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Comparison of Three Commercial Methods for Rapid Detection of *Clostridium difficile* Toxins A and B from Fecal Specimens[▽]

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Three rapid enzyme immunoassays (X/pect *Clostridium difficile* Toxin A/B test, Wampole Tox A/B Quik Chek, and ImmunoCard Toxins A&B) were compared for the diagnosis of *Clostridium difficile* infection. Of the 367 stool specimens tested, 102 (27.8%) were positive for toxigenic *C. difficile* when a combination of direct cytotoxicity assay and cytotoxic culture was used as the gold standard. Sensitivity/specificity values were 49.0%/95.8%, 54.9%/95.5%, and 66.7%/95.1%, respectively. The median times to test five stool specimens were 28, 30, and 24 min, respectively. The ImmunoCard test was the quickest and most sensitive test of the three enzyme immunoassays evaluated.

During the last few decades, the gold standard for the diagnosis of *Clostridium difficile* infection (CDI) has been detection of cytotoxicity from stool specimens (2). However, some studies have demonstrated that cytotoxicity testing performed on *C. difficile* isolates can improve the sensitivity of this diagnostic method by 15 to 22% (1, 8). Unfortunately, this method can take up to 96 h and requires a virology laboratory to supply cells (4). Therefore, most laboratories turn to other procedures for diagnosis of CDI, especially enzyme immunoassays (EIAs) that detect *C. difficile* toxins. Recently, three rapid membrane-based EIAs that detect both A and B toxins in less that 30 min have been commercialized: X/pect *Clostridium difficile* Toxin A/B test (Remel, Lenexa, KS) (X/pect), Wampole Tox A/B Quik Chek (TechLab, Blacksburg, VA) (Quik Chek), and ImmunoCard Toxins A&B (Meridian Bioscience, Cincinnati, OH) (ImmunoCard). Although they have all been compared with cytotoxicity assay and other diagnostic procedures, no comparisons among the three procedures have been published (3, 5–7, 9, 10). The goal of this study was to compare the yields of these rapid EIAs for the diagnosis of CDI in stool specimens.

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The study was carried out during a 6-month period in a 1,750-bed tertiary teaching center serving a population of approximately 715,000 inhabitants. During the first month of study, all stool specimens received in the laboratory for diagnosis of CDI were tested. During the remaining months, the study included those refrigerated stool specimens with a positive result for toxigenic *C. difficile* using the gold standard procedure in order to increase the statistical power of the comparison.

The gold standard was considered the combination of direct cytotoxicity assay from stool specimens and cytotoxicity assay from isolates, so a true positive result was defined as positive by direct cytotoxicity assay or negative by direct cytotoxicity assay but positive by cytotoxic culture (1, 8). Direct cytotoxicity assay was performed by centrifuging stool specimen dilutions (1/40) made with phosphate-buffered saline and filtering 500 μl of supernatant onto monolayers of human MRC-5 fibroblasts. A test result was not considered negative until after 48 h of incubation at 37°C. Specificity of the cytopathic effect was confirmed using a neutralizing high-titer *C. difficile* antitoxin (TechLab) following the manufacturer's instructions. Specimens were also directly cultured in selective medium (*Clostridium difficile* agar; bioMérieux, Marcy l'Etoile, France), incubated at 35 to 37°C in an anaerobic atmosphere, and observed after 48 h of incubation. When the direct cytotoxicity assay was negative and the culture was positive, brain heart infusion broth was inoculated with *C. difficile* colonies, incubated for 24 h in an anaerobic atmosphere, filtered, and tested using the cytotoxicity assay (cytotoxic culture).

EIAs were performed according to the manufacturer's instructions. In order to know the workload for the performance of the EIAs, the median times to test stool specimens with the different procedures were calculated by running four sets of five stool specimens each.

Validity values were calculated with a 95% confidence interval (CI) following an exact binomial distribution. Sensitivities and specificities were compared using the McNemar test for paired samples with two tails. Predictive values were compared using Fisher's exact test with two tails. The level of significance was corrected ($P < 0.017$) with the Bonferroni test in order to acknowledge the existence of three comparisons. Data were analyzed using the SPSS software package, version 15.0 (Chicago, IL).

During the study period, a total of 367 stool specimens from 305 patients were tested. Toxigenic *C. difficile* was detected in 102 stool specimens (27.8%) from 85 patients using the gold standard. The sensitivity of the direct cytotoxicity assay was 79.4%. Sensitivity values for X/pect, Quik Chek, and ImmunoCard were 49.0%, 54.9%, and 66.7%, respectively, while specificity values were 95.8%, 95.5%, and 95.1%, respectively. The

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TABLE 1. Sensitivities, specificities, and positive and negative predictive values of the three EIAs

Test (manufacturer)	Mean % (95% CI)[a]			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
X/pect *Clostridium difficile* Toxin A/B (Remel)	49.0 (39.0–59.1)	95.8 (92.7–97.9)	82.0 (70.0–90.6)	83.0 (78.3–87.0)
Wampole Tox A/B Quik Chek (TechLab)	54.9 (44.7–64.8)	95.5 (92.2–97.6)	82.4 (71.2–90.5)	84.6 (80.0–88.5)
ImmunoCard Toxins A&B (Meridian)	66.7 (56.6–75.7)	95.1 (91.8–97.4)	84.0 (74.1–91.1)	88.1 (83.8–91.6)

[a] *P* values for the comparison of validity values between the X/pect *Clostridium difficile* Toxin A/B test (Remel) and the Wampole Tox A/B Quik Chek test (TechLab) were 0.180 (sensitivity), 1 (specificity), 1 (positive predictive value), and 0.659 (negative predictive value). *P* values for the comparison of validity values between the X/pect *Clostridium difficile* Toxin A/B test (Remel) and the ImmunoCard Toxins A&B test (Meridian) were <0.0001 (sensitivity), 0.839 (specificity), 0.823 (positive predictive value), and 0.081 (negative predictive value). *P* values for the comparison of validity values between the Wampole Tox A/B Quik Chek test (TechLab) and the ImmunoCard Toxins A&B test (Meridian) were 0.012 (sensitivity), 1 (specificity), 0.829 (positive predictive value), and 0.231 (negative predictive value). A *P* value of <0.017 was considered statistically significant.

greater sensitivity of ImmunoCard compared with the other tests was statistically significant ($P < 0.013$), although there were no statistically significant differences between specificities. Predictive values tended to be better with ImmunoCard than with the other tests (Table 1). As most published evaluations of EIA tests use only direct cytotoxicity as the gold standard, we reanalyzed our data using this criterion in order to compare our results with those of other authors (Table 2).

ImmunoCard was the quickest technique, with a median time to test five stool specimens of 23 min 53 s, followed by X/pect (27 min 34 s), and Quik Chek (30 min 24 s). The differences between the median times for the three techniques were statistically significant ($P < 0.0001$).

To our knowledge, only six published reports evaluated any of the three tests (3, 5–7, 9, 10) (Table 2). However, none of them combined the cytotoxicity assay of the specimens and of the isolates as the gold standard, and some used techniques other than cytotoxicity as the gold standard (6, 7). The number of samples studied is very limited in most reports, and the large 95% CIs reflect inaccuracy in the validity values. Our study used the combination of cytotoxicity assays from stools and from isolates as the gold standard and was carried out with a large number of samples.

Diederen et al. (3) reported the only evaluation of X/pect, using 35 positive stool specimens by direct cytotoxicity assay, and like us, they concluded that the sensitivity of this technique was very low (37.1%). Unfortunately, they did not use negative specimens and therefore could not test specificity.

Two studies evaluated the reliability of Quik Chek. The first analyzed the concordance between this test and the ImmunoCard, and neither gold standard was used (6). The results showed that ImmunoCard yielded more positive results than Quik Chek. The gold standard in the second study was a nested PCR that detected the *tcdB* gene. The sensitivity and specificity values (94.7 and 97.2%, respectively) were greater than those obtained in our study. However, the use of a different gold standard in that study prevents us from comparing the values with those of our study (7).

ImmunoCard has been evaluated in five published studies (3, 5, 7, 9, 10). Four of them used direct cytotoxicity assay as a reference, and values of sensitivity and specificity ranged from 86.2% to 96.1% and from 93.8% to 98.9%, respectively. Using the same gold standard, our study showed lower sensitivity and similar specificity.

None of the published works compared the three rapid EIAs. Diederen et al. compared X/pect and ImmunoCard (3) using 35 Vero cell toxin-positive samples and concluded that ImmunoCard has better sensitivity than X/pect. Although the study was limited due to the low number of samples, the authors agree with us as to the significance of the comparison. Samra et al. compared Quik Chek and ImmunoCard using a nested PCR performed directly on the stool specimens as the

TABLE 2. Sensitivities and specificities of the three EIAs as reported in the literature

Reference	Gold standard	No. of stool specimens[a]:		Mean % (95% CI)[b]					
		With toxigenic *C. difficile*	Without toxigenic *C. difficile*	X/pect *Clostridium difficile* Toxin A/B test (Remel)		Wampole Tox A/B Quik Chek test (TechLab)		ImmunoCard Toxins A&B test (Meridian)	
				Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
10	Direct cytotoxicity assay	23	344					91.3 (72.0–98.9)	97.4 (95.1–98.8)
9	Direct cytotoxicity assay	65	81					86.2 (75.3–93.5)	93.8 (86.2–98.0)
6	ImmunoCard Toxins A&B test (Meridian)	17	24			94.1 (71.3–99.9)	100 (85.7–100)		
3	Direct cytotoxicity assay	35	0	37.1 (32–54)				88.6 (73–96)	
5	Direct cytotoxicity assay	76	370					96.1 (88–99)	98.9 (97–99)
7	PCR	94	106			94.7 (88.0–98.3)	97.2 (92.0–99.4)	94.7 (88.0–98.3)	97.2 (92.0–99.4)
Present work[c]	Direct cytotoxicity assay	81	286	61.7 (50.3–72.3)	96.2 (93.2–98.1)	66.7 (55.3–76.8)	95.1 (91.9–97.3)	79.0 (68.5–87.3)	94.1 (90.7–96.5)

[a] According to the gold standard procedure used in the study.
[b] When not provided by the authors in the article, these values were calculated following an exact binomial distribution.
[c] Data are presented using the direct cytotoxicity assay as the gold standard in order to compare the values with those reported by most of the other studies.

gold standard (7). They found similar specificities in both tests, although they did not detect differences in sensitivities.

In conclusion, our findings show that the ImmunoCard Toxins A&B test was the quickest and most sensitive test of the three EIAs for the rapid diagnosis of CDI in clinical specimens. Compared with the gold standard, the sensitivity of all the evaluated EIAs was relatively poor for the diagnosis of CDI. In order to obtain an optimal diagnosis of the disease, a more sensitive test could be used in combination with the EIAs as a toxigenic culture method.

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