[C-305] Performance of TECHLAB® *C. DIFF CHEK*™ in Combination with *C. DIFFICILE TOX A/B II*, Triage® *C. difficile* Panels, and a Cytotoxin Assay for the Diagnosis of *C. difficile*-Associated Diarrhea (CDAD) ASM 2004 New Orleans, LA

Heather Snell, Meredith Ramos, Sue Longo, Michael John, Zafar Hussain
London Health Sciences Centre, London, ON, CANADA.

BACKGROUND AND INTRODUCTION

Clostridium difficile has become the most commonly identified cause of nosocomial diarrhea. The Society for Healthcare Epidemiology of America recommends that in order to achieve maximal diagnostic sensitivity and specificity, cultures as well as a cytotoxin assay should be performed on stool specimens submitted for *C. difficile*-associated diarrhea CDAD(1). Tissue culture detection of toxin B is the most sensitive diagnostic method and is considered the "gold standard". The cytotoxin assay is time consuming, however, with a 72 to 96 hour turn-around-time. Inherent technical complexity and expense of the test also limit its utility. Consequently, a number of rapid enzyme immunoassays have been developed. TECHLAB®, Inc. (Blacksburg, VA) has recently developed an enzyme immunoassay, *C. DIFF CHEK*™, which detects glutamate dehydrogenase (GDH), a common *C. difficile* antigen. TECHLAB® also markets an EIA kit, *C. DIFFICILE TOX A/B II*™, for the detection of toxins A and B. The aim of this study was to evaluate and compare these two kits with another immunoassay test (Triage® , Biosite Diagnostics, San Diego, CA), which detects both GDH and toxin A, with an in-house cytotoxin assay, and stool cultures for *C. difficile*. Furthermore, all *C. difficile* isolates were tested for the toxin A and B genes by PCR.

METHODS

All stool specimens submitted to the Microbiology Laboratory from inpatients suspected of *C. difficile*-associated diarrhea (CDAD) were included in the study. Only informed stools were processed. *C. DIFF CHEK*™ (TL-Ag), *C. DIFFICILE TOX A/B II*™ (TL-Tox) detection, Triage® *C. difficile* test (TR-Ag and TR-Tox), and the cytotoxin assay (CTA) were performed within 48 hours of arrival of specimens in the laboratory. Specimens were kept at 4˚C if not processed immediately. A portion of stool was stored at -70˚C for subsequent culture. If the quantity of a sample was insufficient to perform all tests, the specimen was excluded from the study. *C. DIFF CHEK*™ (TL-Ag) to detect GDH, *C. DIFFICILE TOX A/B II*™ test (TL-Tox) to detect toxins A and B, and Triage® *C. difficile* test to detect GDH (TR-Ag) and toxin A (TR-Tox) were performed according to manufacturer's instructions. Presence of cytotoxin B in fecal samples was determined using an in-house tissue culture of Hep-2 cells. If a toxigenic strain of *C. difficile* was isolated and toxin A or B were detected by any method, the patient was considered to have CDAD.

***C. difficile* Cultures & Identification:** The fecal samples were thawed and planted onto Cycloserine Cefoxitin Fructose Agar (CCFA) (Oxoid, Ottawa, Canada). The plates were incubated for 72-96 hours at 35˚C in an anaerobic glove box. *C. difficile* was identified based on growth on selective media, colonial morphology and a positive reaction with MicroScreen *C. difficile* Latex Slide Agglutination Test (Microgen Bioproducts Ltd. Surrey, UK).

PCR Assay for Toxins A and B Genes: PCR was done using primers NK9 and NK11 derived from the repeating portion of the toxin A gene, and NK104 and NK105 derived from the nonrepeating portion of the toxin B gene as described by Kato et al.

Statistical Analysis: For the purpose of this study, patients with diarrhea from whom a toxigenic strain of *C. difficile* was isolated and whose samples were positive for toxin A or B by any method, were considered to hav CDAD. Sensitivity, specificity, positive and negative predictive values for cytotoxin assay, TL-Tox and TR-Tox of each test was calculated on this basis.

RESULTS

A total of 497 specimens were included in the study. *C. difficile* was isolated from 93/497 (18.7%) specimens. The Toxin A and B genes were not detected in 20/93 (21.5%) of these strains, and these were considered nontoxigenic. All toxigenic strains were positive for toxin A and B genes, and elaborated both toxins. Triage® panels could not be evaluated in 15/497 (3%) if cases because of blackening of panels due to excessive alkaline phosphatase. TL-Ag detected the presence of *C. difficile* common antigen in 87/93 cases and TR-Ag in 79/93 cases. Other results of these tests are shown in Table 1. In all instances were TL-Ag or TR-Ag was falsely positive, TL-Tox and TR-Tox gave a negative result.

Using our definition, 52 of the 497 (10.5%) patients had CDAD. In all of these cases, glutamate dehydrogenase (GDH) tests with both kits were positive. CTA, TECHLAB®, and Triage® were evaluated for their ability to diagnose CDAD. None of the kits could detect all cases of CDAD. CTA detected 50/52, TECHLAB® detected 44/52 and Triage® detected 36/52 cases of CDAD. Both TECHLAB® and Triage® with GDH detection tests and in combination with CTA were able to detect all cases. Results of these tests are shown in Table 2. False positive results for toxins were only seen with specimens that were both culture and GDH negative, or with specimens that yeilded a non-toxigenic strain of *C. difficile*.

Table 1. Comparison of TECHLAB® *C. DIFF CHEK*™ and Triage® Panel for Detection of Antigen by Glutamate Dehydrogenase

Tests	Positive	Negative	False Positive	False Negative	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Culture	93 (18.7%)	404 (81.3%)						
TECHLAB® (TL-Ag)	87	396	8	6	93.5%	98%	91.6%	98.5%
Triage® (TL-Ag)	79	399	5	14	84.9%	98.8%	94%	96.6%

Table 2. Comparison of TECHLAB® *C. DIFFICILE TOX A/B II*™, Triage® Panels and Cytotoxin Assay for Detection of CDAD

Tests	# Positive	# Negative	# False Positive	# False Negative	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
CDAD* cases	52 (10.5%)	445 (89.5%)						
Cytotoxin Assay	50	442	3	2	96.1%	99.3%	94.3%	99.5%
TECHLAB® (TL-Tox)	44	437	8	8	84.6%	98.2%	84.6%	98.2%
Triage® (TR-Tox)	36	445	0	16	69.2%	96.4%	69.2%	100%

*CDAD cases were defined as patients with diarrhea, from whom a toxigenic strain of *C. difficile* was isolated and toxin A or B were detected by any method.

CONCLUSIONS

- As expected, GDH tests, for the detection of *C. difficile*, are less specific, for the diagnosis of CDAD and are unable to differentiate between toxigenic and non-toxigenic strains. In conjunction with toxin testing, however, they are useful screening tests, allowing more rapid turn-around-time than tissue culture.

- In our opinion, the best laboratory approach for the diagnosis of CDAD is to test stool specimens for GDH and subsequently with a toxin detection enzyme immunoassay if GDH is positive. Specimens positive for GDH, but negative for toxin, should be further tested with a cytotoxin assay.

- Using this approach, in the present study, TECHLAB® would have identified 44/52 cases of CDAD, but have necessitated cytotoxin assay in an additional 51 specimens. Triage®, on the other hand, would have identified 36/52 patients with CDAD, but have necessitated cytotoxin assay in an additional 48 specimens.

REFERENCES

1. Gerding, D.N., Johnson, S., Peterson, L.R., Mulligan, M.E., and Silva (Jr) J. 1995. Clostridium difficile-associated diarrhea and colitis. Infect. Control Hosp. Epidemiol. 13:459-477