

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

A. 510(k) Number:

k071711

B. Purpose for Submission:

Addition of sample matrix (serum) to a cleared device

C. Measurand:

Anti- *Saccharomyces Cerevisiae* Antibody (ASCA)

D. Type of Test:

Qualitative ELISA

E. Applicant:

TECHLAB[®]

F. Proprietary and Established Names:

ASCA-CHEK

G. Regulatory Information:

1. Regulation section:

21 CFR 866.5785, Anti-*Saccharomyces Cerevisiae* (ASCA) test system

2. Classification:

II

3. Product code:

NBT, Antibodies, *Saccharomyces Cerevisiae* (*S. Cerevisiae*)

4. Panel:

Immunology 82

H. Intended Use:

1. Intended use(s):

The ASCA-CHEK test is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of human anti-*S. cerevisiae* antibodies (ASCA) in feces and **serum**. The test result is used as an aid in the diagnosis of Crohn's disease in combination with clinical and other laboratory findings.
FOR *IN VITRO* DIAGNOSTIC USE.

2. Indication(s) for use:

Same as intended use.

3. Special conditions for use statement(s):

The devices are for prescription use only.

4. Special instrument requirements:

Microplate reader capable of measuring optical density (OD) at 450 nm or 450/620 nm.

I. Device Description:

Each device contains the following: microplate strips with breakaway microwells (12 strips x 8 wells/strip) coated with antigens of *Saccharomyces cerevisiae*; goat anti-human polyclonal immunoglobulin-horse radish peroxidase conjugate; positive control; wash buffer 20X concentrate; diluent 10X concentrate; tetramethylbenzidine and peroxide substrate; 0.6N Sulfuric acid stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Quanta Lite™ ASCA (S. Cerevisiae) IgG ELISA
2. Predicate K number(s):
k000732
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	ASCA-CHEK	Quanta Lite™ ASCA IgG ELISA
Intended use	To aid in the diagnosis of Crohn's disease	Same
Technology	ELISA	Same
Substrate	TMB	Same
OD reading	450 nm or 450/620nm	Same
Platform	96 well microtiter plates	Same

Differences		
Item	Device	Predicate
Assay Format	Qualitative	Semi-quantitative
Antigen	Purified antigen from <i>Saccharomyces cerevisiae</i>	Partially purified and disrupted <i>S. cerevisiae</i> antigen
Enzyme-Conjugate	HRP goat anti-human polyclonal immunoglobulin conjugate	HRP goat anti-human IgG conjugate
Incubation times	30-30-15	30-30-30
Positive control	Ready to use Positive Sera	Prediluted: ASCA IgG Low and High Positive Sera
Negative control	1X Diluent	Prediluted Negative Serum
Sample type	Feces and Serum	Serum
Sample dilution and sample volume required	1:1000 dilution with sample diluent	1:101 dilution of 5 µL serum with 500 µL HRP Sample Diluent
Sample diluent	10X Phosphate Buffered Protein solution with 0.2% thimerosal	Tris-buffered saline, Tween-20, protein stabilizer and preservative
Wash buffer concentrate	20X Phosphate-buffered saline, detergent, and 0.2% thimerosal	40X Tris-buffered saline and Tween 20
Stop solution	0.6N sulphuric acid	0.344M sulphuric acid
Washing procedure and number of washes	Manual wash using a Squirt bottle with fine- tipped nozzle with ~400 µL of 1X Wash Solution; total of four washes	Microplate washing device (200-300 µL of diluted HRP wash buffer using a repeating, or multichannel pipette, or automated system); total of three washes
OD measurement	Within 2-10 minutes	Within one hour
ASCA Results	(1) 450 nm wavelength: (single	Results in Units:

Differences		
Item	Device	Predicate
Interpretation	wavelength spectrophotometer) Negative: $OD_{450} < 0.110$ Positive: $OD_{450} \geq 0.110$ (2) 450/620 nm wavelength: (dual wavelength spectrophotometer) Negative: $OD_{450/620} < 0.080$ Positive: $OD_{450/620} \geq 0.080$	Negative: 0.0-20.0 Equivocal: 20.1-24.9 Positive: ≥ 25.0

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

CLSI EP7-A Interference Testing in Clinical Chemistry

L. Test Principle:

The microwells are pre-coated with immobilized antigens of *Saccharomyces cerevisiae*. An aliquot of either serum or fecal specimen is emulsified in the diluent. The diluted specimen, the ready to use positive control, and the 1X diluent negative control are transferred to the microwell. If ASCA are present in the specimen, they will bind to the immobilized antigens. After incubation, the wells are washed and the conjugate added. The conjugate binds to the ASCA captured by the immobilized antigens. A second series of wash steps remove any unbound material. Following the addition of the substrate, a color is detected spectrophotometrically due to the enzyme-antibody-antigen complexes that form the presence of ASCA.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision testing was done on a single wavelength spectrophotometer (OD_{450}). The intra-assay reproducibility was determined by testing 8 human serum specimens (4 ASCA positive and 4 ASCA negative) 8 times in the same run. The positive samples comprised of samples with varying concentrations of ASCA. For the analysis, each serum was diluted 1:1000 with the kit diluent. The OD data were reformatted to reflect the reportable result of the assay, which would either be positive or negative. The table below summarizes the OD output as well as the final call for each of the replicates.

#	Test 1 OD_{450}	Test 2 OD_{450}	Test 3 OD_{450}	Test 4 OD_{450}	Test 5 OD_{450}	Test 6 OD_{450}	Test 7 OD_{450}	Test 8 OD_{450}	Final Call for each replicate
13	0.281	0.253	0.280	0.283	0.306	0.318	0.273	0.274	Positive
34	0.287	0.277	0.230	0.295	0.232	0.230	0.264	0.242	Positive
40	0.136	0.129	0.117	0.125	0.127	0.118	0.114	0.112	Positive
46	0.155	0.150	0.127	0.121	0.125	0.127	0.115	0.124	Positive
26	0.045	0.045	0.047	0.046	0.039	0.041	0.042	0.039	Negative
29	0.044	0.048	0.042	0.048	0.040	0.041	0.043	0.041	Negative
32	0.064	0.074	0.065	0.065	0.063	0.062	0.072	0.061	Negative
44	0.047	0.044	0.053	0.044	0.043	0.058	0.060	0.051	Negative

The inter-assay precision was determined by testing 4 ASCA Ig- positive and 4 ASCA Ig-negative serum specimens eight times over a two-day period using a single lot of the ASCA-CHEK test. Results are summarized below.

#	Test 1 OD ₄₅₀	Test 2 OD ₄₅₀	Test 3 OD ₄₅₀	Test 4 OD ₄₅₀	Test 5 OD ₄₅₀	Test 6 OD ₄₅₀	Test 7 OD ₄₅₀	Test 8 OD ₄₅₀	Final call for each replicate
33	0.293	0.277	0.178	0.240	0.256	0.365	0.316	0.282	Positive
34	0.268	0.253	0.198	0.269	0.253	0.366	0.260	0.299	Positive
40	0.134	0.164	0.163	0.126	0.124	0.124	0.144	0.143	Positive
46	0.159	0.189	0.138	0.186	0.132	0.135	0.142	0.168	Positive
24	0.051	0.039	0.042	0.041	0.037	0.053	0.045	0.045	Negative
29	0.052	0.042	0.045	0.040	0.040	0.063	0.048	0.052	Negative
29	0.052	0.044	0.039	0.040	0.047	0.062	0.050	0.054	Negative
31	0.070	0.056	0.041	0.070	0.084	0.103	0.060	0.088	Negative

- b. *Linearity/assay reportable range:*
Not applicable.

Sample dilution

The specimen dilution of 1:1000 was optimized using titrations (1:100 to 1:1000) of known ASCA-positive and ASCA-negative serum specimens. The 1:1000 dilution was then challenged using healthy control serum panel and clinical specimens using 3 different OD_{450nm} cut-offs (0.110, 0.120 and 0.150). The dilution and OD_{450nm} cut-off (1:1000 and ≥ 0.110) that provided the highest range of correlations to Crohn's disease was considered optimal. This was further challenged in the clinical evaluations.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
There is no recognized standard or reference material for ASCA. The calibrator and positive control (confirmed ASCA positive) were prepared in-house and the designated O.D. was assigned during development process.

- d. *Detection limit:*
Not applicable.

- e. *Analytical specificity:*
Interference: Interference studies were performed as follows: Control samples shown below were divided into two aliquots. One aliquot was mixed with equal volumes of high ASCA-Ig-positive serum samples. The other aliquot was mixed with equal volumes of ASCA Ig-negative samples. All samples were tested seven times using ASCA CHEK. All ASCA-Ig-positive samples remained positive and all ASCA Ig-negative samples remained negative. No interference was observed.

Test Substance	Method of Analysis	Concentration
Lipase	Hitachi 917	>20 U/L
Cholesterol	Olympus AU 5000	504 mg/dL
RF	Nephelometry	27.9 IU/mL
ANA	Nephelometry	0.263 ratio
Bilirubin (free)	Beckman LX20	19 mg/dL
Bilirubin (conjugated)	Beckman LX20	18 mg/dL
Hemolyzed blood	Visual red color	Hemolyzed

Cross-reactivity:

Sera from non-Crohn's disease were tested in the ASCA-CHEK test and showed a positivity range of 4% to 13%.

Non-Crohn's disease group	Number tested	Number positive	%Positive
Ulcerative colitis	46	6	13%
Irritable bowel syndrome	30	3	10%
Constipation	1	0	0%
Esophagitis	1	0	0%
<i>H.pylori</i>	1	0	0%
Alcoholic cirrhosis	20	1	5%
ANA-positive	3	0	0%
Healthy	91	4	4%

Distribution of ASCA reactivity in patients with autoimmune liver disease and inflammatory bowel disorders shown in the table below is from a publication by Muratori P, Muratori L, *et.al.*(Clin Exp Immunol 2003;132:473-476).

	No. of patients	ASCA	ASCA IgA	ASCA IgG	ASCA IgA + IgG
AMA-pos. PBC	106	19 (18)	17 (16)	7 (7)	4 (4)
AMA-neg. PBC	17	9 (53)	6 (35)	6 (35)	3 (18)
Type 1 AIH	30	8 (27)	6 (20)	7 (23)	5 (17)
Type 2 AIH	37	4 (11)	2 (5)	4 (11)	2 (5)
PSC	25	11 (44)	8 (32)	7 (28)	4 (16)
Crohn's disease	23	16 (70)	12 (52)	16 (70)	12 (52)
Ulcerative colitis	25	9 (36)	5 (20)	7 (28)	5 (20)
Blood donors	19	1 (5)	1 (5)	0	0
AMAMA, antimitochondrial antibody; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis; PS PSC, primary sclerosing cholangitis.					

f. Assay cut-off:

Two hundred and fifty-seven (257) serum specimens (172 Crohn's disease and 85 non-Crohn's disease) were used to determine the optimal OD cut-off

for a positive result. OD₄₅₀ (single wavelength spectrophotometer) cut-offs at ≥ 0.110 , 0.120 and 0.150 and OD_{450/620} (dual wavelength spectrophotometer) at ≥ 0.080 and 0.110 were evaluated.

Specimens were diluted at 1:1000 and tested by the *ASCA-CHEK* test for ASCA Ig. The optimal cut-off that provided the best correlation to clinically confirmed Crohn's disease was determined. The OD cut-off of ≥ 0.110 at OD₄₅₀ and ≥ 0.080 at OD_{450/620} provided the largest number of samples that correlated to Crohn's disease. The following table shows the results for the *ASCA-CHEK* test. The number of Crohn's and non-Crohn's samples above and below each OD cutoff was determined.

OD ₄₅₀						
Site	OD	N	Crohn's samples		Non-Crohn's samples	
			above cut-off	below cut-off	above cut-off	below cut-off
Children's Hospital of Harvard	0.110	94	49	23	17	5
	0.120	94	41	31	17	5
	0.150	94	35	37	19	3
Riley Hospital	0.110	23	12	8	3	0
	0.120	23	11	9	3	0
	0.150	23	8	12	3	0
Mayo clinic	0.110	63	34	14	15	0
	0.120	63	32	16	15	0
	0.150	63	30	18	15	0
OD _{450/620}						
Kliniken Essen-Mitte	0.080	77	18	14	42	3
	0.110	77	13	19	42	3

2. Comparison studies:

a. Method comparison with predicate device:

A total of 180 (163 Inflammatory Bowel Disease, 17 Irritable Bowel Syndrome/non-IBD) patients were enrolled at 3 clinical sites. Each patient was assessed for active inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). In addition, each patient was diagnosed using in-house procedures and a single serum specimen was collected for analysis with the *ASCA-CHEK* test. For clinical sites #1 – 3, serum specimens were frozen and sent to TECHLAB®, Inc. for analysis for ASCA Ig. Site 1 and site 2 tested only pediatric samples. The following results were obtained:

Pediatric samples:

Site #1 (Children's Hospital of Harvard Medical School) (frozen sera)

N = 94		QuantaLite ASCA IgG		
		Positive	Negative	Total
ASCA Check	Positive	35	13	48
	Negative	9	37	46
	Total	44	50	94

Positive percent agreement = 80% (95%CI 64-90%)

Negative percent agreement = 74% (95%CI 59-85%)
 Overall percent agreement = 77% (95%CI 68-86%)

Site #2 Riley's Children's Hospital (frozen sera)

N = 23		QuantaLite ASCA IgG		
		Positive	Negative	Total
ASCA Check	Positive	9	3	12
	Negative	1	10	11
	Total	10	13	23

Positive percent agreement = 90% (95%CI 54-100%)
 Negative percent agreement = 77% (95%CI 46-94%)
 Overall percent agreement = 83% (95%CI 64-93%)

Adult samples:

Site #3 Mayo Clinic (frozen sera)

N = 63		QuantaLite ASCA IgG		
		Positive	Negative	Total
ASCA Check	Positive	30	4	34
	Negative	8	21	29
	Total	38	25	63

Positive percent agreement = 79% (95%CI 62-90%)
 Negative percent agreement = 84% (95%CI 63-95%)
 Overall percent agreement = 81% (95%CI 70-88%)

Since frozen samples were used in the comparison studies, the sponsor evaluated the effect of a single freeze-thaw cycle on test results in the serum ASCA-CHEK test. For the analysis, 9 ASCA Ig-positive serum samples and 5 ASCA Ig-negative serum samples were tested. An aliquot from each sample was removed and frozen at -20°C followed by a single thaw at room temperature on the day of analysis. The test results for the frozen and unfrozen specimens showed all negative samples remained negative and all positive samples remained positive at all time periods.

b. *Matrix comparison:*

Serum is the only matrix tested in this submission.

3. Clinical studies:

a. *Clinical Sensitivity/ Clinical specificity:*

The same samples used in method comparison were used to assess sensitivity and specificity. In addition, 77 samples from adult patients were tested on Site 4 (Kliniken Essen-Mitte) for clinical assessment. The following results were obtained:

Site #1 (Children's Hospital of Harvard Medical School)

False positive samples were from patients with ulcerative colitis. Sera from non-Crohn's disease tested in the ASCA-CHEK test showed a positivity range

of 4% to 13%. This accounts for a lower specificity for this site.

N = 94 Pediatric samples		Diagnosis		
		Crohn's disease	Ulcerative colitis and non-IBD	Total
ASCA Check	Positive	43	5	48
	Negative	29	17	46
	Total	72	22	94

Sensitivity = 60% (95%CI 48-71%)

Specificity = 77% (95%CI 54-91%)

Site #2 (Riley's Children's Hospital)

N = 23 Pediatric samples		Diagnosis		
		Crohn's disease	Ulcerative colitis and non-IBD	Total
ASCA Check	Positive	12	0	12
	Negative	8	3	11
	Total	20	3	23

Sensitivity = 60% (95%CI 36-80%)

Specificity = 100%

Site #3 (Mayo Clinic)

N = 63 Adult samples		Diagnosis		
		Crohn's disease	Ulcerative colitis and non-IBD	Total
ASCA Check	Positive	34	0	34
	Negative	14	15	29
	Total	48	15	63

Sensitivity = 71% (95%CI 56-83%)

Specificity = 100%

Site #4 (Kliniken Essen-Mitte)

N = 77 Adult samples		Diagnosis		
		Crohn's disease	Ulcerative colitis and non-IBD	Total
ASCA Check	Positive	18	4	22
	Negative	14	41	55
	Total	32	45	77

Sensitivity = 56% (95%CI 38-73%)

Specificity = 91% (95%CI 78-97%)

The combined test results for both pediatric study sites to clinical assessments for disease diagnosis including non-IBD healthy controls is shown below.

N = 136		Diagnosis		
		Crohn's disease	Ulcerative colitis and non-IBD	Total
ASCA Check	Positive	55	6	61
	Negative	37	38	75
	Total	92	44	136

Sensitivity = 60% (95%CI 49-70%)

Specificity = 86% (95%CI 58-76%)

The combined test results for both adult study sites to clinical assessments for disease diagnosis including non-IBD healthy controls is shown below.

N = 215		Diagnosis		
		Crohn's disease	Ulcerative colitis and non-IBD	Total
ASCA Check	Positive	52	7	59
	Negative	28	128	156
	Total	80	135	215

Sensitivity = 65% (95%CI 53-75%)

Specificity = 95% (95%CI 89-98%)

The combined test results for all 4 study sites including samples from 94 healthy persons showed the following:

N = 351		Diagnosis		
		Crohn's disease	Ulcerative colitis and non-IBD	Total
ASCA Check	Positive	107	13	120
	Negative	65	166	231
	Total	172	179	351

Sensitivity = 62% (95%CI 55-69%)

Specificity = 93% (95%CI 88-96%)

- c. 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 value in normal population is negative. Of the 94 healthy persons tested, 4% tested 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 a substantial equivalence decision.