

Comparison of the *C. DIFF QUIK CHEK COMPLETE*[®], a Rapid Combination Test for *Clostridium difficile* GDH Antigen and Toxins A and B in Feces with Bacterial Culture and Cytotoxin Assay

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Introduction

Clostridium difficile is the causative agent of antibiotic-associated diarrhea and pseudomembranous colitis. The syndrome is most often associated with antibiotic use. The cytotoxin B assay has been the "gold standard" for the determination of *C. difficile* disease. However many hospitals elect not to perform the assay. The test is technically difficult to perform, specimen transport may be a problem due to the toxins sensitivity to heat, and the time required to detect a negative sample is 48 hours. For these reasons, many laboratories have elected to assay for toxin using enzyme immunoassays, even though these are often less sensitive than the cytotoxin assay. Algorithms based on initial screening for GDH (glutamate dehydrogenase) and confirmation with a toxin test are becoming more common to increase the sensitivity and speed of diagnosis. All GDH negative results may then be reported, and the presence of *C. difficile* toxins confirmed using toxin-specific assays. The *C. DIFF QUIK CHEK COMPLETE*[®] combines the glutamate dehydrogenase and toxin A and B assays into a single assay that is rapid and simple to perform.

Materials and Methods

Specimens: A total of 456 stool samples were included in the study when a *C. difficile* test was ordered by a physician. Samples were analyzed fresh or were stored at 4° C for fewer than 24 hours prior to testing. The specimens included solid, semi-solid and liquid samples. Patients included in the study ranged from approximately 2 years to 93 years of age. Samples from inpatients and outpatients were included. Test results were not linked to the diagnosis of *C. difficile* disease.

Assays: *C. DIFF QUIK CHEK COMPLETE*[®] - This test is a new membrane test that detects *C. difficile* antigen (glutamate dehydrogenase) and toxins A/B in a single assay. A drop of conjugate was added to kit diluent, followed by the addition of fecal sample. The mixture was applied to the Sample Well and the sample was allowed to migrate past the Reaction Window. Antigen and toxins A/B complexed with antibody-conjugates were captured by immobilized antibodies striped within the Reaction Window. After 15 minutes, the Reaction Window was washed, and substrate was added. The presence of antigen and toxins A/B was determined individually by the appearance of antigen and toxins A/B test lines within the Reaction Window. An internal dotted control line was included in the reaction window to confirm proper migration of reagents.

Cytotoxin Assay – The cytotoxin assay was performed by standard methods using MRC-5 cells. All positives were confirmed by toxin-neutralization, and cultures were held for 48 hours before reporting as negative.

Bacterial culture - Fecal samples either were plated directly on cycloserine cefoxitin fructose agar plates (CCFA, Anaerobe Systems, Morgan Hill, CA) or were processed using an alcohol shock procedure and then plated onto CCFA. Inoculated plates were incubated at 37°C for 3 days. Presumptive colonies were recognized as described by Summanen *et al.*



Fecal specimen is negative for antigen and negative for toxins A/B. The dotted control line demonstrates that the reagents in the test have performed properly and that the procedure was followed correctly.



Fecal specimen is positive for antigen and negative for toxins A/B. The dotted control line demonstrates that the reagents in the test have performed properly and that the procedure was followed correctly.



Fecal specimen is positive for antigen and positive for toxins A/B. The dotted control line demonstrates that the reagents in the test have performed properly and that the procedure was followed correctly.

Results

C. DIFF QUIK CHEK COMPLETE[®] GDH ANTIGEN REACTION VERSUS BACTERIAL CULTURE ASSAY

N = 456	Bacterial Culture Positive	Bacterial Culture Negative
	Antigen Positive	66
Antigen Negative	6	360

Sensitivity	91.7%
Specificity	93.8%
Predictive Positive Value	73.3%
Predictive Negative Value	98.4%
Correlation	93.4%

C. DIFF QUIK CHEK COMPLETE[®] GDH ANTIGEN REACTION VERSUS TISSUE CULTURE ASSAY

N = 456	Tissue Culture Positive	Tissue Culture Negative
	Antigen Positive	42
Antigen Negative	0	366

Sensitivity	100%
Specificity	88.4%
Predictive Positive Value	46.7%
Predictive Negative Value	100%
Correlation	89.5%

C. DIFF QUIK CHEK COMPLETE[®] TOXIN REACTION VERSUS TISSUE CULTURE ASSAY

N = 456	Tissue Culture Positive	Tissue Culture Negative
	Toxin Positive	40
Toxin Negative	2	411

Sensitivity	95.2%
Specificity	99.3%
Predictive Positive Value	93.0%
Predictive Negative Value	99.5%
Correlation	98.9%

Discussion

The *C. DIFF QUIK CHEK COMPLETE*[®], which detects *C. difficile* antigen and toxins A/B directly in fecal specimens, was compared with bacterial culture and tissue culture, which are considered the gold standards for *C. difficile* and its toxins. The test exhibited performance characteristics similar to those of the gold standards. Our findings showed that:

- The antigen portion of the test correlated well with the presence of the organism and detected all tissue culture-positive specimens
- The toxin portion correlated well with the tissue culture assay
- The test offers a turn-around time of <30 minutes, is simple to perform, and the detection of specific lines in a single assay well makes interpretation easy
- The detection of antigen alerts the physicians to the presence of *C. difficile* in situations where there is insufficient toxin to detect

Conclusions

The *C. DIFF QUIK CHEK COMPLETE*[®] is a new rapid membrane test that utilizes an algorithm approach for antigen and for toxins A/B. The antigen portion of the test correlated well with bacterial culture and detected 100% of the tissue culture-positive specimens. The toxins A/B portion of the test exhibited high sensitivity and specificity with the tissue culture assay. These findings support the use of this test as a new *in vitro* diagnostic aid for *C. difficile* disease.

References

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