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Rapid Detection of Toxigenic Strains of *Clostridium difficile* in Diarrheal Stools by Real-Time PCR[▽]

Clostridium difficile is the major causative agent of nosocomial diarrhea and pseudomembranous colitis. The pathogenicity of *C. difficile* is related to the production of the toxins TcdA and TcdB. Currently the "gold standard" for the diagnosis of *C. difficile*-associated infections (CDI) is the cytotoxicity assay. However, this method is time-consuming and labor-intensive and requires an incubation of at least 24 h and cell culture facilities. Several studies have reported in-house real-time PCR for CDI diagnosis with promising performances (1–3, 5). As of October 2007, a real-time PCR assay (BD GeneOhm Cdiff; San Diego, CA) has become commercially available both in the United States and in Europe. The first evaluation of this assay has been recently published in the Journal of Clinical Microbiology, indicating an overall agreement with the stool cytotoxicity assay of 94.8% (4).

We performed a prospective study to evaluate the performances of the BD GeneOhm Cdiff assay in detecting toxin B-producing *C. difficile* in stool specimens. The results were compared to results from the cytotoxicity assay and the toxigenic culture.

Three hundred consecutive diarrheal stool samples (stools taking the shape of the container) from patients suspected of having CDI were included. Stool samples were stored at 4°C until processing and analysis were done within 24 to 36 h of collection. The cytotoxicity assay was performed using MRC-5 cells. Fresh stool specimens were diluted in phosphate-buffered saline buffer (1:10 [wt/vol]) and centrifuged at 2,500 × *g* for 30 min. The supernatant was passed through a 0.45-μm-pore-size filter and inoculated onto confluent monolayers of MRC-5 cells in 96-well microtiter plates that were incubated at 37°C in a 6.5% CO_2 atmosphere for 48 h. The final dilution of the fecal filtrate in each well was 1:100. Samples were considered positive if a characteristic cytopathic effect (cell rounding) was observed for at least 50% of the cells and could be neutralized with anti-*Clostridium sordellii* antiserum (obtained from M. R. Popoff, National Reference Center for Anaerobes, Institut Pasteur, Paris, France). Culture was performed on selective medium (brain heart infusion broth supplemented with 5% defibrinated horse blood, 0.1% taurocholate, 250 μg/ml cycloserine, and 10 μg/ml cefoxitin), and plates were incubated for 48 h in an anaerobic atmosphere. Colonies were identified by use of an enzymatic profile from the RapID32A gallery (bioMérieux, La Balme les Grottes, France). Then, *C. difficile* isolates were incubated in brain heart infusion broth for 5 days, and the supernatant was tested using the cytotoxicity assay. This method is referred as the toxigenic culture.

The BD GeneOhm Cdiff assay was performed according to the manufacturer's protocol. A dry swab was dipped into diarrheal stool samples and resuspended in sample preparation buffer. Genomic DNA was extracted using a lysis buffer (provided by the manufacturer) combining chemical and physical actions. Amplifications were performed using the SmartCycler (Cepheid, Sunnyvale, CA) with primers specific for *tcdB* and for an internal control. Every PCR run included a PCR-positive control (reconstituted DNA from the manufacturer's kit) and a negative control. Discrimination of amplicons was done using two molecular beacons with different fluorimetric properties. In cases of unresolved (internal control invalid due to the presence of an inhibitory specimen or reagent failure) or invalid (failure of the positive or negative controls) results, the tests were repeated. Results of real-time PCR were not known by the technicians performing culture or cytotoxicity assay (blind evaluation).

The prevalence of positive cytotoxicity assay was 8.3% (25/300). The overall agreement between the BD GeneOhm Cdiff assay and the cytotoxicity assay was 92% (276/300). The sensitivity, specificity, and positive and negative predictive values of real-time PCR were 96%, 95.1%, 64.9%, and 99.6%, respectively. A toxin-producing isolate was isolated from 33 stool specimens (11.0%). Compared to the toxigenic culture, the sensitivity, specificity, and positive and negative predictive values were 93.9%, 97.7%, 83.8%, and 99.2% for the real-time PCR and 75.8%, 100%, 100%, and 97.1% for the cytotoxicity assay, respectively. Results of real-time PCR were unresolved in 7.3% (22/300) of cases and remained unresolved in 3.3% (10/300) after the repeated testing. All the unresolved results were actually negative with the cytotoxicity assay and the toxigenic culture.

The overall agreement between the BD GeneOhm Cdiff assay and the toxigenic culture was 94% (282/300) and was very similar to that of 95.3% (385/404) found by Stamper et al. (4). However, we found a higher rate of unresolved results upon initial testing than that reported by Stamper et al. (7.3% versus 0.7%), without any clear explanation. The performance characteristics of the BD GeneOhm Cdiff assay are also in agreement with those of the previous in-house real-time PCR evaluations (1–3, 5), which were shown to have a better sensitivity than the cytotoxicity assay, which is considered so far to be the gold standard for CDI diagnosis.

In conclusion, our results indicate that the BD GeneOhm Cdiff assay is a more rapid (less than 3 h) and more sensitive method than the cytotoxicity assay for the detection of toxigenic *C. difficile* in stool samples. A combination of a quick turnaround time with high performance might result in a better management of CDI and a timely implementation of infection control measures.

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Frédéric Barbut*
Mylena Braun
Béatrice Burghoffer
Valérie Lalande
Catherine Eckert
National Reference Laboratory for Clostridium difficile
Assistance Publique-Hôpitaux de Paris
Hôpital Saint-Antoine
Université Pierre et Marie Curie
75012 Paris, France

*Phone: 33 1 49 28 30 08
Fax: 33 1 49 28 30 09
E-mail: frederic.barbut@sat.aphp.fr

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