

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

K050891

B. Purpose for Submission:

Substantial equivalence determination

C. Measurand:

Clostridium difficile toxins A and B

D. Type of Test:

A qualitative rapid immunoassay

E. Applicant:

TechLab Inc.

F. Proprietary and Established Names:

TOX A/B Quik Chek

G. Regulatory Information:

1. Regulation section:

21 CFR Part 866.2660 Microorganism Differentiation and Identification

2. Classification:

I

3. Product code:

LLH – Reagents, Clostridium difficile toxin

4. Panel:

Microbiology (83)

H. Intended Use:

1. Intended use:

The *TOX A/B QUIK CHEK™* test is a rapid immunoassay for detecting *Clostridium difficile* toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with the patient history.

2. Indication for use:

The *TOX A/B QUIK CHEK™* test is a rapid immunoassay for detecting *Clostridium difficile* toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease. The test is to be used as an aid in the diagnosis of *C. difficile* disease and results should be considered in conjunction with the patient history.

3. Special conditions for use statement:

For prescription use only

4. Special instrument requirements:

Not applicable

I. Device Description:

The *TOX A/B QUIK CHEK™* uses antibodies specific for toxins A and B of *C. difficile*. The device contains a *Reaction Window* with two vertical lines of immobilized antibodies. The test line (“T”) contains antibodies against *C. difficile* toxins A and B. The control line (“C”) contains anti-IgG antibodies. The *Conjugate* consists of antibodies to toxins A and B coupled to horseradish peroxidase. The kit contains:

Membrane Devices –25 pouches, each containing 1 device and a desiccant pack

Diluent (14 mL) – Buffered protein solution containing 0.02% thimerosal

Wash Buffer (10 mL) – A buffered solution containing 0.02% thimerosal

Substrate (3.5 mL) – Solution containing tetramethylbenzidine

Conjugate (2 mL) – Mouse monoclonal antibody specific for toxin A coupled to horseradish peroxidase and goat polyclonal antibody specific for toxin B coupled to horseradish peroxidase in a buffered protein solution containing 0.02% thimerosal

Positive Control (1 mL) – Antigen in a buffered protein solution

J. Substantial Equivalence Information:1. Predicate device names:

Premier toxins A & B, ImmunoCard Toxins A & B, C. difficile TOX A/B II, ProSpecT C. difficile toxin A/B, X/pect C. difficile toxin A/B

2. Predicate 510(k) numbers:

K993914, K041003, K00306 and K030404, K033479 and K041951

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Detection of C.difficile toxins in fecal specimens	Same
Specimen type	Human stool	Same
Technology	Enzyme immunoassay	Same

Differences		
Item	Device	Predicate
Limit of detection	0.63 ng/mL for toxin A and 1.25 ng/ml for toxin B	≥ 1.4 ng/ml of toxin A & ≥ 2.4 ng/ml of toxin B
Clinical sensitivity	90.2 % (84.1 - 94.2% C.I.)	94.7% (88.1 – 98.3% C.I.)
Clinical specificity	99.7 % (98.8 – 99.9% C.I.)	97.3% (95.4 – 98.5% C.I.)

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

“Review Criteria for Devices assisting in the diagnosis of C. difficile associated disease” May 1990, ODE/CDRH Guidance Document

L. Test Principle:

The device contains a *Reaction Window* with two vertical lines of immobilized antibodies (Fig. 1a). The test line (“T”) contains antibodies against *C. difficile* toxins A and B. The control line (“C”) contains anti-IgG antibodies. The *Conjugate* consists of antibodies to toxins A and B coupled to horseradish peroxidase. To perform the test, the

fecal specimen is diluted with *Diluent* and *Conjugate* is added to the diluted sample. The diluted sample-conjugate mixture is added to the *Sample Well* and the device is allowed to incubate at room temperature for 15 minutes. During the incubation, any toxin A and toxin B in the sample bind to anti-toxin antibody-peroxidase conjugate. The toxin-antibody complexes migrate through a filter pad to a membrane where they are captured by the immobilized anti-toxin antibodies in the line. The *Reaction Window* is subsequently washed with *Wash Buffer*, and the test is developed with the addition of *Substrate*. After a 10 minute incubation period, the “T” reaction is examined visually for the appearance of a vertical blue line on the “T” side of the *Reaction Window*. A blue line indicates a positive test. A positive “C” reaction, indicated by a vertical blue line on the “C” side of the *Reaction Window*, confirms that the test is working properly and the results are valid.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The reproducibility of the *TOX A/B QUIK CHEK™* test was determined using known positive (n=6) and negative (n=2) fecal specimens that were coded and masked. Testing was performed at 3 laboratories, which tested the samples on 3 days. The samples produced the expected results 100% of the time.

b. Linearity/assay reportable range:

N/A

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

N/A

d. Detection limit:

The analytical sensitivity was determined using serial two-fold dilutions of highly purified toxins A and B. Toxins were purified using standard in-house procedures. Six separate tests were conducted for each toxin and the test was consistently positive at a concentration of 0.63 ng/ml for toxin A and 1.25 ng/ml for toxin B.

e. Analytical specificity:

CROSS REACTIVITY

Fecal specimens inoculated with the following microorganisms to a final concentration of approximately 10^8 or higher organisms per mL did not react in the *TOX A/B QUIK CHEK*TM:

Bacteria: *Aeromonas hydrophila*, *Bacillus cereus*, *Bacillus subtilis*, *Bacteroides fragilis*, *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter jejuni*, *Candida albicans*, *Clostridium bifermentans*, *Clostridium butyricum*, *Clostridium perfringens*, *Clostridium septicum*, *Clostridium sordellii* (nontoxigenic), *Clostridium sporogenes*, *Enterococcus faecalis*, *Escherichia coli* EIEC, *Escherichia coli*, *Escherichia coli* 0157 H7, *Escherichia coli* ETEC, *Klebsiella pneumoniae*, *Peptostreptococcus anaerobius*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus aureus* (Cowans), *Staphylococcus epidermidis*, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*

Viruses: Adenovirus types 1,2,3,5,40,41, Human coronavirus, Coxsackievirus B2,B3,B4,B5, Echovirus 9,11,18,22,33, Enterovirus type 68,69,70,71.

The only non-*C. difficile* organism to react with the *TOX A/B QUIK CHEK*TM was *C. sordellii* VPI 9048. This strain produces toxins HT and LT, which are homologous to toxins A and B, respectively.

INTERFERING SUBSTANCES

The following substances had no effect on test results when present in feces in the concentrations indicated: mucin (3.5% w/v), human blood (40% v/v), barium sulfate (5% w/v), Imodium[®] (5% w/v), Kaopectate[®] (5 mg/mL), Pepto-Bismol[®] (5% w/v), steric/palmitic acid (40% w/v), Metronidazole (0.25% w/v), Vancomycin (0.25% w/v).

f. Assay cut-off:

The assay was determined to detect Toxin A at 0.63ng/mL and Toxin B at 1.25 ng/mL

2. Comparison studies:

a. Method comparison with predicate device:

N/A

b. Matrix comparison:

N/A

3. Clinical studies:

a. Clinical Sensitivity:

The *TOX A/B QUIK CHEK*TM test was compared with the tissue culture test at three U.S. hospitals and in-house at TECHLAB[®], Inc. Specimens included in the evaluation were submitted to the clinical laboratory for routine testing. The tissue culture test was done according to the in-house procedure. The table below shows a summary of the clinical performance of the *TOX A/B QUIK CHEK*TM test. The test exhibited a sensitivity and specificity of 90.2% and 99.7%, respectively. The predictive positive and negative values were 98.6% and 97.9%, respectively, and the correlation was 98.0%.

Table 1. Correlation of the *TOX A/B QUIK CHEK*TM test with tissue culture.

N = 842	Tiss cult pos	Tiss cult neg
<i>TOX A/B QUIK CHEK</i> TM pos	138	2
<i>TOX A/B QUIK CHEK</i> TM neg	15	687

		95% CI
Sensitivity	90.2	84.1 - 94.2
Specificity	99.7	98.8 - 99.9
Predictive Positive Value	98.6	94.4 - 99.8
Predictive Negative Value	97.9	96.4 - 98.7
Correlation	98.0	97.8 - 98.2

Of the 2 tissue culture-negative/*TOX A/B QUIK CHEK*TM-positive samples, one was negative in a commercial toxin A+B ELISA. Of the 15 specimens that were tissue culture-positive/*TOX A/B QUIK CHEK*TM-negative, 12 were negative in commercial toxin A+B ELISAs. There were nine specimens that were unreadable. Those specimens were negative by PCR analysis for the genes of toxin A (*tcdA*) and toxin B (*tcdB*).

A total of 51 fecal specimens diluted in Cary Blair and 32 fecal specimens diluted in C&S Transport Media were tested in the *TOX A/B QUIK CHEK*TM test and the results were compared to those obtained by routine testing. The test exhibited an agreement of 97.6% for the detection of *C. difficile* toxins in specimens prepared in Transport Media.

b. Clinical specificity:

See Sec. M .3.a.

c. Other clinical supportive data (when a. and b. are not applicable):

N/A

4. Clinical cut-off:

See limit of detection above Sec. M.1.d

5. Expected values/Reference range:

The reported incidence of *C. difficile*-associated disease in patients with antibiotic-associated diarrhea is 10 to 20%. In the studies conducted with this device, the incidence ranged from 10% to 22%. The prevalence of a positive *TOX A/B QUIK CHEK™* test will vary from location to location and hospitals may experience rates lower or higher than those observed at the sites used in this evaluation. *Clostridium difficile* disease is primarily a nosocomial disease of elderly patients, and hospitals that have higher numbers of elderly patients may experience higher rates. It is important to consider any test results in conjunction with clinical symptoms because some healthy adults and large numbers of healthy infants (up to 50%) will be positive for *C. difficile* toxin.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

1. The submitted information in this premarket notification is complete and supports a substantial equivalence decision.