
Wegeners Granulomatosis	56		56
Wegeners Granulomatosis / GN	1		1
Total	**69**	**70**	**139**

Summary of Clinical Results:

	Wegeners Granulomatosis		
AESKU PR3	**Positive**	**Negative**	**Total**
Positive	57	12	69
Negative	0	70	70
Total	57	86	**139**

Sensitivity (57/57) = 100% (95% CI: 93.7 to 100%)
Specificity (70/86) = 85.4% (95% CI: 76.1% to 91.4%)
Overall agreement (123/139) = 91.4% (95% CI: 85.5% to 95.0%)

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

4. Clinical cut-off**:**
See Assay cut-off above.
5. Expected values/Reference range:
The expected value in the normal population is negative although a small portion of the population will have ANCA autoantibodies without clinical disease.

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.