A Cost-Effective Approach for Detection of Toxigenic *Clostridium difficile*: Toxigenic Culture Using ChromID *Clostridium difficile* Agar

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We evaluated the performance and the cost of toxigenic culture using a commercial chromogenic medium (CDIF) for 538 stool specimens. Compared with real-time PCR, this method was found to detect an additional 9% of positive specimens and result in 61% reduction in material costs, with a trade-off increase in turnaround time of 1 day.

Accurate and reliable laboratory diagnosis of *Clostridium difficile* infection (CDI) remains a challenge to microbiologists 35 years after its discovery (1). While toxin enzyme immunoassays (EIA) have unacceptably low sensitivities (2), the cytotoxin neutralization assay (CTN) is too time-consuming and labor-intensive to perform. The performance of more recently developed PCR tests for toxin gene detection is promising, with mean sensitivity ranging from 90% to 100% and specificity ranging from 96% to 99% (3, 4). The hand-on time of some commercial assays can be minimal, since extraction and PCR are all carried out in a self-contained cartridge (4). However, PCR has not been widely utilized in clinical laboratories, presumably due to budgetary issues (5). Alternatively, *C. difficile* isolates can be recovered on selective media. When combined with a sensitive and specific toxin detection method, such as CTN or PCR, toxigenic culture is regarded as one of the gold standards of CDI diagnosis (6). The prototype selective culture medium cycloserine-cefoxitin-fructose-egg yolk agar (CCFA) requires 48 h of incubation and alcohol treatment (7). As a result, toxigenic culture is advocated as part of a diagnostic algorithm to increase the yield, after initial screening by glutamate dehydrogenase (GDH) EIA (8–11). With the availability of a commercial chromogenic selective medium, ChromID *C. difficile* agar (CDIF) (bioMérieux, France), that allows direct

recovery of *C. difficile* within 24 h of incubation (12, 13), coupled with rapid identification by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry and inference of toxigenicity by PCR for the toxin gene (9), toxigenic culture as a routine diagnostic test is no longer impractical. The aim of the present study was to compare the performance and the cost of toxigenic culture against those of real-time PCR performed on stool specimens.

Briefly, 538 soft or liquid stool samples were plated directly onto CDIF medium, which was then incubated in an anaerobic chamber for 48 h according to standard laboratory methods. Suspected flat and irregular colonies were confirmed to be *C. difficile* by MALDI-TOF mass spectrometry (Biotyper 3.0; Bruker Dal-

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FIG 1 Results of the diagnostic algorithm using toxigenic culture.

TABLE 1 Comparison of real-time PCR results with those of toxigenic culture for the diagnosis of CDI^a

PCR result	No. of specimens with toxigenic culture result	
	Positive	Negative
Positive	106	2
Negative	11	416

^a Anaerobic culture was performed on the 538 test stools by plating the specimens onto CDIF medium. All *C. difficile* culture isolates were tested for the presence of toxin production (as evidenced by detection of the *tcdC* gene) by real-time PCR. Real-time PCR for the presence of *tcdC* was performed on 535 stool specimens. The quantities of three specimens were insufficient for real-time PCR, and one of these was found to be toxigenic culture positive. The sensitivity, specificity, positive predictive value, and negative predictive value were 90.6%, 99.5%, 98.1%, and 97.4%, respectively.

tonik, Germany). All *C. difficile* isolates were tested for the presence of a 176-bp fragment of the *tcdC* gene and its 158-bp fragment deletion by real-time PCR (LightMix *C. difficile* kit; TIB MOLBIOL GmbH, Germany), using a suspension of 5 to 10 colonies. All samples were also tested directly for the presence of *tcdC* by the same PCR assay according to the manufacturer's instructions, after DNA extraction (NucliSENS easyMag system; bioMérieux, France).

Overall, the prevalence of positive *C. difficile* cultures was 27.5% (Fig. 1); 79.7% of positive cultures contained the toxin gene. For the subset of specimens ($n = 246$) that were examined daily, 69 had growth of toxigenic *C. difficile* at 24 h of incubation. An additional 6 isolates (8%) were recovered after 48 h of incubation. In comparison, there were 108 (20.2%) real-time-PCR-positive stool specimens. The quantities of three specimens were insufficient for real-time PCR. The difference in detection of positive specimens between toxigenic culture and real-time PCR was significant ($P = 0.02$ by McNemar's test for paired proportions). Using toxigenic culture as the gold standard, the sensitivity, specificity, and positive and negative predictive values of real-time PCR were 90.6%, 99.5%, 98.1%, and 97.4%, respectively (Table 1).

An increase in the detection of patients harboring toxigenic *C. difficile* could have important implications for infection control. A study that used multilocus sequence typing (MLST) to match over 1,000 cases of hospital-acquired CDI revealed that no more than 25% of the cases could be linked to another CDI case epidemiologically (14). The fact that most transmissions came from patients with "undiagnosed" CDI could be partly attributable to the

suboptimal sensitivity of the commonly used diagnostic tests. With improved detection of CDI by toxigenic culture, a significant proportion of cross-transmissions can be averted. Nevertheless, there are concerns about the specificity of toxigenic culture, which does not discriminate between CDI and asymptomatic colonization. A study demonstrated that the presence of free toxins (CTN) but not toxigenic *C. difficile* (toxigenic culture or PCR) correlated with disease severity and 28-day mortality in 6,522 patients with suspected CDI (15). In contrast, CTN missed 45% of clinically significant CDI in another study (16). To enhance the diagnostic accuracy of CDI testing, careful selection of patients in the correct clinical context is crucial.

In our study, over 90% of *C. difficile* isolates were recovered within 24 h of incubation, presumably owing to the superior ability of the medium to stimulate germination. Two percent of specimens might require an additional 24 h of incubation to recover the scant growth of colonies. Chart review of those cases revealed that the patients had mild diarrhea that subsided without treatment. In addition, the quantity of one specimen was insufficient for real-time PCR. In order not to delay the reporting for the majority of specimens, we suggested reporting a negative result after 24 h of incubation with supplementary reporting if necessary. Since subculture of suspected colonies for follow-up testing was not needed, reporting of results could be accomplished within two working days from the receipt of most specimens. Compared with real-time PCR, the relative delay in turnaround time was 1 day. However, if PCR was not performed on a daily basis, the increase in turnaround time would not be significant. For example, real-time PCR was performed on stool specimens thrice per week due to inadequate extraction equipment in our laboratory. Only 6% of the test results could be reported on the same day. In fact, our audit data showed that the turnaround time of toxigenic culture was the same as that of real-time PCR for 80% of the specimens (2.9 days).

The presence of *tcdC* in *C. difficile* isolates is indicative of the presence of both *tcdA* and *tcdB* (17, 18). Although the putative role of *tcdC* as a negative regulator of *tcdA* and *tcdB* has recently been refuted (19), detecting the 18-bp or 39-bp deletion in the gene can rapidly identify the "hypervirulent" PCR ribotype 027 or 078 (20). This is particularly important in regions where molecular typing is not routinely performed on sporadic clinical isolates.

Implementing toxigenic culture to diagnose CDI could result in a 31% cost reduction (Table 2). The reduction in the cost of

TABLE 2 Cost, processing time, and yield of positive results of toxigenic culture and real-time PCR

Assay	No. (%) of specimens ^a		Cost (US\$)						Daily processing time (h)
			Materials		Labor ^b		Total		
	Processed	Positive	Total	Per specimen ^c	Total	Per specimen ^c	Total	Per specimen ^c	
Toxigenic culture									
Culture on CDIF medium	538 (100)	181 (33.6)	1,065	1.98	1,160	2.16	2,225	4.14	0.5
MALDI-TOF on suggestive colonies	181 (33.6)	148 (27.5)	19	0.10	1,160	6.41	1,179	6.51	0.5
Real-time PCR on <i>C. difficile</i> isolates	148 (27.5)	118 (21.9)	1,743	11.78	3,680	24.86	5,423	36.64	1.0
Total	538 (100)		2,827	5.25	6,000	11.15	8,827	16.41	2.0
Real-time PCR	535 (100)	108 (20.2)	7,297	13.64	5,520	10.31	12,817	23.96	1.5

^a Data are based on data from Table 1 and Fig. 1.

^b Labor and benefits were estimated at US\$46/hour for real-time PCR and US\$29/hour for other procedures. The study duration was 16 weeks. Five working days in a week was assumed.

^c Cost per specimen processed.

materials was even more drastic (61%). Taking into account the test volume in 2011 ($n = 3,010$), our laboratory could save up to US\$22,000 in a year. Although there was a slight increase in hand-on time, most of the procedures involved in toxigenic culture were technically simple. In our study cohort, the positive rate of *C. difficile* culture was high (27.5%). The cost could be further reduced for laboratories with a low-prevalence catchment, as the majority of specimens would not require follow-up testing. The machine cost of MALDI-TOF mass spectrometry was great (about US\$190,000), but the cost of consumables was very low (less than US\$1). For laboratories without the hardware for MALDI-TOF mass spectrometry, we proposed preliminary reporting based on the PCR results of suggestive colony isolates, with subsequent identification by conventional tests if needed.

In conclusion, toxigenic culture was a sensitive, convenient, and cost-saving strategy for CDI diagnosis. There was a trade-off increase in turnaround time of 1 day. The availability of isolates for strain typing could facilitate epidemiologic studies and the determination of antimicrobial susceptibility.

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