

Comparative Evaluation of Six Commercially Available Assays for the Detection of *Clostridium difficile* Toxins in Fecal Specimens

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AMENDED ABSTRACT

Clostridium difficile is the primary cause of nosocomial diarrhea and pseudomembranous colitis. Recent evidence suggests an increasing role for the organism as an agent of community-acquired infection, and characterization of increased virulence and antibiotic-resistance (particularly to fluoroquinolone agents) among certain strains is troubling. With protocols for treatment and containment increasingly complicated, early diagnosis becomes key to successful epidemiologic strategies. We evaluated seven commercially available assays designed to detect either toxin A and/or toxin B associated with *C. difficile*-associated disease (CDAD). Results of the seven assays were compared to cell culture cytotoxicity, recognized as the gold standard for the laboratory detection of CDAD. Three hundred fresh fecal specimens were tested yielding a positivity rate of 11% by cell culture cytotoxicity. Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) for each assay are tabulated.

Commercial Assay Sens / Spec / PPV / NPV *C. DIFFICILE TOX A/B II*[™] (TechLab[®], Blacksburg, VA) 86.8 / 100 / 100 / 98.1 *TOX A/B QUIK CHEK*[™] (TechLab[®], Blacksburg, VA) 91.7 / 100 / 100 / 98.9 ProSpecT[®] *C. difficile* Toxin A Assay (Remel, Lenexa, KS) 91.7 / 98.1 / 86.8 / 98.9 ProSpecT[®] *C. difficile* Toxin A/B Assay (Remel, Lenexa, KS) 97 / 94.6 / 68.8 / 99.6 X/pect[™] (Remel, Lenexa, KS) 94.3 / 94.3 / 67.3 / 99.3 Gastro-Tect (Medical Chemical Corp., Torrance, CA) 82.5 / 97.4 / 82.5 / 97.4 Premier[™] Toxins A&B (Meridian, Cincinnati, OH) 91.7 / 98.9 / 91.7 / 98.9

Using cell culture cytotoxicity as the gold standard for all assays, sensitivities and specificities ranged from 82.5 to 97% and 94.3 to 100% respectively, resulting in significant differences in the accuracy of positive results (PPVs 67.3 to 100%). These data, along with final discrepant analysis with a molecular assay, can be combined with clinical diagnosis/outcome, ease of use, cost and volume when selecting the appropriate CDAD assay in the clinical microbiology laboratory.

INTRODUCTION

Automated methods for identifying bacterial isolates and testing antimicrobial susceptibility have, in recent years, become the de facto norm in most clinical laboratories. Laboratories seeking to combine ease of use, rapid turnaround time, and cost savings have driven the demand for new generations of instrumentation. The Phoenix System (Becton Dickinson, Sparks, MD) is the most recent addition to the list of available diagnostic instruments for the clinical microbiology laboratory, and promises greater ease of use, greater reliability, and faster turnaround time, compared to other currently available instruments. The Phoenix system utilizes a combination of fluorogenic and chromogenic substrates for its identification algorithms, a broth-based AST method that utilizes a redox indicator to enhance detection (at 20 minute intervals), and a robust data processing application (the Phoenix EpiCenter).

We compared the Phoenix against our VITEK Legacy (BioMerieux, Durham, NC) for accuracy of bacterial ID and AST using clinical isolates from our institution and a set of known challenge isolates with complex identification and/or susceptibility phenotypes. We tested a total of 200 Gram positive and Gram negative isolates for ID and AST. Accuracy of results was defined as agreement between the two systems or individual agreement to the known reference phenotype if available. Identification discrepancies were resolved by ribotyping and AST discrepancies by MIC determination.

METHODS

Between September 2005 and March 2006, stool specimens submitted for *Clostridium difficile* toxin testing were analyzed by 7 commercially available, FDA approved, enzyme immunoassays (EIA) for the presence of *C. difficile* toxins and compared to cell culture cytotoxicity as the gold standard.

A total of 300 stool specimens were tested. All specimens were fresh, non-formed stools, stored at 5°C for no more than 24 hours before testing.

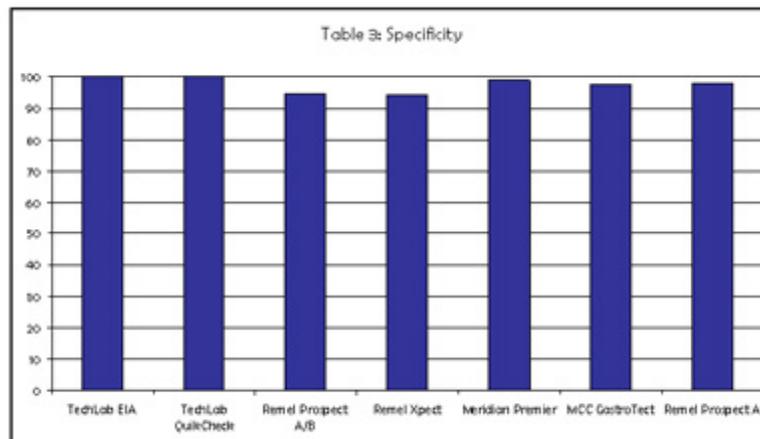
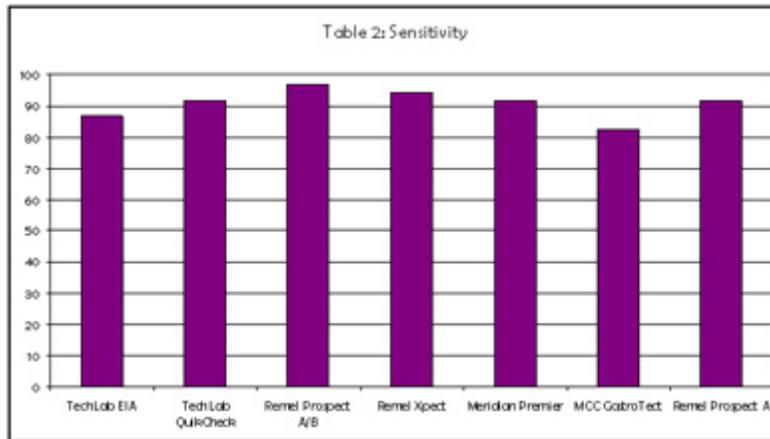
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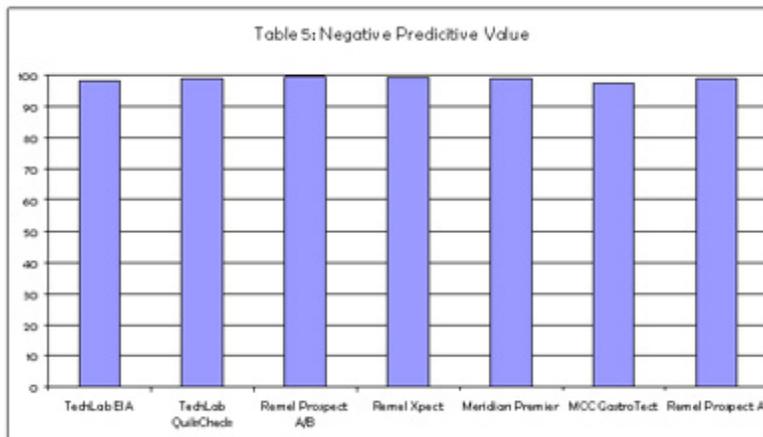
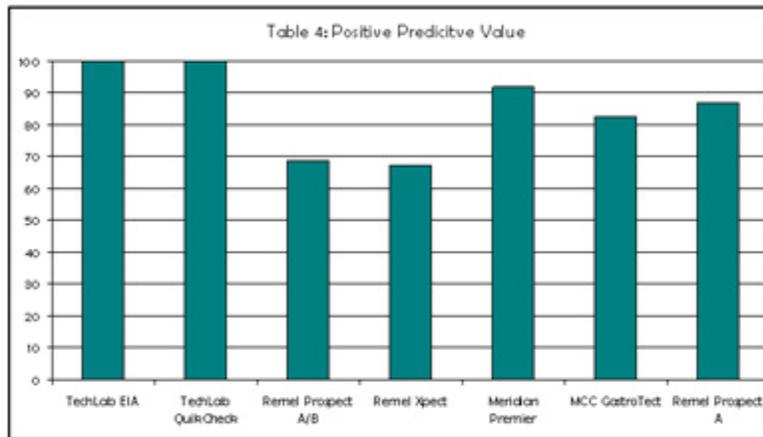
1. *C. DIFFICILE TOX A/B II* (TechLab®, Blacksburg, VA)
2. TOX A/B QUIK CHECK (TechLab®, Blacksburg, VA)
3. ProSpecT *C. difficile* Toxin A Assay (Remel, Lenexa, KS)
4. ProSpecT *C. difficile* Toxin A/B Assay (Remel, Lenexa, KS)
5. X/pect *C. difficile* Toxin A/B (Remel, Lenexa, KS)
6. Gastro-Tect (Medical Chemical Corp., Torrance, CA)
7. Premier Toxins A&B (Meridian, Cincinnati, OH).

Cell culture cytotoxicity was performed using the kit from TechLab (Blacksburg, VA) which uses Human Foreskin Fibroblasts (Diagnostic Hybrids, Inc.) and anti-Toxin B for blocking assay to ensure specificity.

All specimens were tested according to the manufacturers' package insert.

	TechLab EIA	TechLab QuikCheck	Remel Prospect A/B	Remel Xpect	Meridian Premier	MCC GastroTect	Remel Prospect A only
Sensitivity	86.8%	91.7%	97%	94.3%	91.7%	82.5%	91.7%
Specificity	100%	100%	94.6%	94.3%	98.9%	97.4%	98.1%
Positive Predictive Value	100%	100%	68.8%	67.3%	91.7%	82.5%	86.8%
Negative Predictive Value	98.1%	98.9%	99.6%	99.3%	98.9%	97.4%	98.9%





RESULTS

Of the 300 specimens evaluated, 33 exhibited a specific cytotoxic effect in cell culture and therefore considered to be positive by the gold standard.

The positive rate in our institution for this study is 11.0%

The calculated sensitivities, specificities, positive predictive values (PPVs), and negative predictive values (NPVs) for all commercially available kits tested are provided in Tables 1-5.

Sensitivity / NPV:

- Remel Prospect A/B yielded the highest sensitivity at 97%.
- Only the TechLab[®] A/B and MCC GastroTect yielded sensitivities less than 90%.
- Remel Prospect Toxin A only EIA sensitivity was 91.7%
- Overall high sensitivities yielded NPVs at 97.4% or higher.

Specificity / PPV:

- Both TechLab[®] EIAs were 100% specific
- Remel Prospect Toxin A, Meridian Premier and MCC GastroTect yielded specificities between 97.4% and 98.9%.
- Two of the three Remel EIAs were less than 95% specific.
- Overall, PPVs ranged from 67.3% (Remel Xpect) to 100% (both TechLab products).

Two specimens were cell culture cytotoxicity positive and negative for Toxin A by EIA. Both specimens were positive by at least five of six Toxin A and B EIAs evaluated.

CONCLUSIONS

The TechLab[®] EIAs were 100% specific yielded NPVs of 100%

For low volume laboratories, the TechLab[®] QuickCheck offers a very good combined sensitivity and specificity.

The Remel products yielded the overall highest sensitivities, but also decreasing specificities.

The Remel Prospect Toxin A performed as well against the gold standard as most A plus B EIAs.

Our study suggests that FDA approved EIA test kits are an appropriate alternative to cell culture cytotoxicity.

Based on this study our selection criteria will include prevalence of disease in the patient population, statistical analysis of the prospective study, volume of tests run per day and clinician utilization and interpretation of results.

Clinician input on the trade off of sensitivity (and NPV) versus specificity (and PPV) should be considered in the final selection criteria.

Given the high sensitivity of the Prospect Toxin A EIA, the two specimens negative by Toxin A EIA and positive by cell culture cytotoxicity (and at least five of six A plus B EIAs) suggests the presence of Tox A- / Tox B + *C. difficile* strain in our institution. Further analysis by PCR and bacterial culture is required to elucidate this possibility.

Ease of use:

- TechLab A/B and Meridian Premier A/B EIAs yielded most rapid turn around times for result reporting.
- The MCC GastroTect EIA pipettor for sample preparation / dilution was restrictive (small bore opening) for some stool samples.
- Overall, all kits were easy to use.