[C-030] Multicenter Evaluation of a Rapid Test for Detection of *Clostridium difficile* in Fecal Specimens

L. Zheng[1], C. W. Genheimer[1], D. M. Lyerly[1], Z. Hussain[2], J. Van Broeck[3], M. Delmée[3]

[1]TECHLAB®, Inc., Blacksburg, VA, [2]London Health Sciences Centre, London, ON, CANADA, [3]Université Catholique de Louvain Microbiology Unit, Bruxelles, BELGIUM

AMERICAN SOCIETY FOR MICROBIOLOGY
106th General Meeting, May 21-25,2006
Orlando, FL

INTRODUCTION

Clostridium difficile is the leading cause of hospital-acquired antibiotic-associated diarrhea (AAD) and colitis. The two toxins of *C. difficile* are 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 fecal specimens. Bacterial culture is the traditional method for confirming the presence of *C. difficile* in stool. However, this test takes 2 to 3 days and requires specific anaerobic culture equipment and media (1). A sensitive screening test, *C. DIFF CHEK*™, reduces the labor and turn-around time by detecting glutamate dehydrogenase (GDH) (2). GDH is also called the "common antigen" because it is expressed at a high level by all *C. difficile* strains (3). In this study we evaluated a new membrane test, *C. DIFF QUIK CHEK*™, by comparing it to bacterial culture. This test is a rapid membrane test using peroxidase-linked immunoglobulins for detecting *C. difficile* GDH in fecal specimens.

METHODS

The study protocols were approved at each site by respective institutional review boards. Information collected from the specimens were patient age and gender in addition to the test results. The results from this study were not linked to diagnosis. Specimens from infants (<2 years old) were included in this study because only the presence of *C. difficile* was tested.

Fecal specimens involved in this study were: 578 specimens sent to London Health Sciences Center (London, Ontario Canada); 306 samples submitted to Carilion Consolidated Laboratory (Roanoke, VA), Hershey Medical Center (Hershey, PA), and West Virginia University Health Sciences Center (Morgantown, WV) and tested at TechLab, Inc (Blacksburg, VA); and 95 samples were submitted to UCL Microbiology Unit (Brussels, Belgium). Age information was available for 661 patients. The distribution of the age population is shown in Figure 1. The gender was known for 851 patients (Figure 2).

All of these specimens were from AAD patients and were submitted for diagnostic testing for the presence of *C. difficile* and/or its toxins. All of the samples were tested using the *C. DIFF QUIK CHEK*™ test according to the manufacturer's instruction. The bacterial culture protocol was based on in-house protocols at each study site. The results are demonstrated in Table 1.

Excluding the UCL Microbiology Unit study site which uses a definitive bacterial culture method, the samples of discrepant results were resolved using a research polymerase chain reaction (2) or another antibody-based commercial GDH test. The summary is presented in Table 2.

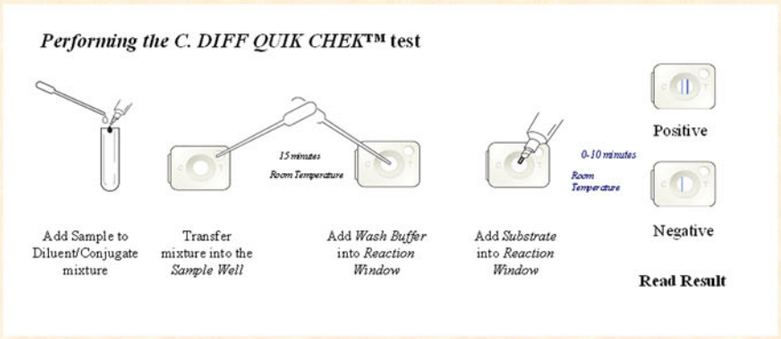

Table 1. Clinical Performance Comparing *C. DIFF QUIK CHEK*™ Test to Bacterial Culture

n=979	Presumptive Bacterial Culture positive	Presumptive Bacterial Culture negative
C. DIFF QUIK CHEK™ positive	206	56
C. DIFF QUIK CHEK™ negative	16	701
		95% Confidence Limits (6)
Sensitivity	92.8%	88.3% - 95.7%
Specificity	92.6%	90.4% - 94.3%
Predictive Positive Value	78.6%	73.1% - 83.3%
Predictive Negative Value	97.8%	96.3% - 98.7%
Correlation	92.6%	91.7% - 93.4%

Table 2. Clinical Performance of *C. DIFF QUIK CHEK*™ Test versus Bacterial Culture Assay after Resolution by another GDH Test

n=979	Resolved Bacterial Culture positive	Resolved Bacterial Culture negative
C. DIFF QUIK CHEK™ positive	231	31
C. DIFF QUIK CHEK™ negative	6	711
		95% Confidence Limits (6)
Sensitivity	97.5%	94.3% - 99.0%
Specificity	95.8%	94.1% - 97.1%
Predictive Positive Value	88.2%	83.5% - 91.7%
Predictive Negative Value	99.2%	98.1% - 99.7%
Correlation	96.2%	95.8% - 96.6%

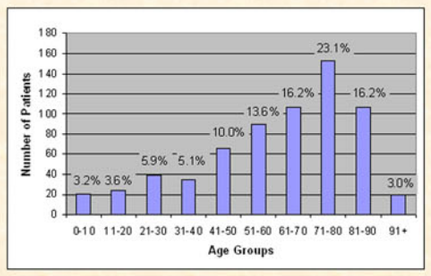

Figure 1. Age distribution of patients whose ages were reported (n=661).

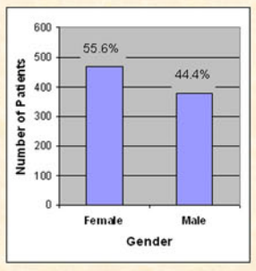

Figure 2. Gender distribution of patients whose genders were reported (n=851).

1-800-TECHLAB www.techlab.com techlab@techlab.com

Limin Zheng, Ph.D., e-mail: zhengl@techlab.com

TECHLAB®

RESULTS

The *C. DIFF QUIK CHEK*™ test was comparable to the resolved bacterial culture test. Of the 979 clinical specimens, 206 tested positive by both tests and 701 were negative by both tests. Twenty-five of the 56 apparent false positive samples were positive by another GDH test, and were considered to be true positives. Thirty-one remained false positive. Ten of the 16 apparent false negative samples were negative by another GDH test, and were considered to be true negatives. Six remained false negative. The resolved sensitivity, specificity, positive predictive value, negative predictive value, and the correlation were 97.5%, 95.8%, 88.2%, 99.2% and 96.2% respectively (Table 2).

DISCUSSION

- The *C. DIFF QUIK CHEK*™ test was comparable to bacterial culture in this study. The high sensitivity and high negative predictive value, along with a rapid turnaround time demonstrated that the *C. DIFF QUIK CHEK*™ test is a suitable rapid screening test for laboratories using the tissue culture assay or PCR for toxin genes. Using the test as a screen would eliminate approximately two-thirds of the samples in less than 30 minutes from further toxin testing, which translates into cost savings on unnecessary patient isolation and extra precaution used for patients with *C. difficile* disease.
- In other studies, only about 55-60% of the fecal specimens positive for *C. difficile* common antigen were positive for toxins either by toxin ELISA or by the neutralizing tissue culture cytotoxicity assay (2,4,5). Although a GDH-positive/toxin-negative result may indicate growth of nontoxigenic isolates of *C. difficile* in the patient, 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 toxin assays. Therefore, these results should alert the physician to monitor the patient closely and to perform additional testing if necessary.

CONCLUSIONS

The *C. DIFF QUIK CHEK*™ test is an excellent screening test for laboratories using bacterial culture to examine the presence of *C. difficile* in stool specimens. The test should be followed with toxin testing, because a positive result from the *C. DIFF QUIK CHEK*™ test does not tell if the *C. difficile* strain present in the sample is toxigenic or non-toxigenic.

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 Assay, Culture, and Latex Agglutination. J. Clin. Microbiol. 34(11):2718-21
6. http://faculty.vassar.edu/lowry/VassarStats.html

ACKNOWLEDGEMENT

We thank Carilion Consolidated Laboratory (Roanoke, VA), Hershey Medical Center (Hershey, PA), and West Virginia University Health Sciences Center (Morgantown, WV) for providing TechLab, Inc. with some of the AAD fecal specimens.