

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

A. 510(k) Number:

k091860

B. Purpose for Submission:

New device

C. Analyte:

Anti-neutrophil cytoplasmic antibody (ANCA)

D. Type of Test:

Semi-quantitative and qualitative ELISA

E. Applicant:

Aesku.Diagnostics

F. Proprietary and Established Names:

AESKULISA[®] MPO 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 MPO is a solid phase enzyme immunoassay employing highly purified myeloperoxidase (MPO) from human peripheral blood polymorphonuclear cells for the semi-quantitative and qualitative determination of antibodies against MPO in human serum. The assay is a tool in the differential diagnosis of autoimmune systemic vasculitis.
2. Indication(s) for use:
Same as intended use.
3. Special conditions for use statement(s):
Prescription use only
4. Special instrument requirements:
Microplate reader capable of reading absorbance values at 450 nm

I. Device Description:

AESKULISA MPO is a solid phase enzyme immunoassay. The test kit includes the following components:

1. 96-well microtiter plate, coated with purified MPO isolated from human neutrophil granular cells.
2. 6 calibrators (0, 3, 10, 30, 100, and 300 U/mL) for semi-quantitative analysis containing human serum, BSA, and buffer components
3. Cut-off control for qualitative analysis, containing human serum, BSA, and buffer components

4. Positive control containing human serum, BSA, and buffer components
5. Negative control containing human serum, BSA, and buffer components
6. Sample buffer concentrate (5x)
7. Wash buffer concentrate (50x)
8. Goat polyclonal anti-human IgG, HRP (horseradish peroxide) conjugate
9. TMB chromogenic substrate
10. Stop solution containing 1M HCl

J. Substantial Equivalence Information:

1. Predicate device name(s):
VARELISA MPO ANCA EIA kit
2. Predicate 510(k) number(s):
k041040
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Name	AESKULISA MPO	VARELISA MPO
Intended Use	A tool in the differential diagnosis of autoimmune systemic vasculitis.	To aid in the diagnosis of certain autoimmune vasculitides such as microscopic polyangiitis and crescentic glomerulonephritis
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 MPO	VARELISA MPO
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

L. Test Principle:

The assay is an indirect noncompetitive enzyme immunoassay. The wells of a microtiter plate are coated with human purified MPO antigen. Antibodies specific for MPO 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 which leads 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 studies were performed on a total of eight serum samples with mean values ranging from 6.2 U/mL to 173.9 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	38.6	1.7	1.6
	2	78.5	3.0	2.5
	3	173.9	5.8	5.7
2	4	6.2	14.4	14.3
	5	7.1	10.8	10.6
	6	10.1	8.8	9.0
	7	14.6	9.3	9.4
	8	25.9	7.7	8.0

A lot-to-lot reproducibility 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	32.3	32.8	33.1	32.7	3.7
2	52.0	53.9	54.7	53.5	8.2
3	161.0	162.0	164.0	162.3	7.4
4	6.3	5.9	6.4	6.2	12.7
5	7.2	6.7	7.0	7.0	10.8
6	10.2	10.2	9.9	10.1	8.8
7	14.7	14.5	13.8	14.3	9.2
8	26.1	25.8	25.0	25.6	6.5

Because the AESKULISA MPO 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. The data are summarized in the table below.

Sample	N, total number of samples run	Mean result (semi-quantitative)	Qualitative results	
			% negative	% positive
Negative	40	10.1	100%	0%
Near cutoff	40	14.6	72.5%	27.5%
Positive	40	25.9	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 – 325 U/mL. A linear regression of the measured vs. expected data in this table produced slope = 1.02, intercept = -0.55, $r^2 = 0.994$.

	dilution	expected	measured	recovery
Sample 1	1/100	78.0	76.5	98%
	1/200	39.0	37.3	96%
	1/400	19.5	19.2	98%
	1/800	9.8	9.4	96%
Sample 2	1/100	33.0	32.8	99%
	1/200	16.5	17.4	105%
	1/400	8.3	9	108%
	1/800	4.1	4.2	102%
Sample 3	1/100	325	342	105%
	1/200	163	178	109%
	1/400	81.3	85.8	106%
	1/800	40.6	42.0	103%
Sample 4	1/100	252	236	93%
	1/200	126	121	96%
	1/400	63.0	60.3	96%
	1/800	31.5	33.7	107%

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

d. *Detection limit:*

The limit of detection was determined to be 1.47 U/mL using the method from CLSI EP-17A.

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. All test results are available in the submission. Recoveries were all in the range 85-115%.

The analytical specificity of the AESKULISA MPO assay was partially addressed in the clinical study by testing 69 samples from patients that were positive for anti-PR3 (c-ANCA) antibodies. Two of these samples were positive for anti-MPO antibodies. Of these two patients with both anti-PR3 and anti-MPO antibodies, one had been diagnosed with SLE and one with acute hearing loss.

The analytical specificity of the AESKULISA MPO 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 MPO 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-MPO 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:

The comparison was made by testing 79 samples that are a subset of the clinical study and that have results in the reportable range of the AESKULISA MPO test. Equivocal results are treated as either positive or negative for the purpose of determining agreement.

		Predicate Assay (Varelisa MPO)			
		+	Equivocal	-	Total
AESKULISA MPO Assay	+	39	4	0	43
	-	0	7	29	36
	Total	39	11	29	79

Treating equivocal results as positive:

Positive agreement (43/50) = 86.0% (95% CI: 73.8% to 93.05%)

Negative agreement (29/29) = 100% (95% CI: 88.3% to 100%)

Overall agreement (72/79) = 91.1% (95% CI: 82.8% to 95.6%)

Treating equivocal results as negative:

Positive agreement (39/39) = 100% (95% CI: 91.0% to 100%)

Negative agreement (36/40) = 90.0% (95% CI: 77.0% to 96.0%)

Overall agreement (75/79) = 94.9% (95% CI: 87.7% to 98.1%)

b. Matrix comparison:

Not applicable.

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

Serum samples from patients with confirmed clinical diagnosis of microscopic polyangiitis (30) or glomerulonephritis (4) were tested using the AESKULISA MPO kit. In addition, 105 serum samples from patients diagnosed with other autoimmune diseases such as Wegener's Granulomatosis (56), reactive arthritis (21), ulcerative colitis (7), Crohn's Disease (6), COPD (3), and other diseases (12) were tested.

		mPAN/GN		
		+	-	Total
AESKULISA MPO	+	32	6	38
	-	2	99	101
	Total	34	105	139

Sensitivity (32/34) = 94.1% (95% CI: 80.9 to 98.4%)

Specificity (99/105) = 94.3% (95% CI: 88.1% to 97.4%)

Overall agreement (131/139) = 94.2% (95% CI: 89.0% to 97.1%)

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

Not applicable.

4. Clinical cut-off:

See Assay cut-off

5. Expected values/Reference range:

The expected value in the normal population is 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.