Comparison of the Xpert *C. difficile*, Verigene *C. difficile*, Simplexa *C. difficile* Universal Direct, and BD MAX Cdiff Assays for the Detection of Toxigenic *Clostridium difficile*

Jeremy J. Gilbreath, Ph.D., MLS(ASCP)
Department of Laboratory Medicine
University of Washington
1959 NE Pacific St
Seattle, WA 98195

E-mail: jeremyjg@uw.edu

UNIVERSITY of WASHINGTON

Jeremy J. Gilbreath[a], Punam Verma[b], April N. Abbott[a], and Susan M. Butler-Wu[a]

[a]Department of Laboratory Medicine, University of Washington, Seattle, WA 98195
[b]Department of Pathology Department of Pathology and Clinical Laboratories, Virginia Mason Medical Center, Seattle, WA 98101

Abstract

We compared the Verigene *C. difficile* (CDF) (Nanosphere, Northbrook, IL), the Simplexa *C. difficile* Universal Direct (Focus Diagnostics, Cypress, CA), the BD MAX Cdiff (Beckton Dickinson, Franklin Lakes, NJ), and the Xpert *C. difficile* (Cepheid, Sunnybrook, CA) assays for the detection of toxigenic *C. difficile.* One hundred and ninety de-identified, remnant diarrheal specimens were included in this study. After resolution of discordant results by toxigenic culture, the Xpert *C. difficile* assay displayed the highest sensitivity (100%), with specificity, positive predictive and negative predictive values (PPV and NPV) of 98.8%, 92.0%, and 100%, respectively. The Verigene CDF test displayed 95.2% sensitivity, 99.4% specificity, 95.2% PPV, and 99.4% NPV. The Simplexa Universal Direct test was 87% sensitive and 100% specific, with a PPV and NPV of 100% and 98.2%, respectively. Finally, the BD MAX Cdiff assay showed sensitivity, specificity, PPV, and NPV of 87%, 98.8%, 90.9%, and 98.2%, respectively.

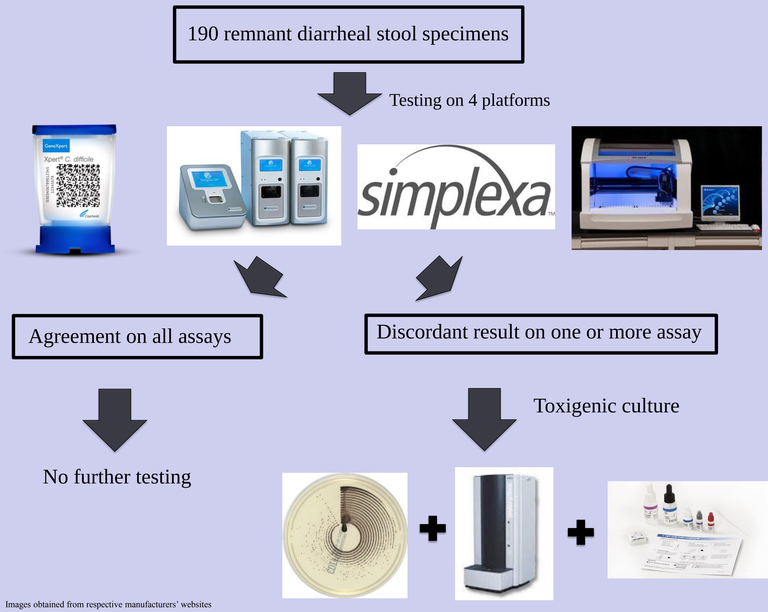
Background

Toxigenic *C. difficile* infection (CDI) is currently the leading cause of hospital-associated infectious diarrhea in the United States (1). The significant morbidity and mortality associated with CDI, as well as increasing disease severity and the emergence of hyper-virulent strains (2, 3) underscores the importance of accurate diagnosis of this infection. Furthermore, with the associated cost of missing a case of CDI exceeding $10,000, rapid diagnosis results in a significant reduction in unnecessary treatment costs. Laboratory methods for diagnosing CDI have traditionally relied on the detection of either *C. difficile* toxin activity (*e.g.* cell culture cytotoxicity assay) or of the toxins themselves (*e.g.* EIA). More recently, detection of toxin genes using molecular methods has increasingly been used by clinical laboratories as either a stand-alone test or performed as part of tiered algorithm that also includes testing for the *C. difficile* glutamate dehydrogenase enzyme by EIA (4). Despite the wide array of molecular tests currently on the market, relatively few studies have directly compared the performance of these assays. Furthermore, the performance of the Verigene CDF test relative to other FDA-cleared assays has not been established. Therefore, the goal of this study was to directly compare the performance of four of the FDA-cleared molecular *C. difficile* assays: the Verigene CDF test, the Simplexa *C. difficile* Universal Direct test, the BD MAX Cdiff assay and the Xpert *C. difficile* assay for the detection of toxigenic *C. difficile* in diarrheal stool specimens.

Table 1. *C. difficile* assay characteristics

Assay	Target(s)	Automated Extraction	Time to result	Platform
Xpert *C. difficile*	*tcdB*	Yes	35-45 min	GeneXpert (Cepheid)
Verigene CDF	*tcdB, tcdA, tcdC* mutation, binary toxin	Yes	110-120 min	Verigene system (Nanosphere)
BD MAX Cdiff	*tcdB*	Yes	~120 min	BD MAX (BD)
Simplexa	*tcdB*	No	~60 min	Integrated cycler (3M)

Study Design



Images obtained from respective manufacturers' websites

Results

Of the 190 specimens tested, we observed concordant results among all four assays for 181 specimens. In all, we observed discordant results for nine specimens (4.7%) with seven of the nine discordant specimens positive by a single assay. A breakdown of the results for each of the discordant specimens is shown in Table 2.

Table 2. Resolution of discordant specimens

Xpert	Verigene	BD MAX	Simplexa	chromID	EIA	*tcdB* PCR	Result
Negative	Negative	Positive	Negative	Negative	N/A	N/A	False positive
Negative	Negative	Positive	Negative	Negative	N/A	N/A	False positive
Positive	Negative	Negative	Negative	Negative[1]	N/A	N/A	False positive
Positive	Negative	Negative	Negative	Negative	N/A	N/A	False positive
Negative	Positive[2]	Negative	Negative	Negative	N/A	N/A	False positive
Positive	Positive	Negative	Positive	Positive	Positive	Positive	True positive
Positive	Indeterminate[3]	Negative	Negative	Positive	Positive	Positive	True positive
Positive	Negative	Negative	Negative	Positive	Positive	Positive	True positive
Positive	Positive	Positive	Negative	Positive	Negative	Positive	True positive

[1] one strain isolated on chromID agar was identified as *Clostridium hathewayi*
[2] specimen was positive for *tcdA* and *tcdC* only by Verigene
[3] as described in the text, the unresolvable indeterminate specimens were not considered discordant

After resolution of discordant results, 23 specimens were determined to be true positives, and 167 were true negatives. The observed a positivity rate for samples enrolled in the study (~12%), was consistent with the historical positivity rate for our institution. Based on these results, we next calculated the sensitivity, specificity, positive predictive value and negative predictive values for each test (Table 3).

Table 3. Assay performance characteristics

	Sensitivity[#]	Specificity[#]	PPV[#]	NPV[#]
Xpert *C. difficile*	100 (82.1-100)	98.8 (95.3-99.9)	92 (72.5-98.6)	100 (97.2-100)
Verigene CDF	95.2 (74.1-99.8)	99.4 (96.2-100)	95.2 (74.1-99.8)	99.4 (96.2-100)
BD MAX Cdiff	87 (65.3-96.6)	98.8 (95.3-99.8)	90.9 (69.4-98.4)	98.2 (94.5-99.5)
Simplexa	87 (65.3-96.6)	100 (97.2-100)	100 (80-100)	98.2 (94.5-99.5)

[#]All values shown are percentages; PPV, positive predictive value; NPV, negative predictive value; 95% confidence intervals are shown in parentheses

As RT-PCR-based assays are generally associated with significant reagent costs, we were interested in analyzing the frequency with which specimen re-testing was required (Table 4). The Simplexa *C. difficile* assay was the only test that did not require repeat testing for any of the specimens. Of the 190 stool specimens in the study, only one (0.5%) had to be re-tested using the Xpert assay. In contrast, five (2.6%) and 11 (5.8%) of the specimens had to be re-tested with the Verigene and BD MAX assays, respectively.

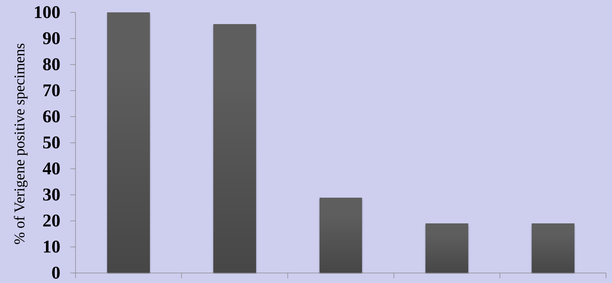
Table 4. *C. difficile* assay repeat testing frequency

	Samples requiring repeat testing	Resolved upon repeat
Xpert *C. difficile*	1 (0.5%)	1 (100%)
Verigene CDF	5 (2.6%)	3 (60%)
BD MAX Cdiff	11 (5.8%)	11 (100%)
Simplexa	0 (0%)	N/A

N/A, not applicable

In contrast to the Xpert, BD MAX, and Simplexa assays, which only detect the presence of the *tcdB* gene, the Verigene CDF also detects *tcdA*, binary toxin, and the point mutation in *tcdC* that is associated with the hyper-virulent PCR ribotype 027 strains. Because the Xpert *C. difficile* assay is currently used for clinical testing in our laboratory, we were interested in determining the distribution of toxin genes in our local population. We therefore examined the distribution of *tcdA* and 027-associated markers in our Verigene CDF positive specimens (Figure 1). The observed prevalence of the 027 PCR ribotype (~20%) was consistent with what has been described previously (5).

Figure 1. Breakdown of Verigene positive specimens



tcdB, toxin B; *tcdA*, toxin A; Binary, binary toxin gene; *tcdC* mut, mutation at base 117 in the toxin regulator gene.

Conclusions

In this study, we compared the performance of four FDA-cleared molecular tests for detecting CDI. The Xpert assay displayed the highest sensitivity (100%), followed by the Verigene (95.5%), BD MAX (87%) and Simplexa (87%) assays . The Simplexa assay was the most specific (100%), followed by the Verigene (99.4%), Xpert (98.8%) and BD MAX Cdiff (98.8%) assays. Despite differences in assays performance, all four tests evaluated in this study are simple to perform a have a relatively rapid in-assay time to result. These data support the utilization of molecular testing for routine laboratory diagnosis of CDI.

Acknowledgements

Use of reagents and instrumentation was provided by Nanosphere, Focus Diagnostics and Becton Dickinson. We thank Anne Cent of the University of Washington clinical virology lab and the staff of the University of Washington clinical microbiology lab for excellent technical assistance. SBW has received research funding from Nanosphere, and JJG received travel support from Nanosphere, Inc.

References

1. Bouza, E (2012) Consequences of *Clostridium difficile* infection: understanding the healthcare burden. *Clin Microbiol Infect*, *18 Suppl 6*, 5-12.
2. Loo, VG, Poirier, L, Miller, MA, Oughton, M, Libman, MD, Michaud, S, Bourgault, AM, Nguyen, T, Frenette, C, Kelly, M, Vibien, A, Brassard, P, Fenn, S, Dewar, K, Hudson, TJ, Horn, R, Rene, P, Monczak, Y, & Dascal, A (2005) A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. *N Engl J Med*, *353*, 2442-2449.
3. Warny, M, Pepin, J, Fang, A, Killgore, G, Thompson, A, Brazier, J, Frost, E, & McDonald, LC (2005) Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. *Lancet*, *366*, 1079-1084.
4. Brecher, SM, Novak-Weekley, SM, & Nagy, E (2013) Laboratory diagnosis of *Clostridium difficile* infections: there is light at the end of the colon. *Clin Infect Dis*, *57*, 1175-1181
5. Carroll, KC, Buchan, BW, Tan, S, Stamper, PD, Riebe, KM, Pancholi, P, Kelly, C, Rao, A, Fader, R, Cavagnolo, R, Watson, W, Goering, RV, Trevino, EA, Weissfeld, AS, & Ledeboer, NA (2013) Multicenter evaluation of the Verigene *Clostridium difficile* nucleic acid assay. *J Clin Microbiol*, *51*, 4120-4125.