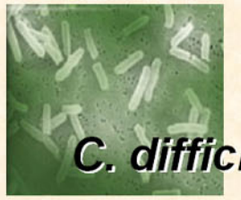

C-029 Detection of *Clostridium difficile* in fecal specimens using a new rapid antigen test, the *C. DIFF QUIK CHEK*™

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INTRODUCTION

Clostridium difficile is the leading cause of hospital-acquired antibiotic-associated diarrhea (AAD) and colitis. *C. difficile* is responsible for about 25% of AAD and most cases of pseudomembranous colitis. The diagnosis of *C. difficile* disease is based on clinical history such as antibiotic treatment, symptoms, and the presence of *C. difficile* toxin in the fecal sample. The tissue culture assay using cultured cells and specific neutralization antiserum is considered by many to be the gold standard for detecting toxin in fecal specimens because of its superior sensitivity and specificity. However, the tissue culture assay takes 48 hours to rule a specimen negative and requires cell culture equipment. The *C. DIFF QUIK CHEK*™ test is a new rapid screening assay that is sensitive and specific. The *C. DIFF QUIK CHEK*™ test detects *C. difficile* glutamate dehydrogenase (GDH), also referred to as the common antigen because it is expressed at a high level by all *C. difficile* strains. The test reduces the labor and turn-around time for reporting negative results. In this study, we evaluated the *C. DIFF QUIK CHEK*™ test by comparing its performance to the tissue culture assay. Our goal was to determine if the test is an effective screen that detects tissue culture-positive specimens and rules out tissue culture-negative specimens.

METHODS

A total of 776 fecal specimens, submitted for routine fecal testing, were collected from the West Virginia University Hospital (Morgantown, WV) and the Carilion Medical Center (Roanoke, VA). The specimens included solid, semi-solid, and liquid samples. Stool specimens from babies (8-months to 2-years) were not excluded from this study because only the presence of *C. difficile* and its toxins were tested and the test results were not linked to the diagnosis of *C. difficile* disease.

These specimens were screened using:

- *C. DIFF QUIK CHEK*™, a rapid membrane test using antibody conjugated with HRP. The test was performed according to the manufacturer's instructions.
- *C. DIFFICILE TOX-B TEST*, which uses a tissue culture format to detect the presence of cytotoxic activity (noted by cell rounding) in fecal specimens and confirms the identification of *C. difficile* toxin using specific antitoxin.

Discrepant specimens were analyzed by:

- *C. DIFF CHEK*™*-60*, an enzyme immunoassay for use as a screening test to detect the GDH in fecal specimens from persons suspected of having *C. difficile* disease.
- PCR using primers specific for the *gdh* gene of *C. difficile*.

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RESULTS

Comparison of the *C. DIFF QUIK CHEK*™ test to the tissue culture assay

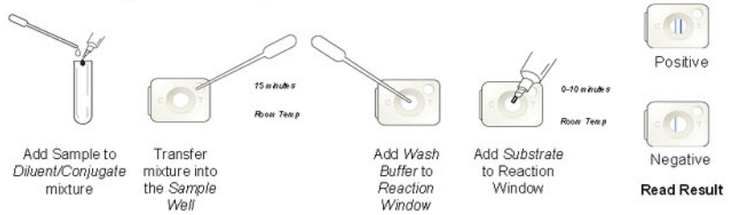
N=776	Tissue culture positive	Tissue culture negative
C. DIFF QUIK CHEK™ positive	167	84
C. DIFF QUIK CHEK™ negative	7	518

Sensitivity	96.0%
Specificity	86.0%
Predictive Pos Value	66.5%
Predictive Neg Value	98.7%
Correlation	88.3%

The *C. DIFF QUIK CHEK*™ test detects both toxigenic and nontoxigenic strains of *C. difficile*. Thus, when compared to the tissue culture assay, the specificity, predictive positive value, and correlation are lower.

- There were 167 specimens that were positive by both tests and 518 specimens that were negative by both tests. Seven specimens were positive only by the tissue culture assay and 84 specimens were positive only by the *C. DIFF QUIK CHEK*™ test.
- The *C. DIFF QUIK CHEK*™ detected 167 of 174 tissue culture-positive specimens, giving a sensitivity of 96.0% with a 95% confidence interval of 91.6 to 98.2%. The predictive negative value for the *C. DIFF QUIK CHEK*™ was 98.7% with a 95% confidence interval of 97.1 to 99.4%.
- The 84 specimens that were tissue culture-negative but positive in the rapid test were tested in the *C. DIFF CHEK*™*-60*, an ELISA for GDH of *C. difficile*. Of these, 66 were confirmed positive for GDH. In addition, 24 of these discrepant specimens were tested by PCR using primers specifically designed for the *C. difficile gdh* gene. Of these, 12 were determined to be positive.

Performing the C. DIFF QUIK CHEK™ Test



DISCUSSION

- The high sensitivity and high predictive negative value, along with the rapid turnaround time demonstrate that the *C. DIFF QUIK CHEK*™ test is a suitable screening assay for laboratories using the tissue culture assay. Using this test as a screen could in less than 30 minutes eliminate approximately >66% of specimens from further toxin testing, which translates into cost savings on unnecessary patient isolation and extra precautions used for patients with *C. difficile* disease.
- In this study, roughly one-third of the fecal specimens positive for *C. difficile* GDH were negative for toxin B by the tissue culture assay. Although a GDH-positive/tissue culture-negative result may indicate the presence of nontoxigenic isolates, we cannot rule out the possibility that some of these specimens were true positives that contained amounts of toxin below the detection limits of the tissue culture assay. Therefore, a GDH-positive/toxin-negative result should alert the physician to monitor the patient closely and to perform additional testing if necessary.
- Like other *C. difficile* antigen tests, the specificity and predictive positive value of the *C. DIFF QUIK CHEK*™ test is lower compared to toxin tests because antigen tests detect both toxigenic and nontoxigenic isolates. This has been reported by other investigators. However, the high sensitivity and predictive negative value demonstrate the value of this test as a screen for patients with AAD.

CONCLUSIONS

The *C. DIFF QUIK CHEK*™ test is an excellent screen for laboratories using the tissue culture assay for detecting *C. difficile* in fecal samples from patients with AAD. The test should be followed with toxin testing because these tests do not distinguish between toxigenic and nontoxigenic strains of *C. difficile*.

REFERENCES

1. Wilkins TD, Lyerly DM (2003) *Clostridium difficile* Testing: after 20 Years, Still Challenging. J. Clin. Microbiol. 41(2):531-4
2. Zheng L, Keller SF, Lyerly DM, Carman RJ, Genheimer CW, Gleaves CA, Kohlhepp SJ, Young S, Perez S, Ye K (2004) Multicenter Evaluation of a New Screening Test that Detects *Clostridium difficile* in Fecal Specimens. J. Clin. Microbiol. 42:3837-3840
3. Lyerly DM, Barroso LA, Wilkins TD (1991) Identification of the latex test-reactive protein of *Clostridium difficile* as glutamate dehydrogenase. J. Clin. Microbiol. 29(11):2639-42
4. Landry ML, Topal J, Ferguson D, Giudetti D, Tang Y (2001) Evaluation of Biosite Triage *Clostridium difficile* Panel for Rapid Detection of *Clostridium difficile* in Stool Samples. J. Clin. Microbiol. 39(5):1855-8
5. Staneck JL, Weckbach LS, Allen SD, Siders JA, Gilligan PH, Goppitt G, Kraft JA, Wills DH (1996) Multicenter Evaluation of Four Methods for *Clostridium difficile* Detection: ImmunoCard *C. difficile*, Cytotoxin Assy, Culture, and Latex Agglutination. J. Clin. Microbiol. 34(11):2718-21