JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2007, p. 2737–2739 Vol. 45, No. 8
0095-1137/07/$08.00+0 doi:10.1128/JCM.00686-07


# Detection of *Clostridium difficile* Toxin: Comparison of Enzyme Immunoassay Results with Results Obtained by Cytotoxicity Assay▽

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Received 28 March 2007/Returned for modification 30 May 2007/Accepted 1 June 2007

**Several kinds of laboratory techniques are available to detect *Clostridium difficile* toxin in fecal samples. Because questions have been raised about the reliability of immunoassays compared to the accepted standard, cytotoxicity assay, we studied three enzyme immunoassays (EIAs) and one rapid EIA, which demonstrated relatively good sensitivities and specificities compared to cytotoxicity assay.**

*Clostridium difficile* colitis has increased in prevalence and severity in hospitals throughout the developed world (4, 7, 10). Prompt, accurate diagnosis and early treatment shorten the duration of diarrhea which, in turn, reduces the spread of infection (6, 12, 14). A cytotoxicity assay (CYTA) has been regarded as the "gold standard" for detecting *C. difficile* toxin in a fecal sample (5), but this test is labor-intensive and requires 18 to 48 h of incubation before a final reading can be made. We compared CYTA with commercially available enzyme immunoassays (EIAs) and one rapid card EIA, all of which detect *C. difficile* toxins A and B.

(These data were presented in preliminary form at the 44th Annual Meeting of the Infectious Disease Society of America, October 2006.)

CYTA (*C. difficile* toxin detection kit; Diagnostic Hybrids, Athens, OH) utilized microwell plates containing cultured human foreskin fibroblasts to detect toxin B. Every study included a toxin-positive and a toxin-negative control, and each fecal sample was studied without or with the addition of antibodies to toxins A and B. Results were read after overnight incubation (16 to 18 h) and again after 48 h of incubation by readers who were blinded to the results of the other studies. For EIA, we initially used a Premier Toxins A&B EIA kit (EIAPrem; Meridian Bioscience, Cincinnati, OH) in accord with the manufacturer's instructions to detect *C. difficile* toxins A and B. Subsequent studies utilized *C. difficile* TOX A/B II (EIATech; TechLab, Blacksburg, VA) and the ProSpecT *Clostridium difficile* toxin A/B microplate assay (EIAPro; Remel, Lanexa, KS), both of which also detect toxins A and B of *C. difficile*. One rapid EIA (REIA) immunochemical detection card (Immuno*Card*; Meridian Bioscience) was also tested in accord with the manufacturer's instructions; each card included positive and negative controls.

In the first phase of this study, we addressed the question of whether EIA or REIA could reliably reproduce results of CYTA, using a single representative EIA and REIA. Accordingly, we initially tested 446 consecutive fecal samples submitted to the Microbiology Laboratory, Michael E. DeBakey VA Medical Center, Houston, TX, for detection of *C. difficile* toxin, comparing CYTA, EIAPrem, and REIA. For the purposes of this study, CYTA was regarded as providing true results. Seventy-six (17.0%) samples were positive by CYTA. In every case in which the result was positive, a correct reading could be made after overnight incubation (16 to 18 h), although the manufacturer's instructions recommend incubation for up to 48 h for a final reading. As shown in Table 1, of these 76 samples, 75 were also positive by EIAPrem (sensitivity, 98.7%; confidence interval [CI], 92 to 99% [Microsoft Excel 2003]). Of the 370 that were negative by CYTA, 10 were positive by EIAPrem (specificity, 97.3%; CI, 95 to 98%). Thus, for EIAPrem, the positive predictive value was 75/85 samples (88.2%; CI, 79 to 94%) and the negative predictive value was 360/361 samples (99.7%; CI, 98 to 99%).

REIA was positive for 73 of 76 CYTA-positive specimens (Table 1) (sensitivity, 96.1%; CI, 88 to 99%). Of the 370 CYTA-negative specimens, 4 were positive by REIA (specificity, 98.9%; CI, 97 to 99%). For REIA, the positive predictive value was 73/77 samples (94.8%; CI, 87 to 98%), and the negative predictive value was 366/369 samples (99.2%; CI, 97 to 99%).

Having shown that results obtained with a representative EIA much more closely resembled those of CYTA than had been suggested by some earlier literature (2, 9, 13, 15, 16), we then compared three EIAs that are commercially available in the United States, utilizing receiver operator curve statistical analysis (True Epistat; Epistat Services, Richardson, TX). For this phase of the study, we used a convenience sample of 131 fresh fecal specimens, 54 of which were CYTA positive and 77 of which were CYTA negative. As shown in Table 2, the sensitivity of EIAPrem and EIATech was 96.3%, and the sensitivity of EIAPro was 90.7%. There were no statistical differences seen in comparing test sensitivities of EIAPrem or EIAPro and EIATech ($P = 0.25$). The specificity of EIAPro was 97.4%, versus 93.5% for EIAPrem and 87.0% for EIATech (Table 2). Although there was no difference in

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▽ Published ahead of print on 13 June 2007.

TABLE 1. CYTA, EIA, and REIA for detecting *C. difficile* toxin in 446 consecutive fecal samples[a]

| Group | No. of samples (total $n$ = 446) | Test result[b] | | |
|---|---|---|---|---|
| | | CYTA | EIA | REIA |
| 1 | 73 | + | + | + |
| 2 | 2 | + | + | − |
| 3 | 1 | + | − | − |
| 4 | 2 | − | + | + |
| 5 | 8 | − | + | − |
| 6 | 2 | − | − | + |
| 7 | 358 | − | − | − |

[a] EIA, Premier Toxins A&B EIA; REIA, Immuno*Card* rapid card EIA.
[b] The sensitivities of EIA and REIA (relative to CYTA) were 98.7% and 96.1%, respectively, and the specificities were 97.3% and 98.9%, respectively.

TABLE 2. Comparison of three EIAs for *C. difficile* toxin in a convenience sample of 131 fecal specimens

| Group | No. of samples (total $n$ = 131) | Test result[a,b] | | | |
|---|---|---|---|---|---|
| | | CYTA | EIAPrem | EIATech | EIAPro |
| 1 | 49 | + | + | + | + |
| 2 | 2 | + | + | + | − |
| 3 | 1 | + | + | − | − |
| 4 | 1 | + | − | + | − |
| 5 | 1 | + | − | − | − |
| 6 | 3 | − | + | + | − |
| 7 | 2 | − | + | − | − |
| 8 | 7 | − | − | + | − |
| 9 | 2 | − | − | − | + |
| 10 | 63 | − | − | − | − |

[a] EIAPrem, Premier Toxins A&B EIA; EIATech, *C. difficile* TOX A/B II; EAIPro, ProSpecT *Clostridium difficile* toxin A/B microplate assay.
[b] The sensitivities of the EIAPrem, EIATech, and EIAPro tests (versus CYTA) were 96.3%, 96.3%, and 90.7%, respectively, and their specificities were 93.5%, 87.0%, and 97.4%, respectively.

test specificities between EIAPro and EIAPrem ($P$ = 0.45), the specificity of EIAPro was significantly lower than that of EIATech ($P$ = 0.04).

The results of this study show that compared to CYTA, three commercially available EIAs and one REIA reliably detect the presence of *C. difficile* toxin in fecal samples. When EIAPrem, REIA, and CYTA were compared with 446 samples, 431 (96.6%) were either positive or negative in all three assays, indicating a high degree of concordance. When EIAPrem, EIATech, and EIAPro were compared, the overall concordance among all three tests was somewhat lower (112 of 131 samples [85.5%]) because of the relatively lower sensitivity but higher specificity of EIAPro. We repeated all tests on every sample that yielded discordant results, leading to a marginally better rate of concordance, but the data presented in this paper were those determined by the initial test, just as would be the case for data provided to clinicians by clinical laboratories.

Earlier studies of EIA for toxins A and B reported sensitivities of 57% to 100%, averaging 83%, compared to CYTA (1–3, 8, 9, 11, 13, 15–17). Many of these studies required that three samples be tested, and a positive result was reported if any of the three was positive. The important implication of our results is that using kits that are presently available, a single EIA is likely to yield a highly reliable result; multiple samples need not be submitted for analysis, and only modest benefits in diagnostic accuracy would be obtained by replacing EIA with CYTA. It appears that manufacturers may have improved the performance of their EIA kits since these kits were introduced.

It is worth noting that, in this study, when discordant results were obtained, they were generally confirmed by repeat testing. In those few instances when repeat testing yielded a different result, we still calculated our data based on the initial results in order to simulate the usual clinical situation. Interestingly, the results in one case illustrate the adage that there is no true "gold standard." One fecal sample that was positive by CYTA was negative by EIA and REIA; the three assays were repeated, yielding the same results. A review of this patient's records showed that he had not received antibiotics and had no clinical findings of *C. difficile*-associated disease (no diarrhea, fever, leukocytosis, or abdominal pain); in fact, the primary physicians were unclear as to why a specimen had been submitted. A new fecal sample from this patient was negative in all assays. The patient received no treatment for *C. difficile* colitis and remained free of symptoms. We regard the initial CYTA result as being falsely positive.

CYTA, the "gold standard" for assaying toxins A and B of *C. difficile*, is labor-intensive, requires tissue-cultured cells and an inverted microscope, and needs overnight incubation before the results are read. EIA is also labor-intensive, requiring several hours of technician time and an EIA reader; batching of specimens increases cost efficiency but may delay reporting of results, especially if tests are not done every day. REIA is more costly for each test but, for laboratories that process only occasional samples, appears to provide prompt, reliable, and cost-effective results.

The Infectious Disease Laboratory of the Michael E. DeBakey VA Medical Center is funded by the Department of Veterans Affairs through the Merit Review Program. The Infectious Disease Laboratory has received funds from Romark Pharmaceuticals, Salix, and Genzyme for studies of agents to treat *C. difficile* colitis.

The present study was conceived and designed by the investigators.

Meridian, TechLab, and Remel graciously provided supplies for use in the experiments.

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