Evaluation of stool specimens with the cobas® Cdiff test for the detection of *Clostridium difficile* toxin B gene compared with direct toxigenic culture

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Introduction and Objectives

Nucleic acid amplification tests have proven to be reliable, sensitive tools for the detection of toxigenic *Clostridium difficile* from unformed (liquid or soft) stool samples. *C difficile* is an anaerobic, toxin producing microorganism known to cause severe diarrhea following antibiotic therapy. The objective of this study was to evaluate performance characteristics of the newly developed **cobas**® Cdiff Test, on the **cobas**® 4800 System (Figure 1), using prospectively collected stool specimens from patients representative of the United States as part of a large, multicenter clinical trial.

Figure 1. cobas® 4800 System (**cobas x** 480 instrument and **cobas z** 480 analyzer)

Methods

The **cobas**® Cdiff Test was evaluated in an IRB-approved, prospective, multisite, investigation comparing the results with direct toxigenic culture using leftover, de-identified, unformed stool samples from subjects suspected of having *Clostridium difficile* infection (CDI). Specimens were collected at five geographically diverse sites across the US from symptomatic eligible male and female subjects. An aliquot of stool from each patient was sent to a reference lab for direct toxigenic culture (Figure 2, path A) and one aliquot was evaluated with the **cobas**® Cdiff Test at 1 of 3 designated sites (Figure 2, path C). Stool specimen was transferred with a polyester swab into **cobas**® PCR media, then loaded directly on the automated **cobas**® 4800 system for processing, PCR setup, amplification and detection. The toxigenic culture included direct culture of stool on pre-reduced selective anaerobic media, cycloserine-cefoxitin-fructose agar with horse blood and taurocholate (CCFA-HT), followed by cytotoxicity testing on *C. difficile* isolates recovered from stool. Briefly, suspected colonies were identified by Gram stain, aerotolerance test, and by the ProDisk test (Hardy Diagnostics, Santa Maria, CA) and then inoculated into anaerobic chopped meat broth, incubated for 5 to 7 days at 35° C and supernatants tested using a cell culture cytotoxicity assay (Figure 2, path A).

Discrepant analysis (DA) included a repeat direct culture plus enrichment culture followed by cytotoxicity testing. In addition, DA included testing an aliquot using a second FDA-cleared nucleic acid amplification test (NAAT)- the Cepheid Xpert® *C difficile*/Epi Test (Figure 2, path B). Enriched toxigenic culture included culture using cycloserine-cefoxitin-manitol broth with taurocholate, lysozyme and cysteine (CCMB-TAL), followed by subculture on *Brucella* agar plates, and with identification and cytotoxicity testing of *C. difficile* recovered from enrichment culture as described.

The positive percent agreement (PPA), negative percent agreement (NPA) and overall percent agreement (OPA) values were calculated by comparing **cobas**® Cdiff Test results with direct toxigenic culture.

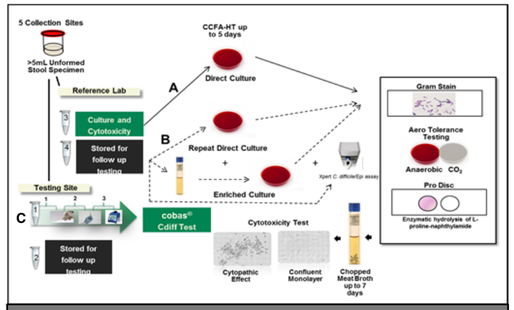

Figure 2. cobas® Cdiff Test clinical study design. A: Direct toxigenic culture; B: discrepant analysis; C: **cobas**® Cdiff Test.

Results

Specimens were collected from 683 subjects: 306 males (44.8%) and 377 females (55.2%) with a mean age of 56 years (range 3 to 99).

Direct toxigenic culture identified 113 positive samples with *C. difficile* . The results are shown in Table 1. The PPA, NPA and OPA of the **cobas**® Cdiff Test was 97.3% (110/113; 95% CI = 92.5% to 99.1%), 94.9% (541/570; 95% CI = 92.8% to 96.4%) and 95.3% (651/683; 95% CI = 93.5% to 96.7%). The prevalence of *C. difficile* observed in the study population was 16.5% based on direct toxigenic culture.

Table 1. Comparison of cobas® Cdiff Test with direct toxigenic culture.

Cdiff		Direct Toxigenic Culture		
		Pos	Neg	Total
cobas® Cdiff Test	Pos	110	29	139
	Neg	3	541	544
	Total	113	570	683
PPA: 97.3% (95% CI: 92.5-99.1%)				
NPA: 94.9% (95% CI: 92.8-96.4%)				
OPA: 95.3% (95% CI: 93.5-96.7%)				

Of the 3 specimens with false negative **cobas**® Cdiff Test results, 1 tested negative for toxigenic *C. difficile* by a second direct (repeat) toxigenic culture plus enriched toxigenic culture and the Xpert® *C difficile* Epi Test. Of the 29 specimens with false positive **cobas**® Cdiff Test results relative to direct toxigenic culture, 24 were *C. difficile* positive by a second toxigenic culture and/or NAAT (9 by culture only, 12 by culture and NAAT, and 3 by NAAT only).

Conclusions

- ❑ The **cobas**® Cdiff Test, on the automated **cobas**® 4800 system, displayed excellent performance compared to direct toxigenic culture for detecting toxigenic *C. difficile* in unformed stool specimens from patients suspected of CDI.*
- ❑ The test is highly suitable for the direct detection of the toxin B (*tcd*B) gene of toxigenic *C. difficile* in unformed stool specimens as an aid in the diagnosis of CDI in humans in conjunction with clinical and epidemiological risk factors.*

Note: this data has not been evaluated by the FDA.

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