

**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 IgG 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)/H₂O₂ 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 \leq 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.

| Anti-GLIA-A | Intra-assay Variation | | |
|--------------------|------------------------------|----------|----------|
| | 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 |

| Anti-GLIA-A | Inter-assay Variation | | |
|--------------------|------------------------------|----------|----------|
| | 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 |

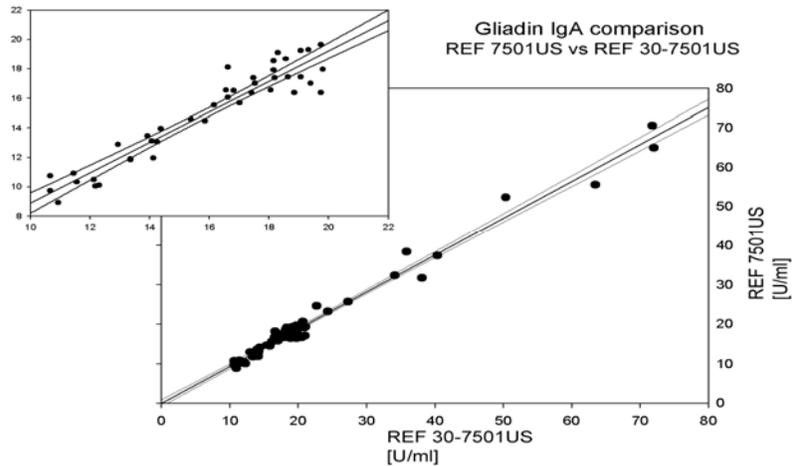
Overall percent Agreement: 74.0% (151/204)
 Positive percent agreement: 56.0% (42/75)
 Negative percent agreement: 84.5% (109/129)

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:

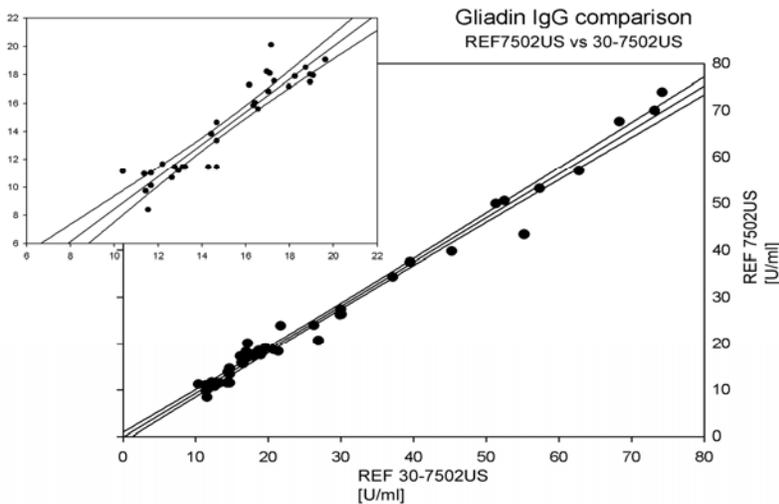
Anti-GLIA-A:

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 Patient Group | n | Anti-Glia-A results | | Anti-Glia-G results | |
|-----------------------------------|----|---------------------|-------------------|---------------------|-------------------|
| | | 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.