

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

A. 510(k) Number:

K082499

B. Purpose for Submission:

Substantial equivalence determination for a new toxin A and B and antigen combined as one test system

C. Measurand:

Clostridium difficile (*C. diff*) toxins A and B and the antigen glutamate dehydrogenase (GDH)

D. Type of Test:

A qualitative rapid immunoassay

E. Applicant:

Techlab, Inc.

F. Proprietary and Established Names:

C. DIFF QUIK CHEK COMPLETE™

G. Regulatory Information:

1. Regulation section:

866.2660

2. Classification:

Class I

3. Product code:

LLH – Reagents, *Clostridium difficile* toxin

MCB- Antigen, *Clostridium difficile*

4. Panel:

83- Microbiology

H. Intended Use:

1. Intended use(s):

The *C. DIFF QUIK CHEK COMPLETE™* test is a rapid membrane enzyme immunoassay for the simultaneous detection of *C. difficile* glutamate dehydrogenase antigen and toxins A and B in a single reaction well. The test detects *C. difficile* antigen, glutamate dehydrogenase, as a screen for the presence of *C. difficile* and confirms the presence of toxigenic *C. difficile* by detecting 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. As with other *C. difficile* tests, results should be considered in conjunction with the patient history.

2. Indication(s) for use:

The *C. DIFF QUIK CHEK COMPLETE™* test is a rapid membrane enzyme immunoassay for the simultaneous detection of *C. difficile* glutamate dehydrogenase antigen and toxins A and B in a single reaction well. The test detects *C. difficile* antigen, glutamate dehydrogenase, as a screen for the presence of *C. difficile* and confirms the presence of toxigenic *C. difficile* by detecting 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. As with other *C. difficile* tests, results should be considered in conjunction with the patient history.

3. Special condition for use statement(s):

For prescription use only

4. Special instrument requirements:

Not applicable.

I. Device Description:

The *C. DIFF QUIK CHEK COMPLETE™* test uses antibodies specific for glutamate dehydrogenase and toxins A and B of *C. difficile*. The device contains a *Reaction Window* with three vertical lines of immobilized antibodies. The antigen test line (“Ag”) contains antibodies against *C. difficile* glutamate dehydrogenase. The control line (“C”) is a dotted line that contains anti-horseradish peroxidase (HRP) antibodies. The toxin A and B test line (“Tox”) contains antibodies against *C. difficile* toxins A and B. The

Conjugate consists of antibodies to glutamate dehydrogenase and antibodies to toxins A and B coupled to horseradish peroxidase.

J. Substantial Equivalence Information:

1. Predicate device name(s):

There are four predicate devices referenced to be substantially equivalent to the *C. DIFF QUIK CHEK COMPLETE™*.

- C. DIFF QUIK CHEK
- TOX A/B QUIK CHEK
- C. DIFF CHEK-60
- C. DIFFICILE TOX A/B II

2. Predicate 510(k) number(s):

- K053572
- K050891
- K030992
- K003306

3. Comparison with predicate:

Similarities					
Item	<i>C. DIFF QUIK CHEK COMPLETE™</i>	<i>C. DIFF QUIK CHEK®</i>	<i>TOX A/B QUIK CHEK®</i>	<i>C. DIFF CHEK™ - 60</i>	<i>C. DIFFICILE TOX A/B II™</i>
Intended Use	Detection of <i>C. difficile</i> toxins A and B and GDH	Detection of <i>C. difficile</i> GDH	Detection of <i>C. difficile</i> toxins A and B	Detection of <i>C. difficile</i> GDH	Detection of <i>C. difficile</i> toxins A and B
Specimen Types	Human Fecal Specimens	Human Fecal Specimens	Human Fecal Specimens	Human Fecal Specimens	Human Fecal Specimens
Technologies	Enzyme Immunoassay - Rapid Membrane	Enzyme Immunoassay - Rapid Membrane	Enzyme Immunoassay - Rapid Membrane	Enzyme Immunoassay – Microassay Plate	Enzyme Immunoassay – Microassay Plate

Differences					
Item	C. DIFF QUIK CHEK COMPLETE™	C. DIFF QUIK CHEK®	TOX A/B QUIK CHEK®	C. DIFF CHEK™ - 60	C. DIFFICILE TOX A/B II™
Limit of Detection (Analytical Sensitivity)	Toxin A \geq 0.63 ng/mL Toxin B \geq 0.16 ng/mL GDH \geq 0.8 ng/mL	GDH \geq 0.8 ng/mL	Toxin A \geq 0.63 ng/mL Toxin B \geq 1.25 ng/mL	GDH \geq 0.8 ng/mL	Toxin A \geq 0.8 ng/mL Toxin B \geq 2.5 ng/mL
Clinical Sensitivity	Toxins A&B: 87.8% GDH: 90.5%	GDH: 92.8%	Toxins A&B: 90.2%	GDH: 70.5%	Toxins A&B: 92.2%
Clinical Specificity	Toxins A&B: 99.4% GDH: 93.1%	GDH: 92.6%	Toxins A&B: 99.7%	GDH: 91.2%	Toxins A&B: 100%

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

This 510(k) Premarket Notification was prepared and referenced the following guidance document and recognized standard:

- “Review Criteria for Devices assisting in the diagnosis of *C. difficile* associated disease” May 1990, ODE/CDRH Guidance Document.
- User Protocol for Evaluation of Qualitative Test Performance*, Clinical Laboratory Standards Institute (CLSI) Approved Guideline, EP12-A (August 2002)

L. Test Principle:

The device contains a Reaction Window with two vertical lines of immobilized antibodies and center dots of immobilized antibody. One line consists of antibodies against *C. difficile* glutamate dehydrogenase and the second line contains antibodies against *C. difficile* toxins A and B. After the incubation period, “Ag” reaction is examined visually for the appearance of a vertical blue line on the “Ag” side of the Reaction Window. A blue line indicates a positive test. If the “Ag” is positive, then the “Tox” reaction should be examined visually for the appearance of a blue line on the “Tox” side of the Reaction Window. A blue line indicates a positive test. A positive “C” reaction, indicated by a vertical dotted blue line under the “C” portion 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 *C. DIFF QUIK CHEK COMPLETE™* test was determined using 12 fecal specimens that were coded to prevent their identification during testing. Testing was performed at 3 independent laboratories, which tested the samples for 3 days. The samples produced the expected results 100% of the time.

An additional 5-day study was performed at 3 sites by running a set of low positive, moderate positive and high negative fecal samples that were spiked with glutamate dehydrogenase, Toxin A and Toxin B. The samples were run in triplicate, twice a day over a 5-day period by multiple technicians at each site. The combined antigen and toxin data from the 5-day reproducibility study is shown in Table 7. The antigen data for Sample 1 was below 90% negative at one site and was consistently negative for antigen at the other two sites. The toxin data for Sample A was below 90% positive at one site, and was consistently positive for toxin at the other two sites. No single site reported results below expectations for both antigen and toxin.

Table 7 Summary of 5-day reproducibility study

Sample ID	Antigen Positive	Antigen Negative	Toxin Positive	Toxin Negative
Sample 1 (95% negative results expected)	15 (16.7%)	75 (83.3%)	0 (0%)	90 (100%)
Sample A (95% positive results expected)	89 (98.9%)	1 (1.1%)	65 (72.2%)	25 (27.8%)
Sample B (mod positive)	87 (96.7%)	3 (3.3%)	86 (95.5%)	4 (4.5%)

The Reproducibility study was satisfactory for this type of assay.

b. Linearity/assay reportable range:

Not applicable.

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

Not applicable.

d. Detection limit:

The cutoff for GDH was determined using highly purified recombinant *C. difficile* glutamate dehydrogenase (rGDH). The cutoff was determined by testing dilutions of purified rGDH in diluent, in replicates of 20. The Limit of Detection (LOD) was established at 0.20 ng/mL. The LOD for Toxin A and B GDH was determined using highly purified *C. difficile* Toxin A and B. The LOD for each toxin was determined by testing dilutions of purified Toxin A and B in diluent, in replicates of 20. The LOD was established at 0.45 ng/mL for Toxin A and 0.06 ng/mL for Toxin B.

The methodologies above supports the product claims for the cutoff for Toxin A at 0.63 ng/mL, 0.16 ng/mL for Toxin B and 0.8 ng/mL for GDH.

The Limit of Detection and Cutoff studies were satisfactory for this type of assay.

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 antigen or toxin portion of the *C. DIFF QUIK CHEK COMPLETE*™ test:

Bacterium or Pathogen: *Aeromonas hydrophila*, *Bacillus cereus*, *Bacillus subtilis*, *Bacteroides fragilis*, *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter jejuni*, *Candida albicans*, *Clostridium butyricum*, *Clostridium clostridiiforme*, *Clostridium haemolyticum*, *Clostridium histolyticum*, *Clostridium novyi*, *Clostridium perfringens*, *Clostridium septicum*, *Clostridium sordellii* (nontoxigenic), *Clostridium sporogenes*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Escherichia coli* EIEC, *Escherichia coli*, *Escherichia coli* O157:H7, *Escherichia coli* ETEC, *Klebsiella pneumoniae*, *Peptostreptococcus anaerobius*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Serratia liquefaciens*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus aureus* (Cowans), *Staphylococcus epidermidis*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*

The non-*C. difficile* organism, *Clostridium bifermentans*, reacted in the antigen portion of the *C. DIFF QUIK CHEK COMPLETE*™ test. The non-*C. difficile* organism, *Clostridium sordellii*, reacted in the toxin portion of the *C. DIFF QUIK CHEK COMPLETE*™ test was VPI 9048. This strain produces toxins HT and LT, which are homologous to toxins A and B, respectively. Appropriate recommendations will be made in the product insert, Limitations of the Procedure section to reflect this cross-reactivity.

The following viruses of $10^{3.3}$ to $10^{8.25}$ TCID units per 0.2 mL did not react in the *C. DIFF QUIK CHEK COMPLETE*™ test:

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, Rotavirus.

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% v/v), Kaopectate® (5% v/v), Pepto-Bismol® (5% v/v), steric/palmitic acid (40% w/v), Metronidazole (0.25% w/v), Vancomycin (0.25% w/v).

f. *Assay cut-off:*

The assay cutoff was determined to be at a concentration of 0.63 ng/mL for toxin A, 0.16 ng/mL for toxin B, and 0.8 ng/mL for glutamate dehydrogenase. This fulfills the requirements of demonstrating a cutoff for the *C. DIFF QUIK CHEK COMPLETE*™.

2. Comparison studies:

a. *Method comparison with predicate device:*

Not applicable.

b. *Matrix comparison:*

Not applicable.

3. Clinical studies:

a. *Clinical Sensitivity:*

Clinical studies were conducted at three external sites in Pennsylvania, Ohio and Virginia using the *C. DIFF QUIK CHEK COMPLETE*™. Clinical truth was determined at Techlab, Inc. by testing samples using the tissue culture assay for analysis of the toxins A and B portion of the test and bacterial culture for analysis of the GDH antigen portion of the test. A total of 1126 fecal samples were collected and tested in the study. The study subjects ranged in age from 5 months–100 years, and 60.1% were female.

The antigen portion of the *C. DIFF QUIK CHEK COMPLETE*™ test was compared to bacterial culture. Specimens included in the evaluation were submitted to the clinical laboratories for routine testing. The bacterial culture test was performed according to in-house procedures. The results are shown in Table 1.

Table 1. Summary of clinical performance comparing *C. DIFF QUIK CHEK COMPLETE*™ test to bacterial culture

N = 1126	Bacterial Culture Positive	Bacterial Culture Negative
<i>C. DIFF QUIK CHEK COMPLETE</i> ™ Antigen Line Positive	201	62
<i>C. DIFF QUIK CHEK COMPLETE</i> ™ Antigen Line Negative	21	842

		95% Confidence Limits
Sensitivity	90.5%	85.7 – 93.9
Specificity	93.1%	91.2 – 94.7
Predictive Positive Value	76.4%	70.7 – 81.3
Predictive Negative Value	97.6%	96.2 – 98.4
Correlation	92.6%	91.8 – 93.4

The antigen portion of the *C. DIFF QUIK CHEK COMPLETE*™ test was compared to the tissue culture assay for the detection of *C. difficile* toxin. Specimens included in the evaluation were submitted to the clinical laboratories for routine testing. The results are shown in Table 2. The antigen portion of the *C. DIFF QUIK CHEK COMPLETE*™ test detected 98.7% of the tissue culture-positive samples.

Table 2. Summary of clinical performance comparing *C. DIFF QUIK CHEK COMPLETE*™ test to the tissue culture assay

N = 1126	Tissue Culture Positive	Tissue Culture Negative
<i>C. DIFF QUIK CHEK COMPLETE</i> ™ Antigen Line Positive	154	109
<i>C. DIFF QUIK CHEK COMPLETE</i> ™ Antigen Line Negative	2	861

Clinical evaluation of the toxin portion of the *C. DIFF QUIK CHEK COMPLETE*™ test

The toxin portion of the *C. DIFF QUIK CHEK COMPLETE*™ test was compared to the tissue culture assay at two clinical laboratories and in-house at TECHLAB®, Inc. Specimens included in the evaluation were submitted to the clinical laboratories for routine testing. The results are shown in Table 3.

Table 3. Summary of clinical performance comparing *C. DIFF QUIK CHEK COMPLETE*™ test to the tissue culture assay

n = 1126	Tissue Culture Positive	Tissue Culture Negative
<i>C. DIFF QUIK CHEK COMPLETE</i> ™ Toxin Line Positive	137	6
<i>C. DIFF QUIK CHEK COMPLETE</i> ™ Toxin Line Negative	19	964
95% Confidence Limits		
Sensitivity	87.8%	81.4 - 92.3
Specificity	99.4%	98.6 - 99.7
Predictive Positive Value	95.8%	90.7 - 98.3
Predictive Negative Value	98.1%	96.9 - 98.8
Correlation	97.8%	97.6 - 98.0

b. Clinical specificity:

See section M.3.a

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

Not applicable.

4. Clinical cut-off:

The cutoff for the assay was established at concentrations of 0.63 ng/mL for toxin A, 0.16 ng/mL for toxin B, and 0.8 ng/mL for glutamate dehydrogenase.

5. Expected values/Reference range:

The reported incidence of *C. difficile* disease in patients with antibiotic-associated diarrhea may range from 5 to 20%, and hospitals may experience rates lower or higher than this range. 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. In addition, *C. difficile* carriage rates of 22% to 32% have been reported in cystic fibrosis patients (1,3). In the studies conducted for this device, using symptomatic patients, the incidence of toxins A and B was 12% and GDH was 18%. A positive result in the antigen portion of the *C. DIFF QUIK CHEK COMPLETE*™ test confirms the presence of *C. difficile* in a fecal specimen; a negative result indicates the absence of the organism. A positive result in the toxin portion of the *C. DIFF QUIK CHEK COMPLETE*™ confirms the presence of *C. difficile* toxin in a fecal specimen; a negative result indicates the absence of toxin or insufficient levels of toxin for detection.

N. Proposed Labeling:

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

O. Conclusion:

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