

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

A. 510(k) Number:

k051927

B. Purpose for Submission:

New Device

C. Measurand:

Anti- *Saccharomyces Cerevisiae* Antibody (ASCA)

D. Type of Test:

Qualitative ELISA

E. Applicant:

TECHLAB[®]

F. Proprietary and Established Names:

TECHLAB[®] 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 TECHLAB[®] ASCA-CHEK test is an ELISA for the qualitative detection of human anti-*S. cerevisiae* antibodies (ASCA) in feces. 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):

INOVA QUANTA Lite™ ASCA IgG

2. Predicate 510(k) number(s):
k000732
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Device
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 Plasma	Prediluted: ASCA IgG Low and High Positive Sera
Negative control	1X Diluent	Prediluted Negative Serum
Sample type	Feces	Serum
Sample dilution and sample volume required	1:10 dilution of 50 µL liquid or 0.05 g solid fecal sample with 450 µL of 1X 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 sulfuric acid	0.344M sulfuric acid
Washing procedure and number of washes	Manual wash using a Squirt bottle with fine-tipped nozzle with ~400 µL of 1X Wash Solution;	Microplate washing device (200-300 µL of diluted HRP wash buffer using a

Similarities		
Item	New Device	Predicate Device
	total of four washes	repeating, or multichannel pipette, or automated system); total of three washes
OD measurement	Within 2-10 minutes	Within one hour
ASCA Results Interpretation	(1) 450 nm wavelength: Negative: OD ₄₅₀ <0.150 Positive: OD ₄₅₀ ≥0.150 (2) 450/620 nm wavelength: Negative: OD _{450/620} <0.110 Positive: OD _{450/620} ≥0.110	Results in Units: Negative: 0.0-20.0 Equivocal: 20.1-24.9 Positive: ≥25.0

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

EP7-A CLSI (NCCLS) Interference Testing in Clinical Chemistry, Vol. 6 No.13

L. Test Principle:

The microwells are pre-coated with immobilized antigens of *Saccharomyces cerevisiae*. An aliquot of 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:*

The intra-assay reproducibility was determined by testing 18 fecal specimens in replicates in one run. Thirteen ASCA positive samples with OD of 0.157 to 0.918 had %CV of 3.6-15.2%. Eight of these samples were close to the cut-off (≤ 0.150 OD) with OD ranged from 0.157 to 0.501 had a CV of 3.6-15.2%. Five ASCA negative samples with OD of 0.026 to 0.048 had CV of 6.0-9.6%.

The inter-assay precision was determined by testing 12 fecal specimens three times over a 6-day period with one kit lot. Eight ASCA positive samples with OD of 0.193 to 1.341 had %CV of 6.0-27.5%. Three of these samples were close to the cut-off (≤ 0.150 OD) had OD ranged from 0.193 to 0.382 had a CV of 18.8-22.0%. Four ASCA negative samples with OD of 0.033 to 0.081 had %CV of 6.6-18.0%.

Day to day ASCA-CHEK fecal test result variation:

A single subject with Crohn’s disease was screened for fecal ASCA using the ASCA-CHEK test and then followed for a 4 day period. A single specimen was obtained each day for the analysis. The fecal ASCA result was positive on day 1 and remained positive during the 4 day test period. The OD₄₅₀ results ranged from 0.893 to 1.371 with a mean ± SD of 0.1.136 ± 0.211 and 95% CI of 0.801 to 1.471 for the 4- day period.

In a second evaluation, a female subject suffering with Crohn’s disease for the presence of fecal ASCA over a 122 day period using the ASCA-CHEK test was followed. A single fecal specimen was collected at sampling points ranging from day 1 to day 122 and tested by the ASCA-CHEK test. A total of 5 fecal specimens, two of which were close to the OD cut-off of 0.150 (0.211 and 0.350), remained ASCA-CHEK test positive during the 4-month period. The OD₄₅₀ results ranged from 0.211 to 0.722 with a mean ± SD of 0.500 ± 0.212 and 95% CI of 0.237 to 0.763 for the 122 day period.

Table 1. ASCA-CHEK test results for samples collected on different days.

Subject ID	Sex	Age range	Disease	Specimen Collection	ASCA-CHEK Test OD ₄₅₀ Result	ASCA-CHEK Test Result
C007	M	20-25 yr	Crohn’s	Day 1	1.237	POSITIVE
				Day 2	1.371	POSITIVE
				Day 3	0.893	POSITIVE
				Day 4	1.043	POSITIVE
HR1	F	15-20 yr	Crohn’s	Day 1	0.599	POSITIVE
				Day 22	0.722	POSITIVE
				Day 80	0.350	POSITIVE
				Day 108	0.211	POSITIVE
				Day 122	0.618	POSITIVE

b. *Linearity/assay reportable range:*

Not applicable.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability: 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 OD values were assigned during development process.

d. *Detection limit:*

Not applicable.

e. *Analytical specificity:*

Interference: Two separate specimen pools (one pool from 3 low ASCA-

positive and one pool from 3 ASCA-negative solid and liquid fecal specimens) were spiked with potential interfering substances and were tested seven times. The interfering substances were listed as follows: mucous, fecal fat, Mylanta®, Blood, Protein, Pepto-Bismol®, Imodium®, Kaopectate®, Leukocytes, and Bilirubin. The studies were designed according to the CLSI EP7-A guidance document. None of the substances listed above interfered with the ASCA-CHEK test results.

Cross-reactivity: No cross-reactivity was observed the ASCA-CHEK test on ASCA-negative fecal specimens spiked with media cultures of the following intestinal organisms: *Aeromonas hydrophila*, *Bacillus cereus*, *Bacillus subtilis*, *Bacteroides fragilis*, *Campylobacter coli*, *Campylobacter jejuni*, *Candida albicans*, *Clostridium butyricum*, *Clostridium difficile* (non-toxigenic) VPI 11186, *Clostridium difficile* (toxigenic) VPI 10463, *Clostridium difficile* (ToxA⁻/ToxB⁺) 8864, *Clostridium perfringens* Type A, *Clostridium septicum*, *Clostridium sordellii*, *Clostridium sporogenes*, *Enterobacter cloacae*, *Escherichia coli* EIEC SD67, *Escherichia coli* ETEC E2348169, *Escherichia coli* 0157:H7, *Escherichia coli*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Porphyromonas asaccharolytica*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Serratia liquefaciens*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *Peptostreptococcus anaerobius*.

The package insert ‘Cross-reactivity Section’ states that “The ASCA-CHEK test was not evaluated for potential cross-reactivity with enteric viruses.”

f. Assay cut-off:

The cut-off at ≤ 0.150 OD was established by analyzing 24 fecal specimens (12 from Crohn’s disease and 12 from non-Crohn’s disease). The 24 fecal specimens were diluted at 1:10 and tested at 2 different OD cut-off values of 0.200 and 0.150. The same 24 fecal specimens were also diluted at 1:20 and were tested at the same 2 OD cut-off values of 0.200 and 0.150. The OD results generated from both specimen dilutions and at the 2 different OD cut-offs at 0.200 and 0.150, were statistically analyzed. The analysis was to determine the optimal combination (specimen dilution and OD cut-off) that provided the best correlation to clinically confirmed Crohn’s disease. The 1:10 dilution in combination of the OD cut-off at 0.150 provided the highest correlation (75%) to Crohn’s disease. The sensitivity and specificity were 58% and 92% respectively.

2. Comparison studies:

a. Method comparison with predicate device:

Comparison was determined against the predicate ASCA EIA kits using 82 samples (45% were from adult patients and 55% from pediatric patients ≤ 18 years old) consisted of: 47 Crohn’s Disease; 23 Ulcerative colitis; 7 IBS/cancer/other; and 5 healthy persons. Results are summarized in the table below:

		Alternative Serum ASCA IgG ELISA Assay		
		Positive	Negative	Total
Fecal ASCA- <i>Check</i> ELISA Assay	Positive	28	5	33
	Negative	12	37	49
	Total	40	42	82

Positive percent agreement 70% (28/40)

Negative percent agreement 88% (37/42)

Overall percent Agreement 79% (65/82)

b. Matrix comparison:

Same as method and fecal/serum sample matrix comparison above in 2a.

3. Clinical studies:

a. Clinical sensitivity and specificity:

ASCA fecal study on 353 samples (142 Crohn's disease, 153 non-Crohn's and 58 healthy persons) were performed in four clinical sites. Results are summarized in the table below:

		Diagnosis		
		Crohn's Disease	Non-Crohn's Disease and healthy persons	Total
ASCA Check	Positive	81	20	101
	Negative	61	191	252
	Total	142	211	353

Sensitivity: 57.0% (81/142)

Specificity: 90.5% (191/211)

Agreement: 77.1% (272/353)

b. Other clinical supportive data (when a is not applicable):

Not applicable.

4. Clinical cut-off:

Same as assay cut-off.

5. Expected values/Reference range:

Expected values in the normal population using the ASCA-CHEK fecal assay should be ≤ 0.150 OD.

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.