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Clinical Evaluation of a New Enzyme Immunoassay (*C. DIFF QUIK CHEK COMPLETE*®) for the Rapid and Simultaneous Detection of *Clostridium* Glutamate Dehydrogenase and *C. difficile* Toxin A/B

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ABSTRACT

A new rapid *C. difficile* test product (*C. DIFF QUIK CHEK COMPLETE*®, Inverness Medical, Princeton, NJ) (*Cd*Q) was evaluated. The product incorporates two separate membrane-bound EIA's: one for a *Clostridium*-associated antigen (glutamate dehydrogenase) and one for *Cd* toxins A and B. The EIA's are in the same device and are performed simultaneously. 218 stool specimens submitted for routine *Cd* testing at a community hospital core laboratory were tested by *Cd*Q and by both the *Cd* toxin EIA and the cell culture toxin neutralization test methods routinely used in this laboratory. A true positive result was defined as one which was positive by either EIA or by CTN. The sensitivities, specificities, PPV, and NPV of the two separate components of the *Cd*Q were the following: *Cd*Q antigen - 100, 87.8, 55.8, and 100%, respectively; *Cd*Q toxin - 86.2, 100, 100 and 97.9%, respectively. Hands-on times were five and 25 minutes for one and 10 specimens, respectively, and incubation time was uniformly 25 minutes. The high sensitivity and NPV of *Cd*Q antigen results, and the high specificity and PPV of *Cd*Q toxin results suggest *Cd*Q has potential to facilitate workflow and to provide accurate, rapid (same day/same shift), and final *C. difficile* test results for 87.6% of tested specimens. *Cd*Q Ag-pos/*Cd*Q toxin-neg specimens could contain nontoxigenic strains of *Cd* and/or lower-than-detectable levels of *Cd* toxins A and B. Such specimens can be tested by alternative methods.

INTRODUCTION AND RATIONALE

Clinical microbiologists are receiving increasing numbers of requests for *C. difficile* tests and are continually looking for *C. difficile* test products which produce rapid results, are easy to perform, reduce turnaround time, facilitate workflow, perform well, and are cost-effective. Many studies have documented the advantages and disadvantages of the major tests for *Clostridium* antigen (glutamate dehydrogenase) and toxins A and B (A/B): toxigenic culture, cell culture toxin neutralization (CTN), enzyme immunoassay (EIA), and molecular methods. The results of several studies have suggested that sequential use of two or three of these tests can result in better statistical performance and shorter turnaround time than does the use of single individual tests.

In the study presented here, a new and novel EIA product (*C. DIFF QUIK CHEK COMPLETE*® [*Cd*Q]) was evaluated. The product allows the simultaneous detection of *Clostridium* antigen and *C. difficile* toxins A/B. The performance of the product was compared with the performance of the routine *C. difficile* EIA and CTN tests used in this laboratory.

Figure 1
Examples of *C. DIFF QUIK CHEK COMPLETE*® Results

Ag- / Tox- Ag+ / Tox+ Ag+ / Tox-

MATERIALS, SPECIMENS, AND PROCEDURES

MATERIALS

Specimen Preparation FECAL-QUIK-PREP™ specimen preparation devices (Inverness Medical, Princeton, NJ)
Routine EIA *C. DIFFICILE TOX A/B II*™ for detection of toxins A/B (Inverness Medical, Princeton, NJ)
Routine CTN HEp2 and MRC-5 cell cultures (Diagnostic Hybrids, Athens, OH; Viromed Laboratories, Minatonka, MN);
C. difficile Toxin-Antitoxin Kit (TechLab®, Blacksburg, VA)
New Product *C. DIFF QUIK CHEK COMPLETE*® for detection of GDH and toxins A/B (Inverness Medical, Princeton, NJ)

SPECIMENS

218 stool specimens submitted for routine *C. difficile* testing at a community hospital core laboratory in Cincinnati, Ohio, were examined. All specimens were tested the day of receipt or the next day after being stored at 4ºC.

PROCEDURES

EIA for *C. difficile* Toxins A/B A portion of each specimen to be tested by the EIA was processed through and simultaneously diluted in a FECAL-QUIK-PREP™ device. The routine EIA used in this laboratory had a typical microwell EIA format and was performed according the manufacturer's instructions. Interpretation of results at our facility: ≤0.029 = negative, 0.030-0.079 = equivocal, and ≥0.080 = positive. In our routine clinical practice, specimens which produce equivocal results are tested with the CTN.
CTN Test for *C. difficile* Toxin B A portion of each specimen to be tested by CTN was diluted in minimal essential medium, centrifuged, and filtered (0.45-µm). One portion of the filtrate was treated with 0.15 ml of distilled water and added to HEp-2 or MRC-5 cell cultures; another portion of the filtrate was treated with 0.15 ml of *C. difficile* antitoxin and added to HEp-2 or MRC-5 cell cultures. The cell cultures were examined at 24 and 48 hours for typical *C. difficile* toxin cytopathic effect which had been neutralized by the antitoxin.
***Cd*Q for *C. difficile* Antigen and Toxins** The *Cd*Q is a small, rapid EIA device which contains a thick absorbent matrix (Figure 1). Direct application of a specimen and visual access to the surface of the matrix is available through a smaller sample well and a larger reaction well, respectively, on the upper surface of the device. The surface of the matrix in the larger well contains three parallel vertical lines of immobilized antibodies to *Clostridium* GDH, horseradish peroxidase, and *C. difficile* toxins A/B. The three lines are labeled Ag (antigen), C (control), and Tox (toxin), respectively.

1. Each specimen was suspended in diluent/conjugate which contained anti-GDH and anti-toxins A/B antibodies, both of which were conjugated to horseradish peroxidase.
2. The diluted specimen solution was added to the sample well and allowed to incubate at room temperature for 15 min. If present, conjugate-target complexes migrated to the reaction well and were captured by appropriate antibodies in the three aforementioned vertical lines of antibodies.
3. Captured conjugate-target complexes in the reaction well were washed with buffer. Substrate (tetramethylbenzidine) was added to the reaction well and allowed to incubate with captured conjugate-target complexes for 10 min. Development of a blue line indicated a positive reaction (Figure 1).

True Positive Results A true positive specimen (the clinical standard routinely used in this laboratory) was defined as one which was positive by either routine EIA or by CTN.

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RESULTS

The *Cd*Q was extremely easy to use and results were easy to read (Figure 1). The total time for the two required incubations was 25 min. Hands-on times were 5 and 25 minutes for one and 10 specimens, respectively.

The results of the 218 *Cd*Q-tested specimens are the following:

- all 166 *Cd*Q Ag-neg specimens (76.1% of the total) were EIA-CTN-neg,
- all 25 *Cd*Q Ag-pos/tox-pos specimens (11.5%) were EIA-CTN-pos, and
- 27 of the specimens (12.4%) were *Cd*Q Ag-pos/tox-neg.

The determined sensitivity, specificity, PPV, and NPV of the separate antigen and toxin EIA components of the *Cd*Q are shown in the tables below.

*Cd*Q ANTIGEN	EIA-CTN	
	Positive	Negative
Positive	29	23
Negative	0	166

Sensitivity	100%
Specificity	87.8%
PPV	55.8%
NPV	100%

*Cd*Q TOXIN	EIA-CTN	
	Positive	Negative
Positive	25	0
Negative	4	189

Sensitivity	86.2%
Specificity	100%
PPV	100%
NPV	97.9%

DISCUSSION

The design of the study precluded *Cd*Q from statistically outperforming our current EIA and CTN methods because these methods were used as the standard. We were looking for a *C. difficile* testing method that not only would perform as well as our EIA-CTN but also reduce the turnaround time and labor involved in daily *C. difficile* testing. The *Cd*Q performance was equivalent to that of EIA and CTN routinely used in our laboratory, and the time required to perform the *Cd*Q was extremely short.

In practice, the antigen and toxin components of the *Cd*Q can be considered a screening test and a confirmation test, respectively. In our laboratory, specimens which yielded either *Cd*Q Ag-neg or *Cd*Q Ag-pos/tox-pos results could have been reported as final *C. difficile* negative and positive, respectively. Specimens which yielded *Cd*Q Ag-pos/tox-negative results could have contained nontoxigenic strains of *C. difficile* and/or lower-than-detectable levels of toxin. Such specimens could have been tested by alternative methods.

The high sensitivity and NPV of the *Cd*Q Ag component and the high specificity and PPV of the *Cd*Q toxin component suggest *Cd*Q has potential to facilitate workflow, and to provide accurate and final results on the same day or during the same shift specimens are received.

CONCLUSIONS

The *C. DIFF QUIK CHEK COMPLETE*® was easy to perform, and results were easy to read. Testing 10 specimens required only 50 minutes of hands-on and incubation time. In our laboratory, the performance of the *Cd*Q was equivalent to that of our routinely used EIA and CTN tests for *C. difficile* toxins A/B. Use of the *Cd*Q could allow us to issue final *C. difficile* results of 87.6% of specimens on the same day or during the same shift specimens are received. Specimens which yield *Cd*Q Ag-pos/tox-neg results can be tested by alternative methods.