

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

A. 510(k) Number:

k062217

B. Purpose for Submission:

New Devices

C. Measurand:

Anti- β 2- glycoprotein IgA, IgG, IgM antibodies and combined IgAGM for screening

D. Type of Test:

Qualitative and Semi-quantitative ELISA

E. Applicant:

AESKU, Inc.

F. Proprietary and Established Names:

AESKULISA® β 2- Glyco-A Protocol 30-30-30 REF 30-7205US

AESKULISA® β 2- Glyco-A Protocol 30-15-15 REF 7205US

AESKULISA® β 2- Glyco-GM Protocol 30-30-30 REF 30-7206US

AESKULISA® β 2- Glyco-GM Protocol 30-15-15 REF 7206US

AESKULISA® β 2- Glyco-Check Protocol 30-30-30 REF 30-7215US

AESKULISA® β 2- Glyco-Check Protocol 30-15-15 REF 7215US

G. Regulatory Information:

1. Regulation section:

21 CFR 866.5660, Multiple autoantibodies immunological test system

2. Classification:

II

3. Product code:

MSV, Antibodies, β 2- Glycoprotein I (β 2-GPI)

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

AESKULISA® β 2- Glyco-A is a solid phase enzyme immunoassay employing native β 2- Glycoprotein I highly purified from human plasma for the semiquantitative and qualitative detection of IgA antibodies against β 2- Glycoprotein I in human serum. The presence of anti- β 2- Glycoprotein I antibodies in conjunction with clinical findings and other laboratory results can be used as an aid in the diagnosis of thrombotic disorders related to primary and secondary antiphospholipid syndrome.

AESKULISA® β 2- Glyco-GM is a solid phase enzyme immunoassay employing native β 2- Glycoprotein I highly purified from human plasma for the semiquantitative and qualitative detection of IgG and/or IgM antibodies against β 2- Glycoprotein I in human serum. The presence of anti- β 2- Glycoprotein I antibodies in conjunction with clinical findings and other laboratory results can be used as an aid in the diagnosis of thrombotic disorders related to primary and

secondary antiphospholipid syndrome.

AESKULISA® β 2- Glyco-Check is a solid phase enzyme immunoassay employing native β 2- Glycoprotein I highly purified from human plasma for the semiquantitative and qualitative detection of IgA, IgG and IgM antibodies against β 2- Glycoprotein I in human serum. The presence of anti- β 2- Glycoprotein I antibodies in conjunction with clinical findings and other laboratory results can be used as an aid in the diagnosis of thrombotic disorders related to primary and secondary antiphospholipid syndrome.

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 measuring OD at 450 nm (reading filter), and optional 620 nm reference filter (600-690 nm)
Microplate washing device (300 μ L repeating or multichannel pipette or automated system).

I. Device Description:

Each device contains: 12x8 microplate strips with breakaway microwells coated with purified β 2- Glycoprotein I antigen; six levels of calibrators (0, 3, 10, 30, 100, 300 U/mL); positive, negative, and cut-off controls (human serum, diluted); wash buffer concentrate; sample buffer concentrate; anti-human immunoglobulin (Ig A/G/M)) horseradish peroxidase conjugate; 3,3',5,5' tetramethylbenzidine (TMB)/H₂O₂ substrate; and 1M hydrochloric acid stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Varelsa β 2- Glycoprotein I IgA Antibodies EIA KIT
Varelsa β 2- Glycoprotein I IgG Antibodies EIA KIT
Varelsa β 2- Glycoprotein I IgM Antibodies EIA KIT
Varelsa β 2- Glycoprotein I Screen Antibodies EIA KIT
2. Predicate 510(k) number(s):
k040450 (IgA), k040449 (IgG), k040451 (IgM), k040452 (Screen Antibodies)
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Device
Technology	ELISA	Same
Capure Antigens	Purified human beta-2 Glycoprotein I	Same
Platform	96 well microtiter plate	Same
Stop Solution	Prediluted	Same
Diluted Sample Volume Required	100 μ l	Same

Differences		
Item	New Device	Predicate
Intended use:	The presence of anti-β2 glycoprotein I antibodies in conjunction with clinical findings and other laboratory results can be used as an aid in the diagnosis of thrombotic disorders related to primary and secondary Antiphospholipid Syndrome.	The presence of β-glycoprotein I antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of thrombotic disorders related to the primary Antiphospholipid Syndrome or occurring secondary to systemic lupus ery-thematosus (SLE) or <i>other autoimmune diseases</i> .
Controls	Positive, Negative and <i>Cut-off Control</i>	Positive and Negative controls
Stop Solution	Ready to use 1 M HCl	Ready to use 0.5M H ₂ SO ₄
Calibrators	6 levels for all AESKULISA (0, 3, 10, 30, 100 and 300 U/mL)	6 levels for all (0, 4, 8, 20, 50 and 100 U/mL)
Assay Format	Qualitative and Semi-Quantitative	Same for single Conjugates, for mixed conjugate only Qualitative
Sample Diluent	5 x concentrated	Ready to use

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

None referenced.

L. Test Principle:

The AESKULISA® β2- Glyco-A, AESKULISA® β2- Glyco-GM and AESKULISA® β2- Glyco-Check devices are solid phase enzyme immunoassays for the semiquantitative and qualitative detection of IgA, IgG and/or IgM (separate), and IgA, IgG and IgM (mixed conjugate) antibodies respectively, against β2- Glycoprotein I in human serum. The wells of a microplate are coated with β2- Glycoprotein I antigen. Antibodies specific to β2- Glycoprotein I present in the patient sample bind to the antigen. Unbound fractions are washed off in the washing step. In the next step, anti-human immunoglobulins conjugated with horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the washing step. Addition of TBM-substrate generates an enzymatic colorimetric blue reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody

complex and is proportional to the initial concentration of the respective antibodies in the patient serum. The results are read spectrophotometrically and are interpreted by comparison to a cut-off control (qualitative) or a standard calibrator curve (semiquantitative).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Three different samples (high, medium, near the cut-off) were assayed 18 times on three microplates of the specific antibody isotype, for two days for the inter-assay study. Three different samples (high, medium, near the cut-off) were assayed 24 times on one microplate of the specific antibody type for the intra-assay study. Both studies were performed on Protocol 30-15-15 incubation time. Target values for the studies were set at %CV \leq 10.

The inter-assay %CV range for AESKULISA® β 2- Glyco-A was from 8.4% to 9.6%, for AESKULISA® β 2- Glyco-GM was 4.4% to 6.5% and for AESKULISA® β 2- Glyco-Check was 8.7% to 9.6%.

The intra-assay %CV range for AESKULISA® β 2- Glyco-A was from 7.6% to 8.7%, for AESKULISA® β 2- Glyco-GM was from 5.1% to 8.8% and for AESKULISA® β 2- Glyco-Check was 7.3% to 8.4%. All the ranges were within the target values.

AESKULISA® β 2- Glyco-A	Inter-Assay Variation		
	Sample 1	Sample 2	Sample 3
CV (%)	8.4	9.6	8.6
Mean (U/mL)	14.4	106.7	124.7
AESKULISA® β 2- Glyco-GM			
CV (%)	4.4	6.9	6.5
Mean (U/mL)	19.6	70.3	172.1
AESKULISA® β 2- Glyco-Check			
CV (%)	8.7	8.5	9.6
Mean (U/mL)	41.1	80.0	174.9

AESKULISA® β 2- Glyco-A	Intra-Assay Variation		
	Sample 1	Sample 2	Sample 3
CV (%)	7.6	8.0	8.7
Mean (U/mL)	13.0	96.5	118.2
AESKULISA® β 2- Glyco-GM			
CV (%)	5.1	8.8	6.6
Mean (U/mL)	17.2	54.3	228.5
AESKULISA® β 2- Glyco-Check			
CV (%)	7.3	7.7	8.4
Mean (U/mL)	30.8	115.3	310.2

b. *Linearity/assay reportable range:*

Study design: Two samples known to contain different levels of β 2-

Glycoprotein I IgA antibodies, two samples known to contain different levels of β 2- Glycoprotein I IgG/M antibodies and two samples known to contain different levels of β 2- Glycoprotein I Ig A/G/M antibodies were chosen and serially diluted to determine the linearity of the assay. From an initial dilution of 1/100, further dilutions of 1:200, 1:400 and 1:800 were made. The **AESKULISA® β 2- Glyco-A** assay had a recovery range of 93.9% to 107.0%. **AESKULISA® β 2- Glyco-GM** assay had a recovery range of 94.0% to 103.9%. **AESKULISA® β 2- Glyco-Check** assay had a recovery range of 90.1% to 106.4% (see tables below).

AESKULISA® β 2- Glyco-A

Sample No.	Dilution Factor	measured concentration (U/ml)	expected concentration (U/ml)	Recovery (%) 90 - 110 %
1	1 / 100	123.7	122.0	101.4
	1 / 200	63.1	61.0	103.4
	1 / 400	29.3	30.5	96.1
	1 / 800	14.7	15.3	96.1
2	1 / 100	126.2	118.0	107.0
	1 / 200	61.1	60.0	103.5
	1 / 400	27.9	29.6	94.6
	1 / 800	13.9	14.6	95.9

AESKULISA® β 2- Glyco-GM

Sample No.	Dilution Factor	measured concentration (U/ml)	expected concentration (U/ml)	Recovery (%) 90 - 110 %
1	1 / 100	191.4	200.0	95.7
	1 / 200	100.7	100.0	100.7
	1 / 400	48.7	50.0	97.4
	1 / 800	24.5	25.0	98.0
2	1 / 100	232.6	236.0	97.3
	1 / 200	124.2	119.5	103.9
	1 / 400	57.9	58.8	96.8
	1 / 800	28.1	29.9	94.0

AESKULISA® β 2- Glyco-Check

Sample No.	Dilution Factor	measured concentration (U/ml)	expected concentration (U/ml)	Recovery (%) 90 - 110 %
1	1 / 100	189.2	199.0	95.1
	1 / 200	105.9	98.5	106.4
	1 / 400	47.0	40.8	94.4
	1 / 800	25.2	24.9	101.2
2	1 / 100	94.0	91.0	103.7
	1 / 200	39.8	40.5	98.3
	1 / 400	19.7	20.3	97.0
	1 / 800	9.1	10.1	99.1

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
There is no reference standard for β 2-glycoprotein I. The standards are

prepared in-house and assigned arbitrary units (U/ml) during the development process. The positive, cut-off, and negative controls are also prepared in-house.

d. Detection limit:

The sample buffer was tested 30 times for each assay and result in an analytical sensitivity of 1.0 U/mL. The value for the analytical sensitivity (detection limit) was calculated as the mean of the optical densities of the sample diluent.

e. Analytical specificity:

Interference by endogenous substances: No data provided. The package insert states that icteric, lipemic, hemolyzed or bacterially contaminated samples should not be used in these assays.

f. Assay cut-off:

The cut-off value for **AESKULISA® β 2- Glyco-A**, **AESKULISA® β 2- Glyco-GM** and **AESKULISA® β 2- Glyco-Check** were determined in serum from 70 healthy donors. As a result, 15 U/mL has been defined as the cut-off value (Mean value +3SD).

2. Comparison studies:

a. Method comparison with predicate device:

One hundred and fifteen (115) sera including 104 samples from patients diagnosed with APS, SLE and other autoimmune diseases (samples were from Germany); and 11 healthy donors were tested on the **AESKULISA® β 2- Glyco-A**, **AESKULISA® β 2- Glyco-GM** and **AESKULISA® β 2- Glyco-Check** and the predicate devices. Results are summarized below.

AESKULISA® β 2- Glyco-A

		Predicate Device		
		Positive	Negative	Total
AESKU β 2GPI IgA	Positive	17	7	24
	Negative	8	83	91
	Total	25	90	115

Positive Percent Agreement 68.8% (17/25)
 Negative Percent Agreement 92.2% (83/90)
 Overall Percent Agreement 87.0% (100/115)

AESKULISA® β 2- Glyco-G

		Predicate Device		
		Positive	Negative	Total
AESKU β 2GPI IgG	Positive	30	18	48
	Negative	1	66	67
	Total	31	84	115

Positive Percent Agreement 96.8% (30/31)
 Negative Percent Agreement 78.6% (66/84)
 Overall Percent Agreement 83.5% (96/115)

AESKULISA® β 2- Glyco-M

		Predicate Device		
		Positive	Negative	Total
AESKU β 2GPI IgM	Positive	14	11	25
	Negative	1	89	90
	Total	15	100	115

Positive Percent Agreement 93.3% (14/15)
 Negative Percent Agreement 89.0% (89/100)
 Overall Percent Agreement 89.6% (103/115)

AESKULISA® β 2- Glyco-Check

		Predicate Device		
		Positive	Negative	Total
AESKU β 2GPI Check	Positive	52	6	58
	Negative	4	53	57
	Total	56	59	115

Positive Percent Agreement 92.9% (52/56)
 Negative Percent Agreement 89.8% (53/59)
 Overall Percent Agreement 91.3% (105/115)

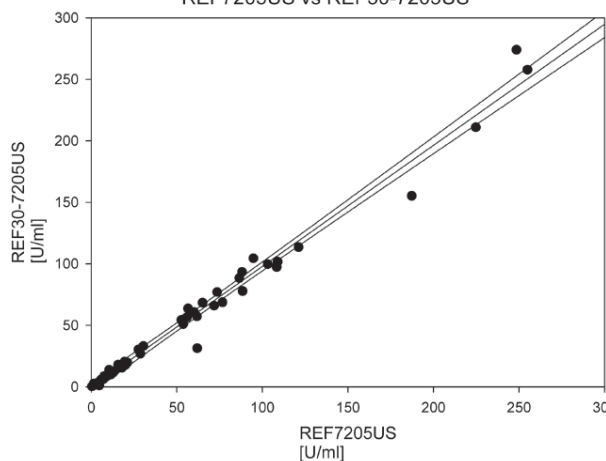
Comparison of Protocol 30-15-15 and Protocol 30-30-30:

AESKULISA β -2 Glyco A (REF7205US)

Comparability of the two protocols was assessed with 52 sera on both REF 7205US (30-15-15 minute protocol) and REF 30-7205US (30-30-30 minute protocol). The linear regression analysis is depicted in the large figure below with an $r^2 = 0.976$. Included in the sera are 26 sera close to the assay cut-off (<30 U/mL).

$Y = b[0] + b[1]X$	value	range (CI95%)
b[0]	-0.47	-5.72 / 4.78
b[1]	0.984	0.940 / 1.028
r^2	0.976	

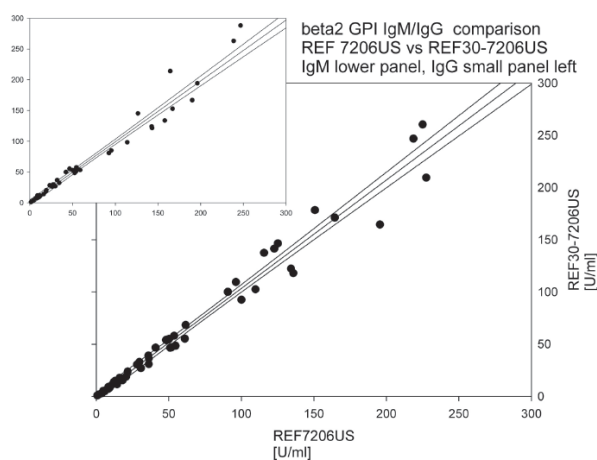
beta2 GPI IgA comparison
REF7205US vs REF30-7205US



AESKULISA β -2 Glyco GM (REF7206US)

Comparability of the two protocols was assessed with 52 IgG sera and 60 IgM sera tested on both REF 7206US (30-15-15 minute protocol) and REF 30-7206US (30-30-30 minute protocol). The linear regression analysis is depicted in the large figure below (IgM) with an $r^2 = 0.973$ and the upper left small figure (IgG) with an $r^2 = 0.968$. Included in these sera are 28/30 sera close to the cut-off (<30 U/mL).

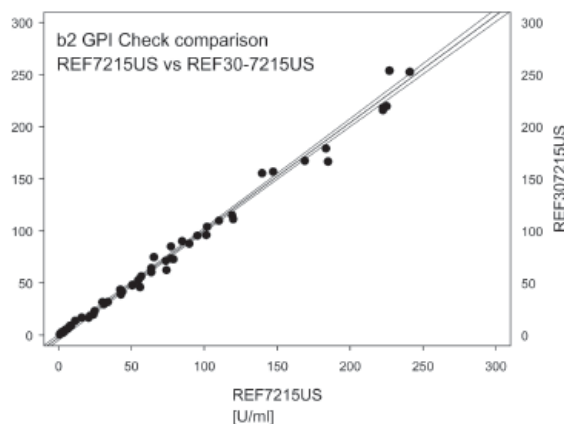
	IgG		IgM	
$Y = b[0] + b[1]X$	value	range (CI95%)	value	range (CI95%)
b[0]	-0.03	-4.87 / 4.93	-0.47	-4.75 / 3.81
b[1]	0.99	0.94 / 1.04	1.04	0.99 / 1.09
r^2	0.968		0.973	



AESKULISA β -2 Glyco Chek (REF7206US)

Comparability of the two protocols was assessed with 60 sera tested on both REF 7215US (30-15-15 minute protocol) and REF 30-7215US (30-30-30 minute protocol). The linear regression analysis is depicted in the figure below with an $r^2 = 0.990$. Included in these sera are 28 sera close to the cut-off (<30 U/mL).

$Y = b[0] + b[1]X$	value	range (CI95%)
b[0]	-2.06	-4.73 / 0.61
b[1]	1.03	1.00 / 1.06
r ²	0.990	



- b. *Matrix comparison:*
Not applicable.
3. Clinical studies:
- a. *Clinical Sensitivity and specificity:*
The clinical sensitivity and specificity study were evaluated on 79 samples from patients with the following diagnosis: 39 APS, 46 SLE, 17 SLE with secondary APS, 1 suspected APS, 1 with indeterminate connective tissue disease with APS and 11 healthy donors. Patients were tested on the AESKULISA® β 2- Glyco-A, AESKULISA® β 2- Glyco-GM and AESKULISA® β 2- Glyco-Check and the results are summarized below.

AESKULISA® β 2- Glyco-A

Disease	# Tested	# positive AESKU (%)	# positive pred dev (%)
Antiphospholipid-Syndrome	39	8 (20.5%)	11 (28.2%)
Systemic-Lupus-Erythematosus	46	7 (15.2%)	5 (10.9%)
SLE with secondary APS	17	9 (52.9%)	9 (52.9%)
suspected APS	1	0 (0%)	0 (0%)
indeterminate connective tissue disease with APS	1	0 (0%)	0 (0%)
healthy donors	11	0 (0%)	0 (0%)

AESKULISA® β 2- Glyco-M

Disease	# Tested	# positive AESKU M (%)	# positive pred dev M (%)
Antiphospholipid-Syndrome	39	18 (46.5%)	10 (25.6%)
Systemic-Lupus-Erythematosus	46	1 (2.2%)	1 (2.2%)
SLE with secondary APS	17	6 (35.3%)	4 (23.5%)
suspected APS	1	0 (0%)	0 (0%)
indeterminate connective tissue disease with APS	1	0 (0%)	0 (0%)
healthy donors	11	0 (0%)	0 (0%)

AESKULISA® β 2- Glyco-G

Disease	# Tested	# positive AESKU G (%)	# positive pred dev G (%)
Antiphospholipid-Syndrome	39	25 (64.1%)	16 (41.0%)
Systemic-Lupus-Erythematosus	46	8 (17.4%)	3 (6.5%)
SLE with secondary APS	17	13 (76.5%)	9 (52.9%)
suspected APS	1	1 (100%)	1 (100%)
indeterminate connective tissue disease with APS	1	1 (100%)	0 (0%)
healthy donors	11	0 (0%)	0 (0%)

AESKULISA® β 2- Glyco-Check

Disease	# Tested	# positive AESKU (%)	# positive pred dev (%)
Antiphospholipid-Syndrome	39	33 (84.6%)	31 (79.5%)
Systemic-Lupus-Erythematosus	46	8 (17.4%)	9 (19.6%)
SLE with secondary APS	17	15 (88.2%)	14 (82.4%)
suspected APS	1	1 (100%)	1 (100%)
indeterminate connective tissue disease with APS	1	1 (100%)	1 (100%)
healthy donors	11	0 (0%)	0 (0%)

Those numbers are in line with the ones found in literature studies.

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.