510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k052439

B. Purpose for Submission:
New Devices

C. Measurand:
AESKULISA® Glia A and AESKULISA® Glia G

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

E. Applicant:
AESKU, Inc.

F. Proprietary and Established Names:
AESKULISA® Glia-A Protocol 30-15-15 REF 7501US
AESKULISA® Glia-A Protocol 30-30-30 REF 30-7501US
AESKULISA® Glia-G Protocol 30-15-15 REF 7502US
AESKULISA® Glia-G Protocol 30-30-30 REF 30-7502US

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.5660, Multiple autoantibodies immunological test system
2. Classification:
   II
3. Product code:
   MST, Antibodies, Gliadin
4. Panel:
   Immunology 82

H. Intended Use:

1. Intended use(s):
   The AESKULISA® GLIA-A is a solid phase enzyme immunoassay for the semiquantitative and qualitative detection of IgA antibodies against gliadin in human serum. The assay is an aid in the diagnosis of celiac disease (gluten-sensitive enteropathy) and should be used in conjunction with other serological tests and clinical findings.

   The AESKULISA® GLIA-G is a solid phase enzyme immunoassay for the semiquantitative and qualitative detection of IgA antibodies against gliadin in human serum. The assay is an aid in the diagnosis of celiac disease (gluten-sensitive enteropathy) and should be used in conjunction with other serological tests 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 nm (and optional 620 nm for dual wavelength readings).
   Microplate washing device (300µL repeating or multichannel pipette or automated system).

I. Device Description:

Each device contains the following: microplate strips with breakaway (12x8) microwells coated with purified alpha-gliadin 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 (IgG or IgA) horseradish peroxidase conjugate; 3,3',5,5' tetramethylbenzidine (TMB)/H_20_2 substrate; and 1M hydrochloric acid stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   ImmuLisa™ IgA Anti-Gliadin (IgA-AGA) Antibody Test Kit
   ImmuLisa™ IgG Anti-Gliadin (IgG-AGA) Antibody Test Kit
2. Predicate 510(k) number(s):
   k964341 (IgA)
   k964344 (IgG)
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Device
Technology	ELISA	Same
Assay Format	Qualitative and semi-quantitative	Same
Stop solution	Ready to use.	Same
Platform	96 well microtiter plates	Same
Diluted sample volume required	100 µL	same

Differences		
Item	Device	Predicate
ELISA: Intended use	To aid in the diagnosis of celiac disease.	To aid in the diagnosis of celiac disease and dermatitis herpetiformis
Antigen	Purified alpha-Gliadin	Gliadin with blocked unreacted sites
Sample dilution	1:101	1:51
Controls	Positive, Negative, and Cut-off Controls	Positive and Negative Controls
Calibrators	6 levels for both GLIA IgA and IgG: 0, 3, 10, 30, 100, 300 U/mL	4 levels for each: IgA-AGA: 19, 30, 52, 104 EU/mL; and IgG-AGA: 18, 37, 50, 77 EU/mL.

Differences		
Item	Device	Predicate
Enzyme-Conjugate	Horseradish Peroxidase	Alkaline phosphatase
Sample buffer/diluent	5X concentrate: Tris buffer, NaCl, BSA	Ready to use
Wash Buffer	50X concentrate: Tris buffer, NaCl, Tween-20	Powder: reconstitute 1 vial to 1 liter
Substrate	TMB Chromogen	pNPP
Incubation times	GLIA-A and GLIA-G (REF 7501US and REF 7502US respectively): 30-15-15 minute protocol. GLIA-A and GLIA-G (REF 7501US and REF 7502US respectively): 30-30-30 minute protocol	IgG-AGA: 30-30-15 IgA-AGA: 30-30-30
Microwell Wash step	3	4
OD reading	450 nm	405 nm
Cut-off	15 U/mL	20 EU/mL

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

None referenced.

L. Test Principle:

The AESKULISA® GLIA-A and the AESKULISA® GLIA-G devices are solid phase enzyme immunoassays for the semiquantitative and qualitative detection of IgA and IgG antibodies respectively, against gliadin in human serum. The wells of a microplate are coated with purified alpha-Gliadin antigen. Antibodies specific to gliadin present in the patient sample bind to the antigen. Unbound fractions are washed off in the washing step. In the next step, the enzyme labeled second antibody (conjugate) of specific isotype (IgA or IgG) binds to the antigen-antibody complex which leads to the formation of an enzyme labeled conjugate-antibody-antigen complex. Unbound conjugate is washed off in the washing step. 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 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 calibrator (qualitative) or a standard 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 24 times on one microplate of the specific antibody type for the intra-assay study.

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. Both studies were performed on Protocol 30-15-15 incubation time. Target values for the studies were set at %CV ≤ 10%. The intra-assay %CV range for Anti-GLIA-A was from 2.8% to 5.9% and for Anti-GLIA-G was from 5.0% to 7.2%. The inter-assay %CV range for Anti-GLIA A was from 3.0% to 8.8% and for Anti-GLIA-G was 1.7% to 4.8%. All the ranges were within the target values.

	Intra-assay Variation		
Anti-GLIA-A	Sample 1	Sample 2	Sample 3
CV (%)	4.7	5.9	2.8
Mean (U/mL)	13.4	32.2	50.4
Anti-GLIA-G			
CV (%)	5.0	7.2	5.9
Mean (U/mL)	12.4	37.0	88.0

	Inter-assay Variation		
Anti-GLIA-A	Sample 1	Sample 2	Sample 3
CV (%)	8.8	4.6	3.0
Mean (U/mL)	14.6	29.0	46.2
Anti-GLIA-G			
CV (%)	4.8	4.5	1.7
Mean (U/mL)	10.6	29.3	66.4

b. Linearity/assay reportable range:

Study design: Two samples known to contain different levels of Anti-GLIA IgA and another two samples known to contain different levels of Anti-GLIA IgG 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 Anti-GLIA-A assay had a recovery range of 92.1% to 109.6%. The Anti-GLIA-G assay had a recovery range of 94.1% to 108.8% (see tables below).

Anti-GLIA A

Sample	Dilution	Measured (U/mL)	Expected (U/mL)	Recovery (%)
1	1/100	102.5	100.9	101.6
	1/200	52.4	50.5	103.8
	1/400	26.3	25.2	104.4
	1/800	13.0	12.6	103.2
2	1/100	53.7	58.3	92.1
	1/200	29.8	29.2	102.1
	1/400	16.0	14.6	109.6
	1/800	7.4	7.3	101.4

Anti-GLIA-G

Sample	Dilution	Measured (U/mL)	Expected (U/mL)	Recovery (%)
1	1/100	117.6	118.0	99.7
	1/200	59.5	59.0	100.8
	1/400	30.2	29.5	102.4
	1/800	14.8	14.8	100.0
2	1/100	85.8	91.0	94.3
	1/200	42.8	45.5	94.1
	1/400	22.3	22.8	97.8
	1/800	12.4	11.4	108.8

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The standards are prepared in-house and arbitrary units are assigned during the development process. The positive, cut-off, and negative controls are also prepared in-house.

d. *Detection limit:*
The sample buffer was diluted according to the directions for use and measured 30 times for each assay. The value for the analytical sensitivity (detection limit) was calculated as the mean of the optical densities of the sample diluent. The analytical sensitivity was 1.0 U/mL.

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 of Anti-GLIA-A (≤15 U/mL) and Anti-GLIA-G (≤15 U/mL) levels were established in serum from 76 healthy donors. For the Anti-GLIA-A two of the 76 samples (2.6%) were above the cut-off at 17 and 18 U/mL. For the Anti-GLIA-G assay one of the 76 samples (1.3%) was above the cut-off at 16 U/mL. This study showed that 97.4% and 98.7% were below the Anti-GLIA-A and Anti-GLIA-G cut-off respectively.

2. Comparison studies:

a. *Method comparison with predicate device:*
Comparison was determined against the predicate Anti-Gliadin IgA and Anti-Gliadin IgG EIA kits using 195 sera clinically confirmed Celiac Disease, Crohn's Disease, Ulcerative Colitis, other related and autoimmune diseases; and 9 healthy donor sera. Results are summarized below.

Anti-GLIA-A

		ImmuLisa Anti-Gliadin IgA		
		Positive	Negative	Total
AESKULISA GLIA-A	Positive	14	20	34
	Negative	2	168	170
	Total	16	188	204

Overall percent Agreement: 89.2% (182/204)
Positive percent agreement: 87.5% (14/16)
Negative percent agreement: 89.4 % (168/188)

The twenty ImmuLisa negative and AESKULISA positive discrepant samples consisted of 11 Celiac Disease, 5 Gluten Free Diet Celiac Disease, 1 Crohn's Disease, 2 RA, and 1 SLE. The two AESKULISA negative and ImmuLisa positive discrepant samples consisted of 1 Celiac Disease and 1 RA.

Anti-GLIA-G

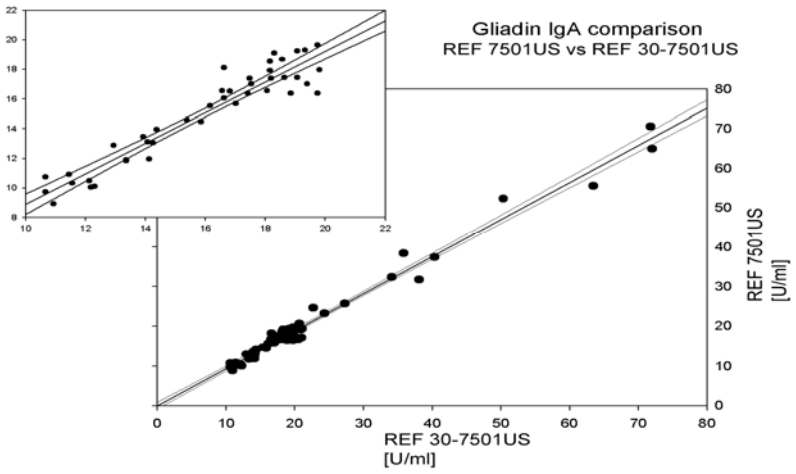
		ImmuLisa Anti-Gliadin IgG		
		Positive	Negative	Total
AESKULISA GLIA G	Positive	42	20	62
	Negative	33	109	142
	Total	75	129	204

Overall percent Agreement: 74.0% (151/204)
Positive percent agreement: 56.0% (42/75)
Negative percent agreement: 84.5% (109/129)

The low positive agreement observed for Anti-GLIA-G was due to discordant results for 33 of the 75 samples that were positive with the predicate ImmuLisa device. Based on the clinical diagnosis, 30 of these 33 samples which included 13 GFD, 7 SLE, 6 RA, 2 Crohn's Disease, 1 Ulcerative Colitis, and 1 Helminthiasis should be negative for anti-gliadin antibody. The 20 discrepant ImmuLisa negative and AESKULISA positive samples were from 10 Celiac disease IgA deficient, 5 Crohn's Disease, 3 Celiac Disease, 1 GFD, and 1 SLE.

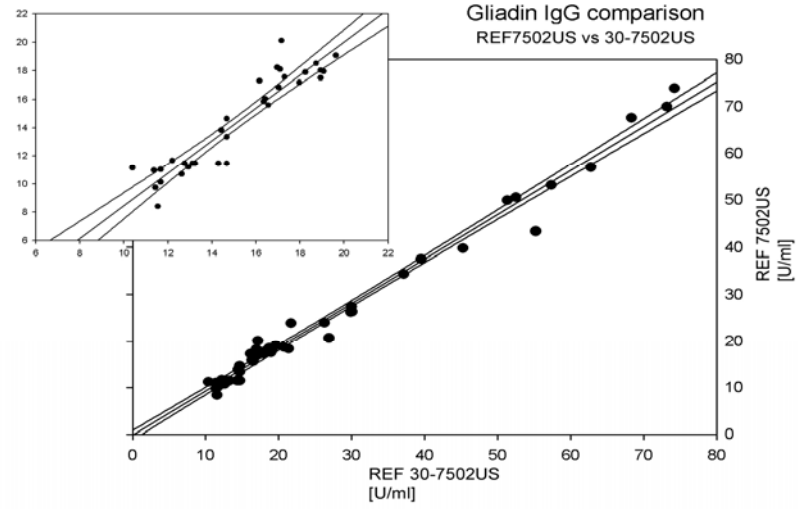
Comparison of Protocol 30-15-15 and Protocol 30-30-30:

Anti-GLIA-A:
Comparability of the two protocols was assessed with 58 sera on both REF 7501US (30-15-15 minute protocol) and REF 307501US (30-30-30 minute protocol). The linear regression analysis is depicted in the large figure below with an $r^2 = 0.99$ and the upper left small figure shows selected 42 results close to the assay 15 U/mL cut-off (10-20 U/mL range).



Anti-GLIA-G:

Comparability of the two protocols was assessed with 52 sera on both REF 7502US (30-15-15 minute protocol) and REF 307502US (30-30-30 minute protocol). The linear regression analysis is depicted in the large figure below with an $r^2 = 0.99$ and the upper left small figure shows selected 33 results close to the assay 15 U/mL cut-off (10-20U/mL range).



b. Matrix comparison:

Not applicable.

3. Clinical studies:

a. Clinical Sensitivity and specificity:

The clinical sensitivity and specificity study were evaluated on 204 clinically defined samples from patients with the following diagnosis: 29 Celiac Disease, 42 Gluten-Free Diet Celiac Disease, 26 IgA deficient Celiac Disease, 25 Crohn's Disease, 6 Ulcerative Colitis, 1 Helminthiasis, 1 Lactose Intolerance, 1 Mixed Connective Tissue Disease, 33 Chronic/Reactive Arthritis, 2 Wegener's Granulomatosis, 29 SLE, and 9 healthy donors. The

following table summarizes the results of both assays for these patient groups.

N= 204		Anti-Glia-A results		Anti-Glia-G results	
Patient Group	n	AESKU positive	ImmuLisa positive	AESKU positive	ImmuLisa positive
Celiac Disease	29	17	7	24	23
Celiac Disease (gluten free diet)	42	5	0	6	18
Celiac Disease (IgA deficient)	26	0	0	19	10
Crohn's Disease	25	5	4	8	5
Ulcerative Colitis	6	2	2	1	2
Helminthiasis	1	0	0	0	1
Lactose Intolerance	1	1	1	1	1
Mixed connective tissue disease	1	0	0	0	0
Arthritis (chronic/reactive)	33	2	1	0	6
Wegener's Granulomatosis	2	0	0	0	0
SLE	29	2	1	2	8
healthy donors	9	0	0	1	1

The sensitivity and specificity for Anti-GLIA-A were 58.6% (17/29) and 88.6% (132/149) respectively when the IgA deficient Celiac Disease patients were excluded from the calculation. The sensitivity and specificity for Anti-GLIA-G were 78.1% (43/55) and 87.2% (130/1149) respectively. Study results are summarized in the tables below.

Anti- GLIA-A:

		Diagnosis		
		Positive	Negative	Total
AESKULISA GLIA-A	Positive	17	17	34
	Negative	12	132	144
	Total	29	149	178

Sensitivity: 58.6 % (17/29)
Specificity: 88.6 % (132/149)

		Diagnosis		
		Positive	Negative	Total
ImmuLisa Anti-Gliadin IgA	Positive	7	9	16
	Negative	22	140	162
	Total	29	149	178

Sensitivity: 24.1 % (7/29)
Specificity: 94.0 % (140/149)

Anti-GLIA-G

		Diagnosis		
		Positive	Negative	Total
AESKULISA GLIA G	Positive	43	19	62
	Negative	12	130	142
	Total	55	149	204

Sensitivity: 78.1% (43/55)
Specificity: 87.2% (130/149)

		Diagnosis		
		Positive	Negative	Total
ImmuLisa Anti-Gliadin IgG	Positive	33	42	75
	Negative	22	107	129
	Total	55	149	204

Sensitivity: 60.0% (33/55)
Specificity: 71.8% (107/149)

b. Other clinical supportive data (when a. and b. are 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.