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A New Rapid Combination Test for *Clostridium difficile* Antigen and Toxins A and B in Feces – Evaluation of the *C. DIFF QUIK CHEK COMPLETE*™

Authors: M. Goodykoontz, J.T. Boone, D. Link, and D. M. Lyerly[1], C. R. Hill[2], L. R. Henry[2];
[1]TECHLAB, Inc., Blacksburg, VA, [2]Carilion Consolidated Laboratories, Roanoke, VA

TECHLAB®, Inc.
2001 Kraft Drive, Blacksburg, VA, 24060
540-953-1664, techlab@techlab.com,
mgoodykoontz@techlab.com

ABSTRACT

C. difficile causes antibiotic associated diarrhea (AAD) and colitis, with estimates approaching 500,000 cases annually in the U.S. Timely and accurate detection of this opportunistic pathogen is a priority for improved patient healthcare. The gold standard methods, which include bacterial culture for the organism and tissue culture assay for toxin, are labor intensive and require 48-72 hours for results. Additionally, the isolation of the organism does not assure that the strain is toxigenic. Recently, an algorithm has been proposed to screen specimens for glutamate dehydrogenase (GDH), a marker for the organism, with confirmation by toxin testing. The *C. DIFF QUIK CHEK COMPLETE*™, a new rapid immunoassay test, utilizes this approach by detecting GDH and toxins A and B in a single device. The *C. DIFF QUIK CHEK COMPLETE*™ test was compared to bacterial culture on cycloserine-cefoxitin-fructose agar and to tissue culture assay using HFF cells. The test was performed according to the package insert, with results for antigen, toxins A and B, and an internal dotted control line obtained in under 30 minutes. The study was conducted using samples (n= 460) submitted for routine *C. difficile* testing at a large hospital with a regional reference laboratory. Specimens were from hospital inpatients (57%), nursing home patients (12%), and outpatients (31%). Patient ages ranged from young children to elderly, with the majority of samples from patients >60 years of age. The antigen portion of the test exhibited a sensitivity and specificity of 92.1% and 92.5%, respectively, compared to bacterial culture, and detected 97.6% of the samples positive by tissue culture assay. The toxin portion of the test had a sensitivity and specificity of 85.9% and 99.5%, respectively, compared to the tissue culture assay.

This is the first commercial test to provide a single assay for the detection of antigen and both toxins A and B. The test is simpler to perform than a stepwise algorithm, offers a rapid turnaround time, and provides more definitive results than single assay tests.

MATERIALS AND METHODS

Specimens: Four hundred sixty specimens that were submitted to a regional reference laboratory were included in this study. Specimens were from hospital inpatients (57%), nursing home patients (12%), and outpatients (31%). Patient ages ranged from young children to elderly, with the majority of samples from patients >60 years of age.

***C. DIFF QUIK CHEK COMPLETE*™**: 750 ul Diluent and 1 drop Conjugate were added to a tube, followed by the addition of 25 ul sample. The contents were mixed well, and 500 ul of the mixture was added to the Sample Well. After 15 minutes at room temp, 300 ul of Wash Solution was added to the Reaction Window, allowed to soak in, and 2 drops of Substrate were added. Reactions were read after 10 minutes.

Tissue culture assay: The tissue culture assay was performed with the *TOX-B TEST* using HFF (human foreskin fibroblast) cells.

Bacterial Culture: Bacterial culture was performed using cycloserine-cefoxitin-fructose agar (CCFA). Cultures were incubated for 72 hours under anaerobic conditions. *C. difficile* colonies were identified by their typical ground glass appearance on CCFA, and verified using an antigen (GDH) EIA test.

RESULTS

Interpretation of C. *DIFF QUIK CHEK COMPLETE*™: After the 10 minute incubation period, the "Ag" reaction was examined for a blue vertical line on the "Ag" side of the Reaction Window, indicating a positive test. If the "Ag" was positive, the "Tox" reaction was examined visually for a vertical blue line on the "Tox" side of the Reaction Window, indicating a positive test. A positive "C" reaction, indicated by a vertical dotted blue line under the "C" portion of the Reaction Window, confirmed the test performed properly.

Test results:
- Antigen-negative, toxin-negative – *C. difficile* is not present
- Antigen-positive, toxin-negative – *C. difficile* is present
- Antigen-positive, toxin-positive – toxin-producing *C. difficile* is present
- Antigen-negative, toxin-positive – Not observed in our studies

Negative result

Positive for Antigen only

Positive for Antigen and Toxin

Performance characteristics: The antigen portion of the test exhibited a sensitivity and specificity of 92.1% and 92.5%, respectively, compared to bacterial culture.

N=460		BACTERIAL CULT	
		Pos	**Neg**
***COMPLETE* GDH pos**		93	27
***COMPLETE* GDH neg**		8	332
Sensitivity	92.1%	(95% CI 84.5-96.3%)	
Specificity	92.5%	(95% CI 89.1-94.9%)	
Pred Pos Val	77.5%	(95% CI 68.1-84.4%)	
Pred Neg Val	97.6%	(95% CI 95.2-98.9%)	
Correlation	92.4%	(95% CI 91.0-93.6%)	

The antigen portion detected 97.6% of the samples positive by tissue culture assay. NOTE: The Pred Pos Val is low, because the *COMPLETE* detects the presence of both toxigenic and nontoxigenic strains, whereas tissue culture detects toxigenic strains only.

N=460		TISSUE CULT	
		Pos	**Neg**
***COMPLETE* GDH pos**		83	37
***COMPLETE* GDH neg**		2	338
Sensitivity	97.6%	(95% CI 91.0-99.6%)	
Specificity	90.1%	(95% CI 86.5-92.9%)	
Pred Pos Val	69.2%	(95% CI 60.0-77.1%)	
Pred Neg Val	99.4%	(95% CI 97.9-99.9%)	
Correlation	91.5%	(95% CI 89.9-92.8%)	

The toxin portion of the test had a sensitivity and specificity of 85.9% and 99.5%, respectively, compared to the tissue culture assay.

N=460		TISSUE CULT	
		Pos	**Neg**
***COMPLETE* TOXIN pos**		73	2
***COMPLETE* TOXIN neg**		12	373
Sensitivity	85.9%	(95% CI 76.2- 92.2%)	
Specificity	99.5%	(95% CI 97.9–99.9%)	
Pred Pos Val	97.3 %	(95% CI 98.8-99.5%)	
Pred Neg Val	96.9%	(95% CI 94.5-98.3%)	
Correlation	97.0%	(95% CI 96.4-97.4%)	

DISCUSSION

The diagnosis of *C. difficile* disease has become more challenging. More samples are being tested at earlier stages in the disease when levels of antigen and toxin are very low. Many samples are from patients who have already started metronidazole or vancomycin treatment, which rapidly reduce antigen and toxin levels, possibly affecting test performance. In addition to inpatients, many samples are from outpatients and nursing home patients, and it still is unclear how diagnostic tests perform in different patient populations. This may be especially confusing with PCR testing, since there are large numbers of asymptomatic carriers in hospitals and because the presence of the organism does not always indicate disease.

We developed a new *in vitro* rapid immunoassay that detects both antigen and toxins A/B, thus utilizing an algorithm approach to provide the physician with more information on the presence of *C. difficile* and whether it is toxigenic, all in under 30 minutes. Our results showed that the antigen portion of the *C. DIFF QUIK CHEK COMPLETE*™ assay correlated well with bacterial culture. In addition, the antigen portion detected >97% of all tissue culture-positive samples. The toxin portion correlated well with the tissue culture assay, and detected typical and atypical toxigenic strains, including the 027 ribotype associated with recent outbreaks.

Based on its performance, our results showed that the *C. DIFF QUIK CHEK COMPLETE*™ is suitable for use as a stand-alone test and as an accurate screen for labs that may consider more time-consuming or expensive tests such as toxigenic culture and PCR.

CONCLUSIONS

- The *C. DIFF QUIK CHEK COMPLETE*™ is a rapid immunoassay for the simultaneous detection of *C. difficile* antigen and toxins A and B in fecal specimens, with results in less than 30 minutes.
- The test exhibits an excellent correlation with bacterial culture and the tissue culture assay.
- The test is suitable as a stand-alone test for clinical labs and an accurate cost-effective screen in labs that pursue additional testing.
- Excludes >90% of samples from further testing with 99% certainty.