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Evaluation of Biosite Triage *Clostridium difficile* Panel for Rapid Detection of *Clostridium difficile* in Stool Samples

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One hundred two stool samples were tested by both the rapid Triage *Clostridium difficile* Panel (Triage Panel) and the cytotoxin cell culture assay. Five samples positive by both the *C. difficile* toxin A (Tox A) and common antigen components of the Triage Panel had cytotoxin titers of ≥10,000. Twenty-three samples were Triage Panel Tox A negative but common antigen positive. Ten of these had cytotoxin titers of 10 to 1,000, but 13 were cytotoxin negative. Bacterial isolates obtained from 8 of these 13 specimens were analyzed for Tox A and B genes by PCR, and only two contained toxigenic bacteria. Thus, the majority of samples positive only for *C. difficile* common antigen contained nontoxigenic bacteria. A Triage Panel Tox A-positive result indicated a sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 33.3, 100, 100, and 88.2%, respectively. A Triage Panel common antigen-positive result indicated a sensitivity, specificity, PPV, and NPV of 100, 82.7, 53.6, and 100%, respectively. The high NPV of the Triage Panel common antigen, together with rapid reporting of results, should prove useful in avoiding unnecessary use of contact precautions and antibiotic treatment for *C. difficile*-negative patients. However, with Triage Panel common antigen-positive patients, a sensitive cytotoxin assay should be used to distinguish true cytotoxin-positive patients from *C. difficile* carriers.

C. difficile is the most important identifiable nosocomial pathogen causing infectious diarrhea in hospitalized patients. Despite reliable diagnostic assays, effective antibiotic therapy, and the use of infection control measures, *C. difficile*-associated diarrhea (CDAD) and colitis remain significant problems. Pathogenic strains of *C. difficile* produce two toxins, toxin A (Tox A), which results in fluid secretion, inflammation, and damage to the intestinal mucosa in animal models, and Tox B, which is a potent cytotoxin in cell cultures but not enterotoxic in animals.

C. difficile infection is usually acquired in the hospital, since environmental contamination is common and health care workers may carry the organism on their hands or on contaminated instruments or equipment. Though colonization of healthy ambulatory adults with *C. difficile* is uncommon, among hospitalized patients, the rate of colonization rises rapidly from 13% for patients hospitalized 1 to 2 weeks to 50% for patients hospitalized >4 weeks. (2). Following colonization with *C. difficile*, the disruption of normal bacterial flora of the colon through exposure to antibiotics, as well as the presence of certain host factors, can result in the release of Tox A and B from toxigenic strains of *C. difficile* (8). Nevertheless, the majority of colonized patients remain asymptomatic and only 20% of antibiotic-associated diarrhea without colitis is due to *C. difficile* (9). Therefore, the need to distinguish CDAD from asymptomatic colonization in a patient with diarrhea due to another source is crucial to prevent inadvertent antibiotic treatment and the unnecessary use of infection control procedures.

However, distinguishing colonization by *C. difficile* from infection with toxin-producing strains is problematic. Culture is slow, requiring bacterial isolation followed by a toxin assay. Detection of cytotoxin (Tox B) in cell culture has been the most sensitive and specific assay to date. Positive results are available as early as 4 h, but negative results require up to 48 h. In addition, cell culture techniques are beyond the expertise of many laboratories. Even with the availability of commercial kits, the sensitivity of cytotoxicity results can vary significantly among laboratories due to differences in cell culture sensitivities (1) and the starting dilutions of stools tested (12). Detection of Tox A and B by enzyme immunoassays (EIA) provides more rapid results, but sensitivity remains suboptimal (8, 12, 13). The reagent in the latex agglutination test for *C. difficile* common antigen reacts with both toxigenic and nontoxigenic strains and also cross-reacts with other anaerobes and other clostridia. PCR can be used to identify toxigenic strains (7, 10) but remains too expensive and specialized for routine use in the laboratory.

The Triage *C. difficile* Panel (Triage Panel) is a new rapid 15-min EIA for the simultaneous detection of both *C. difficile* Tox A and *C. difficile* common antigen. In this report, the Triage Panel was compared with the cytotoxicity assay.

MATERIALS AND METHODS

Stool samples. Stool samples submitted to the Clinical Virology Laboratory at Yale New Haven Hospital for *C. difficile* testing were assayed prospectively by both the standard cytotoxin assay and the Triage Panel (Biosite Diagnostics, San Diego, Calif.) according to the manufacturer's instructions. After chart review, duplicate stools from individual patients and stools submitted for follow-up during or after treatment for CDAD were excluded from analysis.

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TABLE 1. Comparison of Triage Panel and cell culture cytotoxicity results

No. of samples	Triage Panel result		Cell culture result	
	Tox A	Common antigen	Cytotoxin	Titer
5	Positive	Positive	Positive	≥10,000
10	Negative	Positive	Positive	10–1,000
13	Negative	Positive	Negative	NA[a]
1	Negative	Negative	Positive	10
61	Negative	Negative	Negative	NA

[a] NA, not applicable.

Cytotoxicity assay. Stool samples (0.5 ml) were added to 0.5 ml of phosphate-buffered saline with antibiotics (vancomycin, gentamicin, and amphotericin B) and then vortexed, and the toxin was allowed to elute for 5 min. After centrifugation of a sample for 10 min in a microcentrifuge, the supernate was removed and passed through a 0.45-μm-pore-size filter. Then, 20 μl of filtrate was inoculated onto foreskin fibroblast monolayers (MRHF cells; BioWhittaker, Walkersville, Md.) in 96-well plates using serial 10-fold dilutions (1:10 to 1:10,000) *C. difficile* antitoxin (20 μl; TechLab, Inc., Blacksburg, Va.) was added to duplicate wells of the 1:10 and 1:100 dilutions. Monolayers were read at 4, 24, and 48 h after inoculation using an inverted microscope. A known positive control, run with each assay, was required to show cytotoxicity in the expected range. A positive result consisted of cytotoxicity that was neutralized by *C. difficile* antitoxin. Results were given as the highest dilution showing specific cytotoxicity.

Triage Panel. The Triage Panel (Biosite Diagnostics) was performed according to the manufacturer's instructions. Briefly, 0.5 ml of specimen or a level spoonful of sample was transferred to 4.5 ml of specimen diluent in a 15-ml centrifuge tube. After vortexing, a filter was inserted into the centrifuge tube and the sample was centrifuged for 5 min at 1,500 × *g*. Five hundred microliters of the filtrate obtained was added to the center of the detection zone of the test device and allowed to soak in completely. Then, 140 μl of enzyme conjugate was added, followed by wash solution and substrate. The results were read for the one negative and two positive control zones. If a color bar appeared in the negative control zone, the sample was retested using one-quarter of the initial sample volume. A sample was positive for *C. difficile* Tox A and common antigen if the respective sample color bars were positive.

Culture of *C. difficile* from stool specimens. *C. difficile* was cultured from stool specimens in plates containing cycloserine-cefoxitin-fructose agar as previously described (3, 7) by using a dilution method.

DNA amplification and detection of Tox A and B genes. DNA was extracted from bacterial cells, followed by DNA amplification and detection of the Tox A and B genes, as previously described (10).

Patient selection. Patients who had specimens sent for evaluation for *C. difficile* must have had the presence of diarrhea clearly documented in their medical records. The severity of the diarrhea could not be reliably determined. Only one stool specimen per patient within a 7-day period during the period of diarrhea was included in the analysis. Follow-up stool samples from patients already treated for CDAD and stools from patients who did not have diarrhea were excluded from the analysis.

The following demographic data were collected by an epidemiology technician from Yale New Haven Hospital Epidemiology & Infection Control: date of admission, date of discharge, antibiotic history, date of onset of diarrhea, use of contact precautions, antibiotic treatment for *C. difficile*, and the start and stop dates of such antibiotic treatment.

RESULTS

A total of 102 patients were enrolled in the study, and their specimens were evaluated using both the cytotoxicity assay and the Triage Panel. A high level of background staining was observed in 19 of the first 80 samples tested, but improvements in the Triage Panel membrane eliminated this problem. For five specimens, the Triage Panel result could not be read, even after repeat testing, due to diffuse staining of the membrane. After chart review, 7 of the remaining 97 patients did not meet clinical criteria for suspected CDAD and were omitted from the final analysis. These seven specimens were both cytotoxin assay and Triage Panel negative. Consequently, 90 nonduplicate patient results were included in the final analysis. Antibiotic administration in the previous 90 days was documented in the charts of 81 patients and was unknown for 7 patients. It was stated in the charts of two patients that they had not received antibiotics.

Of these 90 stool specimens, 29 (32.2%) were positive by one or both assays, including one positive specimen from a patient with an unknown antibiotic history. As shown in Table 1, the Triage Panel was positive for Tox A only when the cytotoxin titer was ≥10,000. Ten samples that were Triage Panel Tox A negative but common antigen positive had cytotoxin titers of 1,000 ($n = 2$), 100 ($n = 5$), and 10 ($n = 3$). One sample was positive only for cytotoxin, at a titer of 10. Thirteen samples positive for *C. difficile* common antigen only were cytotoxin negative. Ten of these 13 were cultured, as were the 4 samples with cytotoxin titers of 10. Bacterial isolates were analyzed for Tox A and B genes by PCR. The results are shown in Table 2.

Of note, the sample positive by the cytotoxin assay only did not grow *C. difficile*. On initial reading, this sample was recorded as questionable despite neutralization by antitoxin since the pattern of cytotoxicity was atypical. Thus, this was most likely a false-positive cytotoxin result. Two common antigen-positive samples also did not yield bacteria on culture. These two specimens had minimal stool remaining for culture, and thus lack of growth was likely due to insufficient sample for testing. Eight samples positive for common antigen only grew *C. difficile* on culture, but only two (25%) of the eight isolates carried the Tox A and B genes. Thus, the majority of samples positive for *C. difficile* common antigen only contained nontoxigenic bacteria (4).

If the corrected cytotoxin results are taken as the true positives, a Triage Panel common antigen-positive result indicated

TABLE 2. Results of *C. difficile* culture and Tox A and B gene PCR on selected samples

Category	No. of samples	Triage Panel result		Cytotoxin titer	*C. difficile* culture result	Tox A and B gene PCR result
		Tox A	Common antigen			
Low level of cytotoxin	3	Negative	Positive	10	Positive	Positive
	1	Negative	Negative	10	No growth	ND[a]
Common antigen only	2	Negative	Positive	—[b]	Positive	Positive
	6	Negative	Positive	—	Positive	Negative
	2	Negative	Positive	—	No growth	ND

[a] ND, not done.
[b] —, test result was negative.

TABLE 3. Estimated cost savings from same-day reporting of negative *C. difficile* results

Parameter	No. or cost
No. of nonduplicate[a] *C. difficile* specimens per year	1,000
No. of nonduplicate negative *C. difficile* Triage Panel tests[b]	700
No. of patients already requiring contact precautions[c]	−140
No. of patients requiring empiric contact precautions for suspected *C. difficile*	560
No. of days of empiric contact precautions before negative cytotoxin assay result[d]	1,120
Cost of supplies for contact precautions for 2 days[e]	$18.00
Total cost of empiric contact precautions while awaiting cytotoxin result	$20,160.00
Total incremental increase in cost of Triage Panel over that of cytotoxin assay[f]	−$13,000.00
Net cost savings from avoiding empiric contact precautions	$7,160.00
Cost savings per nonduplicate *C. difficile* specimen tested	$7.16

[a] Only one stool per patient per episode of diarrhea; follow-up stools for treated patients are prohibited.
[b] Estimated seventy percent of samples are Triage Panel negative.
[c] For 20% of the patients, contact precautions are used for organisms other than *C. difficile*.
[d] Two days of contact precautions per negative test result.
[e] The cost of a gown and gloves is $0.90. If we assume 10 visits/day by personnel, the cost is $9.00/day in supplies.
[f] Incremental cost of $12/specimen for Triage Panel-negative specimens (70%) and Tox A-positive specimens (5%), plus $16/specimen for specimens positive for Triage Panel common antigen only (25%) that still require cytotoxin tests at $4 per test.

a sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 100, 82.7, 53.6, and 100%, respectively. A Triage Panel Tox A-positive result indicated a sensitivity, specificity, PPV, and NPV of 33.3, 100, 100, and 88.2%, respectively.

An estimate of cost savings based on empiric institution of contact precautions for all patients with suspected CDAD, same-day reporting of Triage Panel results, a $16 list price for the Triage Panel, and a $4 list price for the cytotoxin assay is shown in Table 3. In our setting, an average savings of $7.16 per nonduplicate stool tested was determined. Importantly, submission of duplicate stools, which is common practice for negative samples (13), or follow-up stools for treated patients would result in an overall increase in costs of up to $12 per sample (Triage Panel list price minus the cost of the cytotoxin assay).

DISCUSSION

Although the cytotoxin test is considered the "gold standard," it requires cell culture expertise and 48 h to report negative results. Thus, many hospitals now use a rapid *C. difficile* Tox A or Tox A+B EIA to diagnose CDAD, despite sensitivities in the range of 84 to 92% compared to the cytotoxin assay (12, 13). Since previous studies in our laboratory (M. L. Landry and D. Ferguson, unpublished data) have found Tox A or A-B EIA to miss all cytotoxin assay-positive samples with titers of 10 and many cytotoxin assay-positive samples with titers of 100, we have continued to perform our in-house cytotoxin assay. The Triage Panel test, in contrast, detected all true cytotoxin positives indirectly, by detecting the *C. difficile* bacteria and not the Triage Panel Tox A component. Thus, the greatest advantage of the Triage Panel rapid test over the commonly used *C. difficile* immunoassays was its very high NPV, since the vast majority of samples were ultimately reported as negative. Seventy percent of samples were negative by the Triage Panel in the present study. The rapid reporting of negative results provided by the Triage Panel should reduce the need for private rooms and contact isolation precautions and prevent the occasional delays in hospital discharges incurred while waiting for *C. difficile* test results. Contact precautions require the use of a private room and the donning of a gown and gloves upon entering a patient's room (5). A disposable gown and gloves cost an estimated $0.90. At an average of 10 patient contacts per day, this results in a cost of $18.00 for 48 h until a negative cytotoxin assay result is reported. Furthermore, the rapid negative test result would obviate most of the empiric treatment for CDAD which now occurs with the delay in cytotoxin results. While the cost of oral metronidazole treatment is minimal, exposure to metronidazole has been identified a risk factor for the acquisition of vancomycin-resistant enterococci (6, 11).

The Tox A component of the test had a very low sensitivity for the detection of cytotoxin. Only 5 of 15 samples (33%) considered true positives for cytotoxin were detected by the Tox A component of the Triage Panel, and all of these had cytotoxin titers of ≥10,000. The Triage Panel was most effective in detecting the presence of the *C. difficile* bacteria. Twenty-three samples were positive by Triage Panel for common antigen only. Only 10 of these 23 (44%) were found to be cytotoxin assay positive, and an additional two patients carried toxigenic bacteria. Previous work in DNA fingerprinting of multiple colonies from the same patient, performed by Y. Tang (unpublished data), has shown that the vast majority of patients are colonized with a single strain of *C. difficile*. Thus, it is unlikely that toxigenic strains were missed. Without the use of the cytotoxin assay, or culture followed by PCR, it would not be possible to distinguish asymptomatic carriers from those with toxin-producing bacteria. Treating all common antigen-positive, Tox A-negative patients with antibiotics and contact precautions would double the number of positive patients that would be treated based on the cytotoxin assay results. Besides the added expense, unnecessary treatment may increase the risk of acquiring vancomycin-resistant enterococci (6, 11). Finally, asymptomatic carriers of *C. difficile* appear to have a lower risk of CDAD than noncarriers (14); thus, overtreatment of carriers may increase the incidence of future CDAD cases.

Therefore, if the Triage Panel is used, we envision a two-step approach. The Triage Panel would be used to screen all stools submitted for *C. difficile* testing. Triage Panel-negative samples and samples positive for both Triage Panel Tox A and common antigen would be reported immediately. However, samples positive for common antigen only would then be tested by the cytotoxin assay to prevent inadvertent treatment of carriers. In our patient population, approximately 25% of samples would require both assays.

The Triage Panel is expensive at $16 per test (list price), plus repeats, although some discount can be anticipated depending on the volume of testing performed. In our laboratory, the in-house cytotoxin assay costs only $3.67 per sample. The increase in cost for the Triage Panel can be justified if savings are

gained on the ward from avoiding empiric contact precautions, unnecessary antibiotic therapy, and occasional delays in hospital discharges. These savings can be realized, however, only if hospital policy requires that empiric contact precautions are routinely implemented when stools are sent for *C. difficile* testing and if duplicate stool specimens are not submitted.

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