510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k051492

B. Purpose for Submission:
New Device

C. Measurand:
Anti-*Saccharomyces cerevisiae* antibodies (ASCA), IgA and IgG

D. Type of Test:
Solid phase ELISA

E. Applicant:
AESKU Diagnostics

F. Proprietary and Established Names:
AESKULISA® ASCA-A, REF 7507, REF 30-7507
AESKULISA® ASCA-G REF 7508, REF 30-7508

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.5785

2. Classification:
   Class II

3. Product code:
   NBT, Anti-*Saccharomyces cerevisiae* (*S. cerevisiae*) antibody (ASCA) test systems.

4. Panel:
   Immunology 82

H. Intended Use:

1. Intended use(s):
   The **AESKULISA® ASCA-A** is a solid phase enzyme immunoassay (ELISA) employing purified mannan for the semi-quantitative and qualitative detection of IgA anti-*Saccharomyces cerevisiae* antibodies (ASCA) in human serum. ASCA recognize specifically mannan, a component of the outer cell wall of yeast. The assay is an aid in the diagnosis of Crohn's disease and should be used in conjunction with other serological tests and clinical findings. The AESKULISA® ASCA-A kit should not be used as a screening test for ASCA, since some Crohn's disease patients do not have ASCA IgA antibodies. The AESKULISA® ASCA-A kit should be used to compliment, but not to replace or to substitute for ASCA IgG antibody testing.

   The **AESKULISA® ASCA-G** is a solid phase enzyme immunoassay (ELISA) employing purified mannan for the semi-quantitative and qualitative detection of IgA anti-*Saccharomyces cerevisiae* antibodies (ASCA) in human serum. ASCA recognize specifically mannan, a component of the outer cell wall of yeast. The assay is an aid in the diagnosis of Crohn's disease and should be used in conjunction with other serological tests and clinical findings.

2. <u>Indication(s) for use:</u>
   Same as Intended Use.
3. <u>Special conditions for use statement(s):</u>
   For prescription use only.
4. <u>Special instrument requirements:</u>
   Microplate reader capable of measuring OD 450 nm.

I. Device Description:

The AESKULISA® ASCA-A and AESKULISA® ASCA-G assays each consist of a microplate coated with *S. cerevisiae* purified mannan antigen, positive, negative and cut-off control materials, 6 calibrators, peroxidase-conjugated goat anti-human immunoglobulins (IgA or IgG, respectively), enzyme (TMB) substrate, stop solution, wash and sample buffers, frame and instructions for use.

J. Substantial Equivalence Information:

1. <u>Predicate device name(s):</u>
   INOVA QUANTA Lite ASCA (*S. cerevisiae*) IgA and
   INOVA QUANTA Lite ASCA (*S. cerevisiae*) IgG
2. <u>Predicate 510(k) number(s):</u>
   k000733 and k000732
3. <u>Comparison with predicate:</u>

Similarities		
Item	New Device	Predicate Device
Intended use & Indications for Use	Aid in the diagnosis of Crohn's disease	same
Technology	Indirect, non-competitive ELISA	same
Antigen	purified mannan	same
Controls	Positive, negative and cut-off controls, calibrators	High and Low Positive, negative and cut-off controls

Differences		
Item	New Device	Predicate Device
Calibrators	6 levels (0, 3, 10, 30, 100, and 300 U/mL)	2 levels (High positive and Low positive)
Incubation times	30-15-15-5; 30-30-30-5 (min)	30-30-30 (min)
Read time	within 30 min of stopping reaction	within 1 hr of stopping reaction
Cut-off	15 U/mL	20 U/mL
Equivocal range	none	20.1-24.9 U/mL
Assay Format	Semi-quantitative and Qualitative	Semi-quantitative only

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

Class II Special Control Guidance Document for Anti-*Saccharomyces cerevisiae* antibody (ASCA) Premarket Notifications.

L. Test Principle:

The AESKULISA® ASCA-A and G kits are both solid phase enzyme immunoassays for the semi-quantitative and qualitative detection of IgA and IgG antibodies, respectively, against anti-*S. cerevisiae* antibodies (ASCA) in human serum. The wells of the microplate are coated with purified mannan, a component of the outer cell wall of yeast, which ASCA recognizes. Antibodies specific to ASCA present in the patient sera bind to the antigen. In a second step, the enzyme-labeled secondary antibody (conjugate) of specific isotype (IgA or IgG, respectively), bind to the antigen-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 is proportional to the initial concentration fo 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 (semi-quantitative).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   For each kit (ASCA-A and ASCA-G), three samples (high, medium, and "near the cut-off"/low) were assayed 18 times on three different microplates on different days for the inter-assay study. The same samples were assayed 24 times on a microplate for the intra-assay variation study. Both studies were performed using the 30-15-15 kits. The acceptance %CV criteria for both the inter- and intra-assay studies were set at $\leq 10\%$.

	Inter-assay Precision		
ASCA-A	Sample 1	Sample 2	Sample 3
CV (%)	5.56	5.05	2.88
Mean (U/mL)	18.18	52.45	112.56
ASCA-G			
CV (%)	7.94	5.97	2.89
Mean (U/mL)	15.5	36.1	69.82

	Intra-assay Precision		
ASCA-A	Sample 1	Sample 2	Sample 3
CV (%)	4.44	4.35	3.42
Mean (U/mL)	19.03	48.45	115.47
ASCA-G			
CV (%)	3.94	9.15	3.12
Mean (U/mL)	16.41	33.5	74.12

   b. *Linearity/assay reportable range:*

   i. Study design: Two samples known to contain different levels of Anti-ASCA IgA and another two samples known to contain different levels of Anti-ASCA 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, providing a (calculated) range of 15.6-125 U/mL and 36.9-295 U/mL for ASCA-A and 38.2-305.4 U/mL and 13.3-106 U/mL for ASCA-G.

    ii. Results/Acceptance criteria: The Anti-ASCA A assay had a recovery range of 91.3% to 106.9%. The Anti-ASCA G had a recovery range of 90.1% to 108.1%. Based on this study, the dynamic range of the assay is 1 to 300 U/mL.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):* There is no reference standard available. The standards are prepared in-house and values are assigned during the development process. Positive and negative controls are prepared in-house.

d. *Detection limit:* The sample diluent was diluted according to the directions for use and measured 40 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:*
    i. Interference by endogenous substances: No data provided. The package inserts states not to use icteric, lipemic, hemolysed or bacterially contaminated samples in the assays and sera with particles should be cleared first by low speed centrifugation.

    ii. Cross-reactivity to other autoantibodies: The ASCA-A and ASCA-G assays were tested with sera from patients with other autoimmune diseases.

        1. ASCA-A: Two hundred sixty-four samples were tested with the 30-15-15 ASCA-A kit for the presence of ASCA. Sixteen subjects, with diseases other than Crohn's Disease, demonstrated the presence of ASCA.

Diseases	# subjects	# Pos	# Neg
Crohn's Disease	100	59	41
Ulcerative colitis	55	9	46
Healthy	50	0	50
Celiac Disease	30	2	28
Systemic Lupus erythematosus	10	2	8
Wegener's granulomatosis	2	0	2
Sjogren's Syndrome	4	2	2
Reactive arthritis	11	1	10
Mixed connective tissue disease	1	0	1
Chronic arthritis	1	0	1
Total # tested	264	75	189

        2. ASCA-G: Two hundred seventy-one samples were tested with the 30-15-15 ASCA-G kit for the presence of ASCA. Twenty-one subjects, with diseases other than Crohn's Disease, demonstrated the presence of ASCA, including three healthy
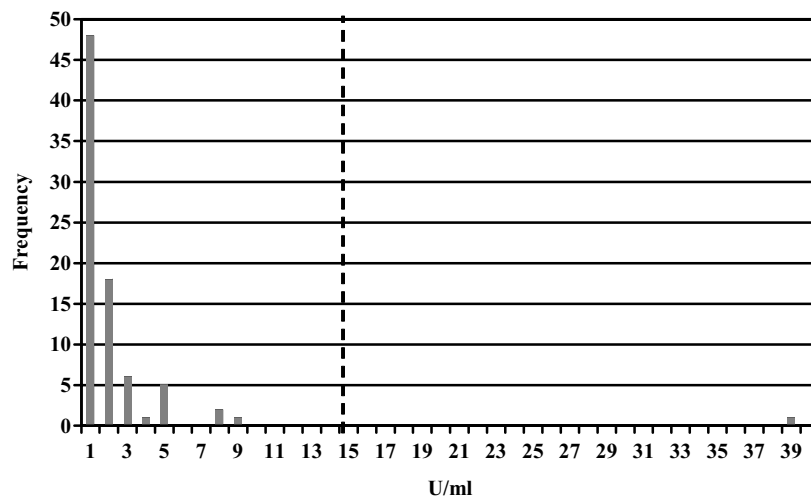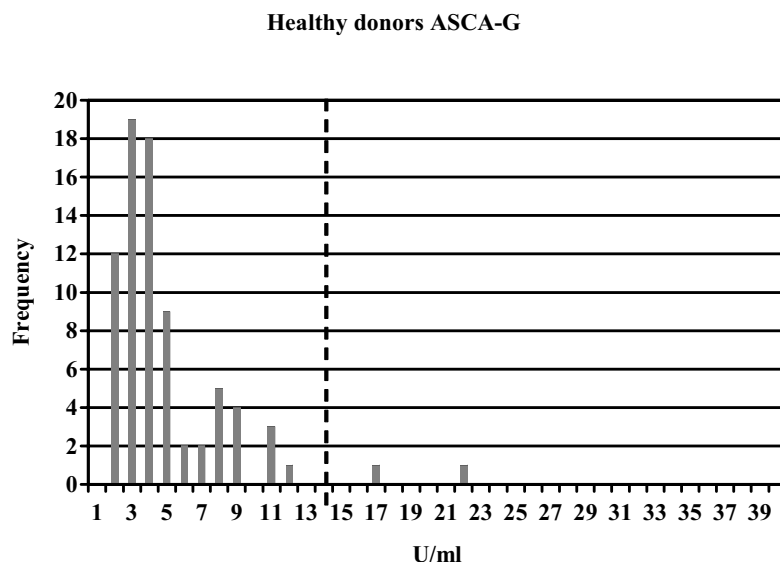
donors.

Diseases	# subjects	# Pos	# Neg
Crohn's Disease	103	81	22
Ulcerative colitis	59	9	50
Healthy	50	3	47
Celiac Disease	30	5	25
Systemic Lupus erythematosus	10	1	9
Wegener's granulomatosis	2	0	2
Sjogren's Syndrome	4	2	2
Reactive arthritis	11	1	10
Mixed connective tissue disease	1	0	1
Chronic arthritis	1	0	1
Total # tested	271	102	169

f. *Assay cut-off:*

The cut-off value of 15 U/mL for the ASCA-A and ASCA-G devices was based on the testing of 77 healthy donor samples. Of the 77 donor subjects, 98.7% and 97.4% were negative for the ASCA-A and ASCA-G assays respectively. The results are depicted in the two graphs below. The ASCA-A graph, shows 76 of 77 subjects had results equal to or below 9 U/mL, where the ASCA-G graph shows 75 of 77 subjects had results equal to or below 12 U/mL. The three subjects above the cut-off demonstrated results of 39 (ASCA-A), and 17 and 22 U/mL (ASCA-G).

Healthy donors ASCA-A

Healthy donors ASCA-G

U/ml

2. Comparison studies:
    a. *Method comparison with predicate device:* The AESKULISA® ASCA-A and ASCA-G kits were compared with their respective predicate devices using sera obtained from patients having various autoimmune diseases. The percentage of cases from male donors was 43.2% and from female donors, 56.8%. In addition, 18.5% of the sera were from donors younger than 26 years, 55.7% were between 26-44 years, and 25.8% were older than 45 years of age. The samples were tested using the 30-15-15 assay protocols and analysis was performed according to the instructions for use.
        i. ASCA-A assay – Testing was performed on 264 samples including 100 confirmed Crohn's Disease (CD); 55 Ulcerative colitis (UC); 50 Healthy; 30 Celiac Disease; 10 Systemic Lupus Erythematosus (SLE); 2 Wegener's granulomatosis (WG); 4 Sjögren's syndrome (SS); 11 reactive arthritis, 1 mixed connective tissue disease (MCTD)and 1 chronic arthritis. Seven cases (3 CD and 4 UC) cases were assayed on the QUANTA Lite IgA predicate but not on the AESKULISA ASCA-A device; therefore they were not included in the comparison.

    ASCA-A Comparison data:

		Quanta Lite IgA predicate		
		(+)	(-)	Total
AESKULISA	(+)	74	1	75
ASCA-A	(-)	0	189	189
	Total	74	190	264

Positive percent agreement: 100% (74/74)
Negative percent agreement: 99.5% (189/190)
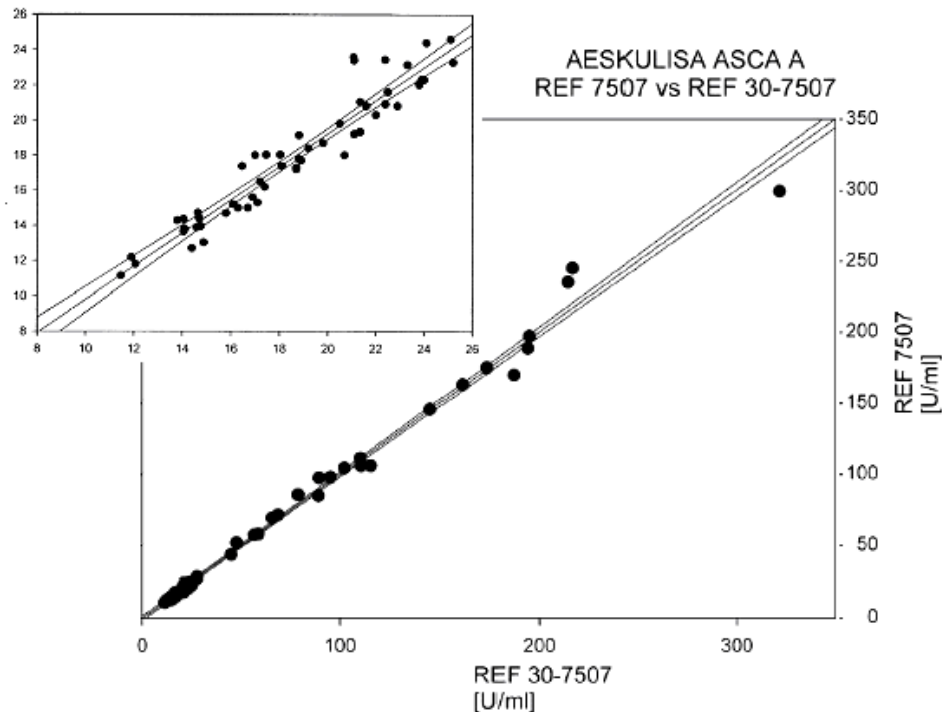Overall agreement: 99.6% (263/264)

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ii.   ASCA-G assay – Testing was performed on 271 samples including 103 confirmed CD; 59 UC; 50 Healthy; 30 Celiac Disease; 10 SLE; 2 WG; 4 SS; 11 reactive arthritis, 1 MCTD and 1 chronic arthritis.
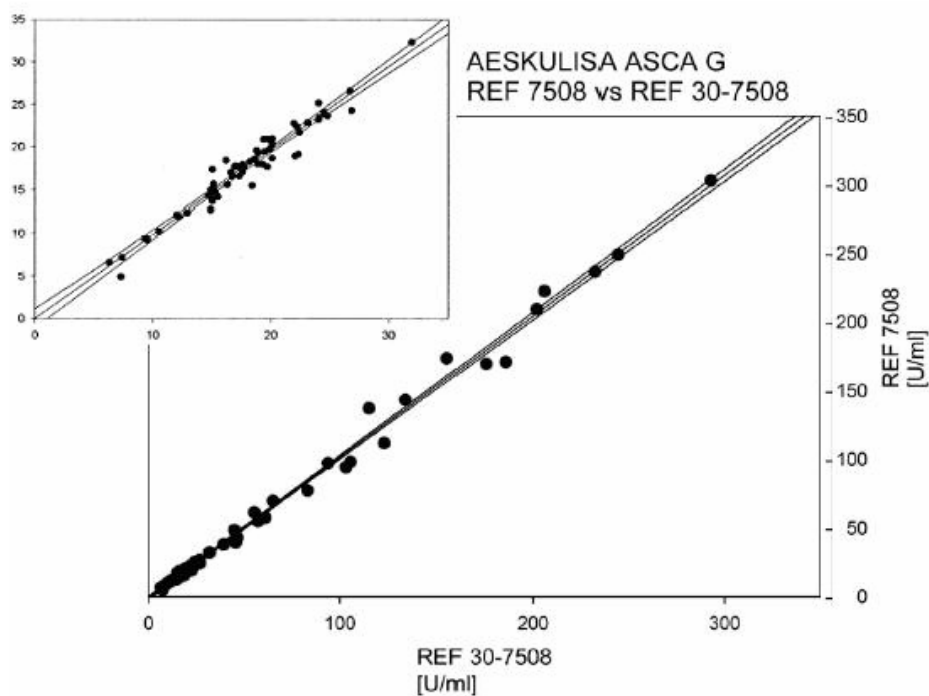
ASCA-G Comparison data:

		Quanta Lite IgG predicate		
		(+)	(-)	Total
AESKULISA	(+)	84	18	102
ASCA-G	(-)	0	169	169
	Total	84	187	271

Positive percent agreement: 100% (84/84)
Negative percent agreement   90.4% (169/187)
Overall agreement: 93.3% (253/271)

iii.   Protocol comparison studies: The comparability of the two ASCA-A assays were assessed with 78 sera tested on both REF 7507 (30-15-15 minute protocol) and REF 30-7507 (30-30-30 minute protocol). The linear regression analysis is depicted in the large figure below with $r^2 = 0.99$. The upper left small figure shows selected results close to the 15 U/ml cut-off ($r^2 = 0.92$).

AESKULISA ASCA A
REF 7507 vs REF 30-7507

The comparability of the two ASCA-G assays were assessed with 85 sera tested on both REF 7508 (30-15-15 minute protocol) and REF 30-7508 (30-30-30 minute protocol). The linear regression analysis is depicted in the large figure below with $r^2 = 0.99$. The upper left small figure shows selected results close to the 15 U/mL cut-off ($r^2 = 0.95$).

AESKULISA ASCA G
REF 7508 vs REF 30-7508

b. *Matrix comparison:*
   Both assays use serum as the matrix.
3. Clinical studies:
   a. *Clinical Sensitivity and specificity:*
   The tables below show the same samples mentioned in the above comparison data, but the results are according to the diagnosis.

Sera with Diagnosis with the AESKULISA® ASCA-A kit

		Diagnosis		
		(+)	(-)	Total
AESKULISA	(+)	59	16	75
ASCA-A	(-)	41	148	189
	Total	100	164	264

Clinical sensitivity: 59% (59/100)
Clinical specificity: 90.2% (148/164)

Sera with Diagnosis with the AESKULISA® ASCA-G kit

		Diagnosis		
		(+)	(-)	Total
AESKULISA	(+)	81	21	102
ASCA-G	(-)	22	147	169
	Total	103	168	271

Clinical sensitivity: 78.6% (81/103)
Clinical specificity: 87.5% (147/168)

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4. Clinical cut-off:
    See assay cut-off.
5. Expected values/Reference range:
    Expected value in the normal population is negative. However, the sponsor cited literature where 7% of the normal population tested was positive for ASCA.

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 the substantial equivalence decision.