

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

A. 510(k) Number:

K032469

B. Analyte:

Anti-U1-70, anti-snRNP-C, anti-Sm, anti-SS-A, anti-SS-B, anti-Scl-70, anti-Cenp-B and anti-Jo-1 antibodies

C. Type of Test:

Qualitative and semi-quantitative, EIA

D. Applicant:

AESKU, Inc.

E. Proprietary and Established Names:

AESKULISA[®] ANA 8Pro Test Kit

AESKULISA[®] ENA 6Pro Test Kit

AESKULISA[®] U1-70 Test Kit

AESKULISA[®] snRNP-C Test Kit

AESKULISA[®] Sm Test Kit

AESKULISA[®] SS-A Test Kit

AESKULISA[®] SS-B Test Kit

AESKULISA[®] Cenp-B Test Kit

AESKULISA[®] Scl-70 Test Kit

AESKULISA[®] Jo-1 Test Kit

F. Regulatory Information:

1. Regulation section:

21 CFR 866.5100, Antinuclear antibody immunological test system

2. Classification:

Class II

3. Product Code:

LLL, Extractable antinuclear antibody, antigen and control

LJM, Antinuclear antibody (enzyme-labeled), antigen and control

4. Panel:

Immunology (82)

G. Intended Use:

AESKULISA ANA 8Pro is a solid phase enzyme immunoassay for the separate qualitative detection of IgG antibodies against eight cellular and nuclear antigens in human serum. The wells are separately coated with recombinant 70 kDa U1 snRNP, SS-B, SS-A 52 kDa, Scl 70, centromere protein B (CenpB), Jo-1 and highly purified native human snRNP/Sm, Sm and SS-A 60 kDa. The assay is an aid in the differential diagnosis of systemic rheumatic diseases and should be used in conjunction with other serological tests and clinical findings.

AESKULISA ENA 6Pro is a solid phase enzyme immunoassay for the separate semi-quantitative detection of IgG antibodies against six cellular and nuclear antigens in human serum. The wells are coated with recombinant SS-B, SS-A 52 kDa, Scl 70,

Jo-1 and highly purified native human snRNP/Sm, Sm and SS-A 60 kDa. The assay is an aid in the differential diagnosis of systemic rheumatic diseases and should be used in conjunction with other serological tests and clinical findings.

AESKULISA U1-70 is a solid phase enzyme immunoassay employing recombinant human 70 kDa protein of the U1-snRNP complex for the semi-quantitative and qualitative detection of antibodies against the 70 kDa U1-RNP in human serum. The assay is an aid in the diagnosis of mixed connective tissue diseases (MCTD) and systemic lupus erythematosus (SLE) and should be used in conjunction with other serological tests and clinical findings.

AESKULISA snRNP-C is a solid phase enzyme immunoassay for the qualitative and semi-quantitative detection of antibodies against the snRNP complex in human serum. The assay employs native human U1-snRNP complex purified from the cell-line HeLa. The U1-snRNP complex comprises of the Smith antigen (Sm) and RNPs, the 70kDa U1-specific protein plus protein A and C. The assay is an aid for the diagnosis of mixed connective tissue diseases (MCTD) and systemic lupus erythematosus (SLE) and should be used in conjunction with other serological tests and clinical findings.

AESKULISA Sm is a solid phase enzyme immunoassay with purified native Smith antigen (Sm) from human eukaryotic cells (HeLa) for the qualitative and semiquantitative detection of antibodies against Sm in human serum. Anti-Sm antibodies recognize specific conformational epitopes only accessible on native human Sm. The assay is an aid in the differential diagnosis of systemic lupus erythematosus (SLE) and should be used in conjunction with other serological tests and clinical findings.

AESKULISA SS-A is a solid phase enzyme immunoassay for the semi-quantitative and qualitative detection of antibodies against Ro/ SS-A in human serum. The assay employs human Ro/SS-A antigen composed of purified native 60kDa and recombinant human 52 kDa Ro/SS-A protein. Anti-SS-A antibodies preferentially react with the native 60kDa molecule where as most antibodies to the 52 kDa protein prefer the denatured molecule. The assay is an aid in the diagnosis of Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE) and should be used in conjunction with other serological tests and clinical findings.

AESKULISA SS-B is a solid phase enzyme immunoassay employing human recombinant La-antigen/ SS-B for the qualitative and semi-quantitative detection of antibodies against La-antigen / SS-B in human serum. The assay is an aid in the diagnosis of Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE) and should be used in conjunction with other serological tests and clinical findings.

AESKULISA Scl-70 is a solid phase enzyme immunoassay with human recombinant 70 kDa fragment of DNA topoisomerase I for the qualitative and semi-quantitative detection of antibodies against Scl-70 (70 kDa scleroderma antigen) in human serum.

The assay is an aid in the differential diagnosis of systemic sclerosis and should be used in conjunction with other serological tests and clinical findings.

AESKULISA CENP-B is a solid phase enzyme immunoassay employing purified recombinant human 80 kDa centromere protein B (Cenp-B) for the qualitative and semi-quantitative detection of IgG antibodies against Cenp-B in human serum. The assay serves as an aid in the diagnosis of systemic sclerosis and CREST syndrome and should be used in conjunction with other serological tests and clinical findings.

AESKULISA Jo-1 is a solid phase enzyme immunoassay with recombinant human histidyl-tRNA-synthetase (HRS) for the semi-quantitative and qualitative detection of antibodies against Jo-1 in human serum. The assay is an aid in the diagnosis of polymyositis and dermatomyositis and should be used in conjunction with other serological tests and clinical findings.

1. Indication(s) for use:
Same as Intended Use.
2. Special condition for use statement(s):
For prescription use only
3. Special instrument Requirements:
None

H. Device Description:

Each device consists of antigen coated 96 well microtiter plate, horseradish peroxidase conjugated anti-human IgG, TMB substrate, cut-off control, positive control, negative control, calibrators, washing buffer concentrate (5x), sample buffer concentrate (5x) and stop solution. The calibrators and the positive and cut-off controls are diluted human sera. The negative control is BSA/PBS.

I. Substantial Equivalence Information:

1. Predicate device name(s):
Varelisa ANA Profile EIA kit
2. Predicate K number(s):
K951205
3. Comparison with predicate:

| DEVICE | PREDICATE |
|--|---|
| A. Similarities | |
| Intended Use. Determination of antibodies against nucleic antigens (U1-70, snRNP-complex, Sm, SS-A, SS-B, Scl-70, Cenp-B and Jo-1) in human serum to aid in the diagnosis of rheumatological disorders like systemic lupus erythematosus (SLE), rheumatoid arthritis or Sjögren's syndrome. | Determination of antinuclear antibodies (U1-snRNP, Sm, RNP-Sm, SSA, SSB, Scl-70, Centromere, and Jo-1 antibodies) in human serum or plasma to aid in the diagnosis of rheumatological disorders |
| Assay type – ELISA | Same |
| Antigens - Same | Same |

| | |
|---|------------------|
| Reporter conjugate - Horseradish peroxidase | Same |
| Substrate - TMB | Same |
| B. Differences | |
| Assay Format – Qualitative and semi-quantitative | Qualitative |
| Sample Type – Serum | Serum and plasma |
| Incubation Time – 30/15/15 minutes | 30/30/10 minutes |

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

None referenced.

K. Test Principle:

Purified native or recombinant autoantigens are coated separately in wells of microtiter plates. Diluted patient serum is added to the microtiter well and antibody specific to the antigen will bind to the immobilized antigen if present. Unbound sample is washed away and an enzyme labeled anti-human IgG antibody is added to each well and bind to the antigen/antibody complex. After washing away any unbound enzyme conjugate, the chromogenic substrate is added. The color intensity in the wells is proportional to the amount of each autoantibody in the sample.

L. Performance Characteristics (if/when applicable):1. Analytical performance:*a. Precision/Reproducibility:*

AESKULISA ANA 8Pro – Intra-assay reproducibility was determined by assaying 8 specimens 9 times on one plate and for inter-assay reproducibility, 8 specimens were assayed 9 times for 4 days. Results were summarized below.

| Intra-Assay | | | Inter-Assay | | |
|-------------|---------------|--------|-------------|---------------|--------|
| ANA 8 Pro | Mean OD Ratio | CV (%) | ANA 8 Pro | Mean OD Ratio | CV (%) |
| UIRNP | 3.1 | 0.8 | UIRNP | 3.2 | 0.8 |
| Sn RNP | 2.1 | 0.4 | Sn RNP | 4.2 | 1.0 |
| Sm | 1.8 | 1.0 | Sm | 4.1 | 1.1 |
| SSA | 3.5 | 1.2 | SSA | 3.9 | 0.9 |
| SSB | 2.8 | 1.0 | SSB | 1.5 | 0.7 |
| Scl-70 | 1.2 | 1.5 | Scl-70 | 1.4 | 1.9 |
| CenpB | 1.5 | 1.1 | CenpB | 2.5 | 1.2 |
| Jo-1 | 1.0 | 1.2 | Jo-1 | 2.4 | 0.8 |

AESKULISA ENA 6Pro - Intra-assay reproducibility was determined by assaying 6 specimens 12 times on one plate and for inter-assay reproducibility, 6 specimens were assayed 12 times for 3 days. Results were summarized below.

| Intra-Assay | | | Inter-Assay | | |
|-------------|-----------|--------|-------------|-----------|--------|
| ANA 6 Pro | Mean U/mL | CV (%) | ANA 6 Pro | Mean U/mL | CV (%) |
| SnRNP | 20.0 | 3.1 | Sn RNP | 21.7 | 2.8 |
| Sm | 51.6 | 1.7 | Sm | 54.6 | 3.9 |
| SSA | 45.7 | 1.5 | SSA | 44.2 | 1.3 |
| SSB | 124.8 | 2.6 | SSB | 123.3 | 2.4 |
| Scl-70 | 19.3 | 3.1 | Scl-70 | 22.4 | 3.7 |
| Jo-1 | 65.5 | 4.2 | Jo-1 | 68.4 | 1.7 |

For the individual devices, intra-assay reproducibility was determined by assaying 3 specimens (high, medium and low titer) 24 times on one plate and for inter-assay reproducibility, 3 specimens were assayed 18 times for 3 days. Results were summarized below.

AESKULISA U1-70

| Sample | Intra-Assay | | Inter-Assay | |
|--------|-------------|--------|-------------|--------|
| | Mean U/mL | CV (%) | Mean U/mL | CV (%) |
| 1 | 151 | 2.3 | 148 | 2.6 |
| 2 | 72 | 3.6 | 70 | 4.1 |
| 3 | 26 | 4.5 | 24 | 3.9 |

AESKULISA snRNP

| Sample | Intra-Assay | | Inter-Assay | |
|--------|-------------|--------|-------------|--------|
| | Mean U/mL | CV (%) | Mean U/mL | CV (%) |
| 1 | 19.1 | 1.0 | 19.1 | 1.6 |
| 2 | 50.5 | 3.3 | 51.3 | 3.7 |
| 3 | 85.1 | 6.2 | 83.6 | 6.4 |

AESKULISA Sm

| Sample | Intra-Assay | | Inter-Assay | |
|--------|-------------|--------|-------------|--------|
| | Mean U/mL | CV (%) | Mean U/mL | CV (%) |
| 1 | 64.2 | 4.6 | 50.4 | 3.3 |
| 2 | 41.5 | 3.3 | 29.6 | 2.3 |
| 3 | 20.4 | 1.9 | 12.7 | 1.4 |

AESKULISA SS-A

| Sample | Intra-Assay | | Inter-Assay | |
|--------|-------------|--------|-------------|--------|
| | Mean U/mL | CV (%) | Mean U/mL | CV (%) |
| 1 | 78.2 | 8.1 | 102.4 | 3.8 |
| 2 | 44.3 | 2.8 | 62.5 | 5.5 |
| 3 | 22.9 | 2.0 | 26.3 | 1.9 |

AESKULISA SS-B

| Sample | Intra-Assay | | Inter-Assay | |
|--------|-------------|--------|-------------|--------|
| | Mean U/mL | CV (%) | Mean U/mL | CV (%) |
| 1 | 20.7 | 1.8 | 21.6 | 1.8 |
| 2 | 55.5 | 3.1 | 58.9 | 4.2 |
| 3 | 87.0 | 6.0 | 88.5 | 5.2 |

AESKULISA Scl-70

| Sample | Intra-Assay | | Inter-Assay | |
|--------|-------------|--------|-------------|--------|
| | Mean U/mL | CV (%) | Mean U/mL | CV (%) |
| 1 | 66.5 | 7.7 | 57.5 | 4.4 |
| 2 | 37.7 | 4.3 | 52.2 | 4.2 |
| 3 | 19.4 | 1.9 | 17.7 | 1.3 |

AESKULISA CenpB

| Sample | Intra-Assay | | Inter-Assay | |
|--------|-------------|--------|-------------|--------|
| | Mean U/mL | CV (%) | Mean U/mL | CV (%) |
| 1 | 265 | 1.5 | 261 | 1.4 |
| 2 | 122 | 2.3 | 119 | 2.5 |
| 3 | 44 | 4.1 | 46 | 3.8 |

AESKULISA Jo-1

| Sample | Intra-Assay | | Inter-Assay | |
|--------|-------------|--------|-------------|--------|
| | Mean U/mL | CV (%) | Mean U/mL | CV (%) |
| 1 | 15.3 | 1.0 | 15.3 | 1.0 |
| 2 | 63.3 | 7.5 | 63.3 | 7.5 |
| 3 | 112.9 | 9.3 | 112.9 | 9.3 |

Originally the sponsor had submitted individual devices for SS-A subtypes (SS-A60 and SS-A52). Since devices for autoantibody subtypes have never been cleared by the agency due to lack of information to support their use in differential diagnosis and patient management, the sponsor was asked to withdraw the SS-A60 and SS-A52 from the 510(k) submission and the sponsor agreed.

b. Linearity/assay reportable range:

AESKULISA ANA-8Pro - A medium and a high titer Scl-70 serum were diluted to 1:100, 1:200, 1:400 and 1:800. Percent recovery for the high sample ranged from 93.4% to 100% and for the medium sample, 94.1% to 95.4%.

AESKULISA ENA 6Pro - A medium and a high titer U1-70 serum were similarly diluted and tested as described for the AESKULISA ANA 8Pro. Percent recovery for the high sample ranged from 97.3% to 99.3% and for the medium sample, 93.5% to 98.7%.

For the individual devices, a medium and a high titer antigen specific serum sample were similarly diluted and tested as described for the AESKULISA ANA 8Pro. Percent recoveries for the high and medium samples were summarized below.

| Analyte | High Titer Sample % Recovery | Medium Titer Sample % Recovery |
|---------|---------------------------------|-----------------------------------|
| snRNP | 97.0 - 100 | 91.2 - 105.3 |
| Sm | 97.0 - 100 | 91.2 - 105.3 |
| SS-A | 96.6 - 102 | 92.2 - 105 |
| SS-B | 94.3 - 98.0 | 90.0 - 106.6 |
| Scl-70 | 90.7 - 101.2 | 93.3 - 99.8 |
| CenpB | 98.0 - 100.3 | 98.8 - 101.5 |
| Jo-1 | 94.3 - 97.1 | 91.3 - 98.3 |

c. Traceability (controls, calibrators, or method):

Controls and calibrators are traceable to CDC reference ANA sera. A negative, a positive and a cut-off control are included in each device. The calibrators are supplied in 4 or 6 concentrations depending on the device.

d. *Detection limit (functional sensitivity):*

Not applicable.

e. *Analytical specificity:*

The devices were also tested for cross-reactive with other autoantibodies namely anti-gliadin, anti-cardiolipin, anti-thyroid peroxidase, anti-thyroglobulin and anti-proteinase 3. All results were negative. Interference testing with hemolyzed, lipemic or icteric samples were not performed but the package insert specified that these types of samples should not be used.

f. *Assay cut-off:*

To determine the cut-off value, serial dilutions (1:3) of an antibody specific patient serum are tested in triplicates. The OD₄₅₀ value for each dilution is plotted (linear-log with a 4 parameter fitting) against the dilution factor to determine the linear range. The dilution in the linear range with an OD value of ~2 is defined as Calibrator F and assigned an arbitrary unit of 300 U/mL. Calibrator F is diluted and calibrated to the respective CDC ANA reference serum (the antibody concentrations of the CDC ANA reference sera were determined by indirect immunofluorescence on Hep2 cells and are expressed as antibody titers). The selected cut-off is equivalent to an OD of 0.5 to 0.6 of that of the reference serum. To validate the cut-off of the 9 analytes in the AESKULISA ANA 8 Pro, 40 healthy subjects from two German hospitals were tested. Results were summarized below.

| | OD Ratio Values | | | | | | | | |
|-------------|-----------------|-------|-------|-------|--------|-------|-------|--------|-------|
| | U1-70 | snRNP | Sm | SSA | SSA-52 | SSB | CenpB | Scl-70 | Jo11 |
| Mean | 0.261 | 0.276 | 0.244 | 0.246 | 0.166 | 0.245 | 0.254 | 0.310 | 0.231 |
| SD | 0.019 | 0.048 | 0.038 | 0.038 | 0.033 | 0.041 | 0.049 | 0.051 | 0.044 |
| Mean + 3 SD | 0.409 | 0.419 | 0.357 | 0.359 | 0.264 | 0.367 | 0.402 | 0.464 | 0.363 |

The other devices in the submission were similarly tested. The cut-off value for semi-quantitative determination is >15 U/mL for all assays.

2. Comparison studies:

a. *Method comparison with predicate device:*

In the original submission, only 57 clinically defined patient samples were tested on the predicated device, the new device and IFA. These samples were from two German hospitals and included 16 SLE, 1 SLE/CREST Overlap, 5 Progressive Systemic Sclerosis, 6 Sjogren's Syndrome, 2 SHARP Syndrome, 3 CREST Syndrome, 3 anti-Jo-1, 5 Polymyositis-Sjogren's Overlap Syndrome, 1 Polymyositis, 7 Rheumatoid Arthritis and 8 sera for other diseases (HLA-B27 disease, suspected CREST or PSS, MCTD, psoriasis arthritis, fever

of unknown origin and with elevated ANA after tick bite). This limited dataset is insufficient to support substantial equivalence claim for the individual autoantibody devices. The sponsor was asked to provide data on additional positive samples and the sponsor supplemented data from 16 U1-70, 42 snRNP-C, 45 Sm, 28 SS-A, 45 SS-B, 47 Scl-70, 46 CenpB and 14 Jo-1 patient samples. The table below summarizes the number of samples tested and % agreement with the predicate device:

| Device | # samples | % Total Agreement | % Positive Agreement | % Negative Agreement |
|---------|-----------|-------------------|----------------------|----------------------|
| U1-70 | 73 | 95.8% | 87.0% | 100% |
| snRNP-C | 99 | 97.0% | 96.2% | 97.9% |
| Sm | 102 | 95.1% | 96% | 94.2% |
| SS-A | 86 | 84.7% | 80.3% | 95.8% |
| SS-B | 102 | 91.1% | 84.7% | 100% |
| Scl-70 | 104 | 97.1% % | 100% | 94.3% |
| CenpB | 103 | 97.1% | 96.1% | 98.1% |
| Jo-1 | 71 | 100% | 100% | 100% |

But when the analytes were combined together as in the AESKULISA ANA 8Pro, the overall agreement with the predicate device was only 54.4% based on comparative results of the original 57 patient samples. The majority of the discrepancy was due to positive reactivity to SS-A when tested with the predicate device and when compared to the clinical diagnosis, would be considered in some cases false positives and in others nonspecific.

b. Matrix comparison:

Not applicable.

3. Clinical studies:

a. Clinical sensitivity:

The clinical sensitivity was 100% for all of the individual autoantibody devices except AESKULISA SS-A which was 98.8%.

b. Clinical specificity:

The clinical specificity was 100% for all of the individual autoantibody devices.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

Not performed. The sponsor referred the expected values in published literature.

M. Conclusion:

Based on the review of information provided in this 510 (k), the analytical performance of the AESKULISA ANA 8Pro which includes analytes of the individual autoantibody devices (AESKULISA U1-70, AESKULISA snRNP-C, AESKULISA Sm, AESKULISA SS-A, AESKULISA SS-B, AESKULISA Scl-70, AESKULISA CenpB and AESKULISA Jo-1) and the ENA panel, AESKULISA ENA

6Pro correlated with the performance of the Varelisa ANA Profile EIA kit and therefore, demonstrate that the new device is substantially equivalent to the marketed device.