Clinical Comparison of Simplexa™ Universal Direct and BD GeneOhm™ Tests for Detection of Toxigenic Clostridium difficile in Stool Samples

Frederick S. Nolte# and Danielle G. Ribeiro-Nesbitt

Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC

Running Title: Comparison of Simplexa and BD GeneOhm Tests for C. difficile

#Correspondent footnote: nolte@musc.edu
We compared the performance characteristics of the Simplexa Universal Direct (Focus Diagnostics, Cypress, CA) and BD GeneOhm (BD Diagnostics/GeneOhm Sciences, San Diego, CA) tests for detection of toxigenic Clostridium difficile in 459 stool samples (9.4% positive). The observed agreement for the results of the two tests with 452 samples with valid test results was 98.2% (kappa, 0.9; McNemar test p, 0.73). When samples with discordant or invalid results were retested, the agreement increased to 100%.
Toxigenic strains of *Clostridium difficile* are primary pathogens in antibiotic-associated colitis, and account for 15% to 20% of cases of nosocomial antibiotic-associated diarrhea. Illness ranges from mild watery diarrhea to potentially fatal pseudomembranous colitis and toxic megacolon (1). In recent years diagnostic testing for *C. difficile* infection (CDI) has been dramatically transformed by the development and application of nucleic acid amplification tests (NAAT) for toxigenic *C. difficile*. NAATs combine sensitivity, specificity, speed and convenience in a single test to diagnose CDI (2). Currently there are twelve FDA-approved NAAT tests for *C. difficile* using different amplification and detection techniques, including real-time PCR, endpoint PCR with gold nanoparticle probe detection, loop-mediated amplification, and blocked primer thermophilic helicase-dependent amplification (http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm). To our knowledge this is the first published evaluation of the Simplexa *C. difficile* Universal Direct Test.

In this study we compared the performance characteristics of two real-time PCR tests for *C. difficile*, the Simplexa Universal Direct and the BD GeneOhm tests. Both tests target the *tcdB* gene and include internal controls to detect the presence of PCR inhibitors. The tests differ in the types of probes used to detect the amplicons and the instruments used for the analysis. The BD GeneOhm test uses molecular beacons and the Simplexa test uses bi-functional fluorescent probe-primers (scorpion probes) to detect the amplicons. The BD GeneOhm test is performed on a SmartCycler® (Cepheid, Sunnyvale, CA) with 45 amplification cycles and the Simplexa test is performed on a 3M™ Integrated Cycler (Focus Diagnostics) with 40 amplification cycles. Both tests
employ a simple preparation method entailing dilution of the stool specimen in a sample buffer and heating the diluted sample to lyse bacteria and release the nucleic acids. In addition the sample preparation for the BD GeneOhm test includes 3 vortex steps and brief centrifugation step prior to heating the sample.

A total of 459 unformed stool samples that were submitted to our Molecular Pathology Laboratory for detection of *C. difficile* over a 3-month period were enrolled in the study. The laboratory’s policy was to reject samples submitted within a 7 day time period from the same patient unless approved by the Laboratory Director. Samples were stored at 4°C prior to analysis.

Samples were tested in parallel with the BD GeneOhm and Simplexa tests in daily batches according to the manufacturers’ instructions by a single operator for a total 51 runs of each test. In addition to the positive and negative controls provided in the kits, a previously positive patient sample was included with each run as an external control. It was stored at -70°C in single use aliquots until needed. We also inspected all of the PCR amplification curves from both tests to ensure that the computer algorithms employed correctly identified positive reactions (3). Three different lots of Simplexa and eight different lots of BD GeneOhm reagents for each test were used during the study. All samples that gave invalid results with the Simplexa test were re-prepared and re-tested the following day. The lysates of samples with invalid BD GeneOhm test results were subjected to a freeze and thaw cycle and re-tested. The different treatments of samples with invalid results were in accordance with the manufacturers’ instructions. All samples with discordant results were also re-prepared and re-tested with both tests the following day. Any samples with remaining discordant results after repeat
testing were to be frozen at -70°C for toxigenic culture at a referral laboratory (Focus Diagnostics). In addition timing studies were conducted to estimate the labor component associated with each test.

The 459 stool samples were collected from 372 patients. Their ages ranged from 2 mo. to 91 yr. with a median of 57 yr. Forty eight patients (12.9%) were ≤18 yr. and 11 (3%) patients were ≤1 yr. A total of 39 patients had more than one sample included in the study. Thirty-one patients with 2 samples, 7 patients with 3 samples and 1 patient with 4 samples tested. Only 7 (1.9%) of 392 patients had duplicate samples tested within this time window and none converted from negative to positive status.

Table 1 shows the initial test results for all samples. The Simplexa test identified 43 (9.4%) samples as positive, 410 (89.3%) as negative and 6 (1.3%) as invalid due to failed internal controls. The BD GeneOhm test identified 45 (9.8%) samples as positive, 413 (90%) as negative and 1 (0.2%) as invalid due to failed internal control. Re-testing of all samples with invalid test results yielded valid negative results. After resolution of the invalid results the overall agreement between the two test results was excellent at 98.2% (kappa, 0.9; McNemar test p, 0.73). All positive discordant results were found with samples with high cycle threshold (Ct) values near the test cutoffs (Table 1).

When the 3 samples that were initially Simplexa positive/BD GeneOhm negative were re-tested one was positive in both tests and 2 were negative in both tests. Two of the 5 samples that were initially BD GeneOhm positive/Simplexa negative re-tested as positive in both tests and the remaining 3 re-tested as negative in both tests. After re-testing the overall agreement between the results of the two tests was 100% with 43 (9.4%) positive and 416 (90.6%) negative results. Consequently, none of the samples
required toxigenic culture to adjudicate discordant NAAT results. The initial
discrepancies were most likely explained by poorer reproducibility near the assays’
cutoffs.

The $C_t$ values ranged from 26.9 to 41.5 (mean, 33.67) in the BD GeneOhm test
and from 25.5 to 39.9 (mean, 32.91) in the Simplexa test. The association between the $C_t$
values of the two tests was moderately strong with a $R^2$ value of 0.76; however, on
average the $C_t$ values were 0.77 lower with the Simplexa test.

The reproducibility of the two tests was compared with two different external
positive controls. External positive control no. 1 was used for 28 runs and the mean $C_t$
and coefficient of variation (CV) with the BD GeneOhm test were 28.83 and 4.2%,
respectively, and were 26.51 and 2.4%, respectively, with the Simplexa test. Similar
results were obtained for external positive control no. 2 that was used for the remaining
23 runs. Mean $C_t$ and CV were 35.7 and 2.3%, respectively with the BD GeneOhm test
and were 32.91, and 1.9%, respectively, with the Simplexa test.

The BD GeneOhm test required a total of 45 min. and 24 sec. of hands on time to
complete a run of 11 samples and 3 controls, a typical batch size in our laboratory. The
Simplexa test required only 32 min. and 16 sec. to complete the same batch size which
represented a 13 min. savings of a medical technologist’s time achieved primarily
through a more streamlined sample preparation method with no vortexing or
centrifugation required.

In conclusion, we found that the Simplexa and BD GeneOhm tests for *C. difficile*
had very similar performance characteristics with 100% agreement between tests when
discordant samples with late $C_t$ values were re-tested. However more invalid tests results
were obtained with Simplexa test, but all samples gave valid results when re-tested. A limitation of our study is that no adjunctive tests for *C. difficile* were performed to resolve the discrepancies between the two NAATs. However the performance characteristics of the BD GeneOhm test are well established with reported sensitivity from 82.1 to 100% and specificity ranging from 90.6 to 99.2% for detection of *C. difficile* infection when compared to toxigenic culture or cell culture cytotoxicity neutralization assay (4).

The Simplexa test offers several advantages over the BD GeneOhm test including higher throughput/instrument (96 vs. 16 tests), less labor, and easy access to amplification curves. Although PCR amplification curves can be accessed with the BD GeneOhm test it must be specially requested from the manufacturer, is cumbersome, and examination of the amplification curves is not included as part of the interpretation of the results as it is with the Simplexa test. The software algorithm used for the BD GeneOhm test software occasionally miscalls positives with atypical amplification curves (3, personal observation). However no atypical amplification curves were observed with either assay during the course of this study. In addition the Focus Diagnostics offers a wide variety of analyte-specific reagents and FDA-cleared tests that can be run simultaneously on the same instrument which could substantially improve laboratory workflow.
ACKNOWLEDGEMENTS

Focus Diagnostics supplied equipment and reagents used in this study. FSN has received speaker’s fees from BD GeneOhm.
REFERENCES


TABLE 1. Initial results obtained with the Simplexa Universal Direct and BD GeneOhm tests for *C. difficile*.

<table>
<thead>
<tr>
<th></th>
<th>Simplexa</th>
<th>BD GeneOhm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Invalid (^a)</td>
</tr>
<tr>
<td>Positive</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Invalid (^a)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>5 (^c)</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\)Internal control failure.

\(^b\)C\(_t\) values of 38.3, 39.6 and 39.8.

\(^c\)C\(_t\) values of 40.5, 40.6, 40.7, 40.8 and 41.8.