Multicenter Evaluation of the Verigene Clostridium difficile Nucleic Acid Assay


Published Ahead of Print 2 October 2013.
Multicenter Evaluation of the Verigene *Clostridium difficile* Nucleic Acid Assay

Karen C. Carroll,a,b Blake W. Buchan,c Sokha Tan,b Paul D. Stamper,a Katherine M. Riebe,c Preeti Pancholi,d Cheryl Kelly,d Arundhati Rao,a Robert Fader,e Robert Cavagnolo,f Wendy Watson,f Richard V. Goering,g Ernest A. Trevino,h Alice S. Weissfeld,h Nathan A. Ledeboerf

Department of Pathology, the Johns Hopkins University School of Medicine,a and Medical Microbiology Laboratory, the Johns Hopkins Hospital,b Baltimore, Maryland, USA; Medical College of Wisconsin, Dynacare Laboratories, Milwaukee, Wisconsin, USA;c Ohio State University, Columbus, Ohio, USA;d Scott and White Hospital, Temple, Texas, USA;e Medfusion, Lewisville, Texas, USA;f Creighton University Medical Center, Omaha, Nebraska, USA;g Microbiology Specialists, Houston, Texas, USAh

The Verigene *Clostridium difficile* Nucleic Acid Assay (Verigene CDF test) (Nanosphere, Northbrook, IL) is a multiplex qualitative PCR assay that utilizes a nanoparticle-based array hybridization method to detect *C. difficile* tcdA and tcdB in fecal specimens. In addition, the assay detects binary toxin gene sequences and the single base pair deletion at nucleotide 117 (Δ 117) in tcdC to provide a presumptive identification of the epidemic strain 027/NAP1/BI (referred to here as ribotype 027). This study compared the Verigene CDF test with anaerobic direct and enriched toxigenic culture on stool specimens from symptomatic patients among five geographically diverse laboratories within the United States. The Verigene CDF test was performed according to the manufacturer’s instructions, and the reference methods performed by a central laboratory included direct culture onto cycloserine cefoxitin fructose agar (CCFA) and enriched culture using cycloserine cefoxitin mannitol broth with taurocholate and lysozyme. Recovered isolates were identified as *C. difficile* using gas liquid chromatography and were tested for toxin using a cell culture cytotoxicity neutralization assay. Strains belonging to ribotype 027 were determined by PCR ribotyping and bidirectional sequencing for Δ 117 in tcdC. A total of 1,875 specimens were evaluable. Of these, 275 specimens (14.7%) were culture positive by either direct or enriched culture methods. Compared to direct culture alone, the overall sensitivity, specificity, positive predictive value, and negative predictive value for the Verigene CDF test were 98.7%, 87.5%, 42%, and 99.9%, respectively. Compared to combined direct and enriched culture results, the sensitivity, specificity, positive predictive value, and negative predictive values of the Verigene CDF test were 90.9%, 92.5%, 67.6%, and 98.3%, respectively. Of the 250 concordantly culture-positive specimens, 59 (23.6%) were flagged as “hypervirulent”; 53 were confirmed as ribotype 027, and all 59 possessed Δ 117 in tcdC. Time to results was approximately 2.5 h per specimen. The Verigene CDF test is a novel nucleic acid microarray that reliably detects both *C. difficile* toxins A and B in unformed stool specimens and appears to adequately identify ribotype 027 isolates.

The optimum testing method for the diagnosis of *Clostridium difficile* is still a matter of debate. A variety of testing methods are available and include enzyme immunoassays (EIAs) for detection of toxins A and B, cell culture cytotoxicity neutralization assays (CCNAs), anaerobic toxigenic culture, detection of glutamate dehydrogenase, and nucleic acid amplification tests (NAATs). Although EIAs provide same-day results and are relatively inexpensive and easy to perform, there is general agreement in the United States and in some European countries that EIAs should not be performed as stand-alone tests due to their lack of sensitivity (1, 2). CCNAs and anaerobic toxigenic culture do not provide timely results and have never been standardized. Laboratories are beginning to adopt nucleic acid amplification tests (NAATs) alone or as a component of multiset algorithms that include screening with glutamate dehydrogenase (GDH) (3–5). Such strategies are endorsed by various professional societies in their *C. difficile* testing and treatment guidelines (1, 6).

Nucleic acid amplification tests (NAATs) have evolved as improved fecal extraction protocols, and real-time PCR methods have overcome inhibition accompanied with improved assay performance (4, 7). Currently, there are nine FDA-cleared NAATs available in the United States, with several more in development (8–14). The majority of these are real-time PCR assays that detect the toxin B gene (tcdB) and include the BD GeneOhm Cdiff assay (BD-GeneOhm, San Diego, CA), the GeneXpert Cdiff and Cdiff Epi tests (Cepheid, Inc., Sunnyvale, CA), the Prodesse ProGastro CD assay (Hologic Gen-Probe, Inc., San Diego, CA), and the Simplex Universal Direct assay (Focus Technologies, Inc., Cypress, CA). The GeneXpert Epi assay is a multiplex PCR method that not only detects tcdB but also detects binary toxin genes and the single base pair deletion at nucleotide 117 (Δ 117) in tcdC to provide presumptive identification of the epidemic strain 027/NAP1/BI.

Several tests are based upon other novel chemistries, including loop-mediated isothermal amplification such as the illumigene assay (Meridian Bioscience, Inc., Cincinnati, OH) (11, 12), helicase-dependent amplification in the Portrait *C. difficile* assay (Great Basin, Inc., Salt Lake City, UT) (13), and the AmpliVue *C. difficile* assay (Quidel Molecular, Inc., San Diego, CA), and upon array technologies such as the Verigene Cdiff assay (Nanosphere, Inc., Northbrook, IL). Several of these assays detect conserved regions of the toxin A gene (tcdA) either alone (illumigene) (11, 12) or in combination with other genetic targets (Verigene *Clostridium difficile* Nucleic Acid test [Verigene CDF test]). One manufacturer,
Luminex, Inc., Austin, TX, has included *C. difficile* in a multiplex assay that detects other enteric pathogens (xTAG Gastrointestinal Pathogen Panel).

The Verigene CDF test is a multiplex qualitative assay that utilizes PCR-amplified DNA in a nanoparticle-based microarray to detect *C. difficile* tcdA and tcdB in fecal specimens. In addition, the assay detects binary toxin gene sequences and the single base pair deletion at nucleotide 117 in *tcdC* to provide presumptive identification of the epidemic strain 027/NAP1/BI (referred to here as ribotype 027). This study evaluated and established the analytical performance of the Verigene CDF test using the Verigene system compared to the performance of anaerobic toxigenic culture, ribotyping, and bidirectional sequencing as the reference methods.

(This research was presented in part at the 113th General Meeting of the American Society for Microbiology, Denver, CO, and in part at the 23rd European Congress of Clinical Microbiology and Infectious Diseases, Berlin, Germany).

**MATERIALS AND METHODS**

**Participating centers and overall study design.** This was a prospective study from 28 February 2012 until 27 June 2012 to assess the analytical performance of the Verigene CDF test among five geographically diverse clinical microbiology laboratories. These included The Johns Hopkins Hospital Microbiology Laboratory, Baltimore, MD; The Medical College of Wisconsin, Milwaukee, WI; Ohio State University, Columbus, OH; Scott and White Hospital, Temple, TX; and Medfusion Laboratories, Inc., Lewisville, TX. Each site obtained appropriate institutional review board approval or a waiver consistent with local human subject research requirements. All sites performed Verigene CDF testing on leftover unidentified stool samples submitted to the clinical laboratory specifically for *C. difficile* testing according to the institution's routine practices. In addition, all sites reserved an aliquot of the stool for shipping to a central reference laboratory, Microbiology Specialists, Inc. (MSI; Houston, TX), for performance of anaerobic toxigenic culture methods (see "Reference culture methods" section below). Toxin-producing isolates were sent to independent laboratories for PCR ribotyping and bidirectional sequencing (see "PCR ribotyping" and "Bidirectional sequencing" sections below).

**Specimen collection and handling.** A minimum of 2 ml of liquid stool or a marble-size soft stool specimen was required for testing. Each study site deidentified the fresh prospectively collected specimen submitted to the laboratory for clinical testing by assigning each a unique specimen identification number. Duplicate specimens from the same patient were not enrolled. The collection time and date, patient age, and consistency of the specimen were recorded for each specimen. Enrolled specimens were thoroughly mixed in the original container for 5 s, and then 2 ml of specimen was pipetted into a 5-ml conical tube (tube A) for culture and molecular testing within 48 h of collection. Specimens were kept at 2 to 8°C until tested. A 0.5-ml aliquot of liquid stool or a pea-size soft stool specimen in tube A was transferred to Anaerobe Tissue Transport Medium (ATTM) at the time of Verigene testing (Anaerobe Systems, Inc., Morgan Hill, CA). The ATTMT was stored at room temperature and shipped each weekday in an insulated shipping container to MSI for anaerobic toxigenic culture.

**Reference culture methods.** Upon arrival at MSI, the stool was inoculated onto prereduced cycloserine cefoxitin fructose agar (CCFA [CCFA-D]) and cycloserine cefoxitin mannitol broth with taurocholate and lysyme (CCMB-TAL) (Anaerobic Systems). The CCFA and CCMB-TAL were incubated anaerobically for 48 h. All TAL broths were then subcultured to CCFA and CCFA-HT (CCFA with horse blood and taurocholate [CCFA-E]). These plates were subsequently incubated under anaerobic conditions for 48 h. On CCFA, colonies of *C. difficile* exhibit a yellow, ground glass appearance and chartreuse fluorescence under a Wood’s lamp. On CCFA-HT, colonies of *C. difficile* exhibit a gray, ground glass appearance. A Gram stain assay was performed on suspicious colonies; large Gram-positive rods were further tested for aerotolerance and susceptibility to vancomycin.

Any vancomycin-susceptible, strictly anaerobic, large Gram-positive rods were placed in chopped meat carbohydrate (CMC) broth for 48 h. Part of the broth was then extracted for gas liquid chromatography (GLC); the presence of isovaleric, isocaproic, and isobutyric acids in the volatile product represented a definitive identification for *C. difficile*. Another portion of the CMC was centrifuged, and the supernatant was then removed and used to determine the presence of toxin by inoculation onto human fetal foreskin cells. Toxin was neutralized with specific antitoxin to *C. difficile* (Diagnostic Hybrids, Athens, OH) to confirm the presence of toxicogen *C. difficile* (10).

For the purposes of this study, if *C. difficile* was isolated from the CCFA-D plate and the isolate was positive by the cell cytotoxicity assay, the specimen was classified as "toxicigenic *C. difficile* positive" and the CCFA-E plate was not further analyzed. If no *C. difficile* was isolated from the CCFA-D plate or if the isolate was negative by the cell culture cytotoxicity assay, the CCFA-E plate was further analyzed. If CCFA-E was positive for *C. difficile* and the isolate was positive by the cell cytotoxicity assay, the specimen was classified as "toxicigenic *C. difficile* positive." The specimen was reported as "negative" if CCFA-E was negative for *C. difficile* or the isolate tested negative by the cell cytotoxicity assay.

**Description of the Verigene CDF assay.** The Verigene system is a bench-top sample-to-result platform consisting of two modules: the Verigene Processor SP and the Verigene Reader. The Processor SP automates specimen nucleic acid extraction, PCR amplification, and amplicon hybridization. The Processor SP utilizes four single-use consumables per test: an extraction tray, an amplification tray, a test cartridge, and a pipette tip holder assembly. The user tests a specimen by loading the single-use consumables into the Processor SP, pipetting the test specimen into the sample well of the extraction tray, and initiating the test protocol on the Verigene Reader by scanning the test cartridge barcode and specimen information. Upon test completion, the user inserts the microarray slide from the test cartridge into the Verigene Reader for optical analysis and generation of test results.

For *C. difficile* testing on the Verigene system, 100 μl of the prepared stool sample is loaded into the sample well of the extraction tray. The test specimen is prepared from a soft or liquid stool sample by swabbing the stool with the provided flocked swab and transferring the evenly coated swab to the provided stool prep buffer tube, which is subsequently vortexed for 15 s and centrifuged for 30 s. Following test initiation on the Processor SP, sample bacteria are lysed and target DNA is extracted using magnetic bead-based extraction. Extraction is followed by multiplex PCR-based amplification, which generates target-specific amplicons. Finally, amplicon hybridization to target specific capture DNA occurs in a microarray setting using mediator and gold nanoparticle probe hybridization to the captured amplicons. Silver enhancement of the bound gold nanoparticle probes at the capture sites results in gold-silver aggregates that are assessed optically on the Verigene Reader to produce the final test result.

**Controls.** A negative control is included in the array to detect whether the melt temperature of the hybridization chamber is too low due to an instrument malfunction. The Verigene Reader automatically checks the target oligonucleotide signal relative to the negative-control oligonucleotide signal. If the target signal is not adequately higher than the negative-control signal, a "no call" result is generated.

An artificial DNA construct serves as a hybridization control and is referred to as processing control 1 (PC1). This control material, along with the primers, enzymes, and amplification buffers, is included within the amplification tray. If the process control is not valid, a "no call" result is obtained and the test should be repeated. *Bacillus subtilis* serves as a specimen preparation and amplification control and is referred to as processing control 2 (PC2). This control is automatically added by the Processor SP to each specimen prior to the extraction step. If the process
control is not valid, a “no call” result is obtained and the test should be repeated. Additional positive controls are immobilized on the test slide and are used to determine that hybridization was performed correctly. The Verigene CDF test algorithm requires that these controls be valid before decisions regarding the presence or absence of any other target on the panel can be made. If these controls are not detected, a “no call” result is obtained and the test is repeated.

Verigene CDF test performance. For liquid samples, 150 μl of the specimen from tube A was transferred to a small screw-cap microcentrifuge tube containing stool lysis buffer. For soft stool specimens, a sterile swab was fully immersed in the specimen and, once evenly coated, was transferred to the stool lysis buffer tube and the swab was broken at the preformed score breakpoint. In both cases, the screw cap was gently tightened by hand. All specimen transfers were performed in such a manner as to ensure a minimum risk of contamination. Gloves were changed between pipetting and transfer of specimens, and only one specimen was open and processed at a time. All sample buffer tubes containing specimen were vortexed for 15 s and microcentrifuged for 30 s, after which 100 μl of supernatant was inoculated into the specimen well of the extraction tray. Testing was performed in a blind manner on the deidentified aliquots according the manufacturer’s protocol and product package insert.

Three external positive controls were supplied to each test site for quality control purposes. These external positive controls were run on an alternating basis once every 24 h during the period of time that clinical testing was conducted (e.g., CDF control 1 was run on day 1 of testing, CDF control 2 on day 2, and so on), with the goal being an approximately equal distribution of CDF control runs during the study at each site. Several test-specific negative controls are immobilized on the test slide and are used to guide test decisions (see “Description of the Verigene CDF assay” above). A single external negative control (which is a nontoxigenic C. difficile control) was run every 24 h during the period when clinical specimen testing was conducted.

Interpretation of results. The Verigene CDF assay provides a qualitative result for the presence (detected) or absence (not detected or not applicable) of tcdA, tcdB, binary toxin genes, and the Δ117 deletion in tcdC. Table 1 demonstrates the possible outcomes for valid test results. Invalid results may be generated by failure to image the array slide (“no call—no grid”) or from internal control failures or because the Verigene Reader could not read the target-specific signals due to variability. Other sources of invalid results include preanalytical errors. All tests producing invalid results were repeated within 48 h of specimen collection using a refrigerated reserved aliquot of the original stool sample.

PCR ribotyping. Following the culture testing at MSI, the toxigenic C. difficile-positive isolates were sent for strain identification by PCR ribotyping to an external third-party site (Creighton University Medical Center, Omaha, NE) per the study protocol. In addition to conventional agarose-gel electrophoresis, for added discrimination, PCR products were analyzed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Clara, CA). The strains were classified into two PCR ribotyping categories, 027 and non-027. Strain type assignments were based on a comparison of isolate profiles with profiles of known C. difficile reference strains from the C. difficile collection of the Cardiff-European Centre for Disease Prevention and Control (ECDC) obtained from Ed Kuijper, Leiden University Medical Center, Netherlands, using BioNumerics software, version 6.6 (Applied Maths, Belgium).

Bidirectional sequencing. During the study, DNA from the extraction step of the Verigene CDF test was saved and stored frozen at −20°C until needed. If the PCR amplification for tcdC failed for a DNA sample, the DNA extracted during the PCR ribotyping was tested. Sequencing templates were prepared by PCR amplification of the stored DNA using sequencing primers. The same set of primers was also used for sequencing the amplified material. In parallel, following central culture testing, DNA from the culture-confirmed C. difficile-positive isolates (that was extracted during the Verigene CDF test) was sent to an external third-party site (ACGT, Inc., Wheeling, IL) for tcdC bidirectional sequencing per the protocol. A 295-bp fragment of tcdC was sequenced.

Data analysis and statistical methods. Results of the Verigene CDF test were compared to direct culture results on CCFA, with isolates confirmed and analyzed independently by ribotyping and bidirectional sequencing. In addition, the Verigene CDF test was also compared to enriched culture, with positives verified by ribotyping and bidirectional sequencing (see “Reference culture methods” above).

Results were entered into Excel (Microsoft Corporation, Redmond, WA), and the sensitivity values, specificity values, positive predictive values (PPV), and negative predictive values (NPV) were calculated. The exact binomial methods in SAS 9.1.3 (SAS Institute, Inc., Cary, NC) were used to calculate the lower and upper two-sided confidence limits.

RESULTS

Among the five study sites, 1,877 specimens met the inclusion criteria. Seventy-one specimens (3.8%) required repeat testing: 46 specimens (2.4%) had an initial “no call” test result due to assay internal control errors; 17 specimens (1.0%) had an initial “indeterminate” call (“no call—IND”); and eight specimens (0.4%) had preanalytical errors (four motor stalls, two tip failures, one cracked slide, and one cartridge not detected). The eight specimens that experienced preanalytical errors and the 46 “no call” specimens all produced a valid call upon repeat testing per the protocol. However, two of the “no call” specimens required a second repeat test. Repeat testing of the 17 “no call—IND” specimens called all but two specimens. Therefore, two specimens had a final “indeterminate” call and were not included in the clinical data analysis of evaluable results. Thus, 1,875 specimens were analyzed in this clinical evaluation.

Table 2 summarizes the overall Verigene CDF test clinical performance. Of the 1,875 specimens that were analyzed in this trial,
there were 275 specimens positive for *C. difficile* and 1,600 specimens negative by either direct or enriched culture methods after all repeat testing. The majority (98.8%) of positive specimens contained genes for both toxins A and B (data not shown).

The overall agreement between direct culture and the Verigene CDF test for detection of toxigenic *C. difficile* in stool was 88.4%. The overall sensitivity, specificity, positive predictive values, and negative predictive values of the Verigene CDF test compared to direct culture on CCFA are listed in Table 2. Overall agreement between the combined direct and enriched culture results and the Verigene CDF assay was 92.3% (Table 2).

Table 3 lists the performance characteristics of the Verigene CDF test compared to those of the combined culture methods stratified by investigational site. There were no major differences in the performance of the Verigene assay by investigational site.

There were 72 nontoxigenic *C. difficile* isolates recovered by culture during the study. Eleven of these were detected as toxigenic by the Verigene CDF test and were classified as false positives. Nine of the 11 false-positive results were confirmed positive for *tcdC* by bidirectional sequencing. Two of the 11 false-positive results were negative for *tcdC* and *tcdB* by bidirectional sequencing (data not shown).

Table 4 shows the distribution by Verigene CDF test results for strain characterization. Of the 250 concordantly culture- and Verigene-positive specimens, 59 (23.5%) were flagged as “hyperproducer.” Fifty-three were confirmed as ribotype 027, and all 59 possessed the Δ117 mutation in *tcdC*. One isolate that was ribotype 027 and contained the Δ117 *tcdC* mutation was incorrectly identified as non-O27 by the Verigene CDF test. Compared to ribotyping, the Verigene CDF test correctly assigned the ribotype in 89.7% of the cases; compared to sequencing, the agreement was 100%.

**DISCUSSION**

*Clostridium difficile* remains a formidable pathogen that continues to cause significant morbidity and mortality among hospitalized patients (15). Between 2001 and 2006, the incidence of *C. difficile* infection (CDI) in the United States more than doubled, and the incidence continues to rise in North America and Europe (15). In part, this increase was due to the appearance of hypervirulent strains such as ribotype 027. In addition to being more transmissible and a hyperproducer of toxins A and B, this ribotype is notably more drug resistant than historical strains (15). Increased severity of illness has resulted in more colectomies, prolonged hospitalizations, and an increase in the observed number of recurrences (16). More significantly, mortality rates have also increased and in North America and Europe are reported to be as high as 22% to 37% (15). The economic burden is substantial, as was shown in a systematic review of health care costs associated with patients with primary and recurrent CDI (17). In 2008 U.S. dollars, the additional costs for treating relatively mild primary CDI varied from $9,822 to $13,854 (17). In Europe, the incremental increase in costs ranges from €4,067 to €9,276 (15).

Over the last decade, the emergence of hypervirulent strains such as ribotype 027, ribotype 078, and the newly described ribotypes 176 and 198 (19, 20) has spurred many clinical laboratories to abandon insensitive EIAs for more sensitive methods such as NAATs. The published performance characteristics among the nine FDA-cleared NAATs vary depending upon the comparative method, but in general, those studies that used toxigenic culture as the reference method have demonstrated sensitivities ranging from 77% to 100% and specificities that ranged from 90% to 100% (4, 7, 8, 11–14, 18). The Verigene CDF test is one of the newer assays to obtain FDA 510(k) clearance and is novel. The assay uses multiplex PCR combined with silver enhancement of gold nanoparticle probe hybridization to capture amplicons in a microarray format. The Verigene CDF test is the only FDA-cleared assay that detects both toxins A and B and identifies ribotype 027 by identifying both the binary toxin gene and the Δ117 deletion of *tcdC*. In terms of performance characteristics, the overall sensitivity and specificity of 98.7% and 87.5%, respectively, compared to direct toxigenic culture and 90.9% and 92.5%, respectively, compared to enriched culture are comparable to the published clinical performance of other NAATs (8–14). As has been noted for other NAATs, the NPV for the Verigene assay ranges from 98% to 99.9% and essentially excludes *C. difficile* as a cause of diarrheal disease, whereas the PPV is low, ranging from 42% to 67% compared to toxigenic culture methods. Some of the false-positive samples were confirmed by bidirectional sequencing as containing toxin-producing strains, indicating possible infection with mixed toxigenic and nontoxigenic isolates. A limitation of this study is that no clinical data were collected and included in the assessment of a true positive or negative specimen.

The proportion of combined “no call” and invalid assessments and preanalytical problems of 3.9% necessitating repeat testing is also similar to the BD GeneOhm assay’s reported invalid rate of 0.5% to 7.3% (8, 12, 14), the Prodesse ProGastro CD’s reported invalid rate of 2.7% (9, 14), and the illumigene assay’s reported rate of 0.8% to 4.4% (11, 12). This is slightly higher than what was reported for the Quidel AmpliVue *C. difficile* assay (0.5%) (18).

The usefulness of detecting ribotype 027 in any *C. difficile* assay remains controversial. While some studies have demonstrated more severe disease and increased mortality associated with ribotype 027 (16, 19), more recent studies have challenged this observation (21, 22). In the latter studies, when other covariates were considered, the ribotype of the infecting organism was not found.

<table>
<thead>
<tr>
<th>Method</th>
<th>No. of specimens</th>
<th>No. positive/no. tested (%), (% CI)*</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct culture</td>
<td>1,875</td>
<td>156/158 (98.7), (95.5–99.9)</td>
<td>1,502/1,717 (87.5), (85.8–89.0)</td>
<td>1,658/1,875 (88.4), (86.9–89.8)</td>
<td>156/371 (42.1), (37.0–47.3)</td>
<td>1,502/1,504 (99.9), (99.5–100)</td>
<td></td>
</tr>
<tr>
<td>Direct plus enriched culture</td>
<td>1,875</td>
<td>250/275 (90.9), (86.9–94.0)</td>
<td>1,480/1,600 (92.5), (91.1–93.7)</td>
<td>1,730/1,875 (92.3), (91.0–93.4)</td>
<td>250/370 (67.6), (62.5–72.3)</td>
<td>1,480/1,505 (98.3), (97.6–98.9)</td>
<td></td>
</tr>
</tbody>
</table>

* CI, percent confidence interval.
TABLE 3 Direct culture results and direct culture plus enriched culture results stratified by laboratory site

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of specimens</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct culture</td>
<td>Direct plus enriched culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>557 (100)</td>
<td>55/55 (100)</td>
<td>83/83 (100)</td>
<td>55/13 (341.4)</td>
<td>424/424 (100)</td>
<td>98/106 (92.5)</td>
</tr>
<tr>
<td></td>
<td>(91.5–100)</td>
<td>(81.0–87.5)</td>
<td>(83.2–89.1)</td>
<td>(32.9–50.2)</td>
<td>(99.1–100)</td>
<td>(85.7–96.7)</td>
</tr>
<tr>
<td>2</td>
<td>482 (100)</td>
<td>36/101 (35.6)</td>
<td>381/381 (100)</td>
<td>36/101 (35.6)</td>
<td>381/381 (100)</td>
<td>58/61 (95.1)</td>
</tr>
<tr>
<td></td>
<td>(90.3–100)</td>
<td>(82.8–88.2)</td>
<td>(82.8–89.1)</td>
<td>(26.4–45.8)</td>
<td>(99.0–100)</td>
<td>(86.3–94.8)</td>
</tr>
<tr>
<td>3</td>
<td>299 (100)</td>
<td>25/47 (53.2)</td>
<td>252/252 (100)</td>
<td>25/47 (53.2)</td>
<td>252/252 (100)</td>
<td>58/61 (95.1)</td>
</tr>
<tr>
<td></td>
<td>(86.3–100)</td>
<td>(87.7–94.6)</td>
<td>(88.7–95.1)</td>
<td>(38.1–67.9)</td>
<td>(98.5–100)</td>
<td>(86.3–94.8)</td>
</tr>
<tr>
<td>4</td>
<td>371 (100)</td>
<td>25/30 (83.3)</td>
<td>310/312 (99.4)</td>
<td>25/30 (83.3)</td>
<td>310/312 (99.4)</td>
<td>72/72 (99.1)</td>
</tr>
<tr>
<td></td>
<td>(75.9–100)</td>
<td>(87.6–95.5)</td>
<td>(88.6–93.1)</td>
<td>(29.6–55.9)</td>
<td>(97.7–99.9)</td>
<td>(72.7–94.8)</td>
</tr>
<tr>
<td>5</td>
<td>166 (100)</td>
<td>150/166 (90.4)</td>
<td>135/135 (100)</td>
<td>150/166 (90.4)</td>
<td>135/135 (100)</td>
<td>20/24 (83.3)</td>
</tr>
<tr>
<td></td>
<td>(78.2–100)</td>
<td>(83.4–93.8)</td>
<td>(84.8–94.4)</td>
<td>(30.2–66.9)</td>
<td>(97.3–100)</td>
<td>(62.6–95.3)</td>
</tr>
<tr>
<td>Total</td>
<td>1,875a</td>
<td>156/158 (98.7)</td>
<td>1,502/1,717 (87.5)</td>
<td>1,502/1,717 (88.4)</td>
<td>1,502/1,717 (88.4)</td>
<td>250/275 (90.9)</td>
</tr>
<tr>
<td></td>
<td>(95.5–99.9)</td>
<td>(85.8–89.0)</td>
<td>(86.9–89.8)</td>
<td>(37.0–47.3)</td>
<td>(99.5–100)</td>
<td>(86.9–94.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of strains with indicated ribotype 027</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not 027 59, 027 191</td>
</tr>
<tr>
<td>2</td>
<td>Not 027 53, 027 188</td>
</tr>
<tr>
<td>3</td>
<td>Not performed 1, 027 3</td>
</tr>
<tr>
<td>4</td>
<td>Not performed 1, 027 3</td>
</tr>
<tr>
<td>5</td>
<td>Not performed 1, 027 3</td>
</tr>
</tbody>
</table>

a Of the 1,875 specimens evaluated, 7 specimens were culture positive but were either not PCR ribotyped or sequenced or both but are included in the performance characteristics presented above.

b Of the 120 specimens that were toxigenic C. difficile by Verigene CDF testing but toxigenic C. difficile by direct/enriched toxigenic culture, 90 were positive by bidirectional sequencing for tcdC, 6 were positive by bidirectional sequencing for tcdB, and 24 were negative by bidirectional sequencing for both tcdC and tcdB.

We thank ACGT, Inc., under the direction of Semyon Rubinchik for performing the PCR ribotyping for our aerotubid strains.}

### ACKNOWLEDGMENTS

We thank ACGT, Inc., under the direction of Semyon Rubinchik for providing the PCR ribotyping for our aerotubid strains. The authors are grateful to the following individuals for their contributions: [List of contributors and their roles]. The study was supported by [funding agencies].
Niki Falandino from Nanosphere, Inc., who assisted with the data analysis. In addition, we are all grateful to the staff of our clinical laboratories for their support of this study.

This study was sponsored by Nanosphere, Inc.

N.A.L. and B.W.B. have served as consultants to Nanosphere.

REFERENCES


