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Comparison of Illumigene, Simplexa, and AmpliVue *Clostridium difficile* Molecular Assays for Diagnosis of *C. difficile* Infection

E. Deak, S. A. Miller, R. M. Humphries
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We compared the performance of the Simplexa Universal Direct (Focus Diagnostics) and AmpliVue (Quidel Corporation) assays to that of the Illumigene assay (Meridian Bioscience, Inc.) for the diagnosis of *Clostridium difficile* infection. Two hundred de-identified remnant diarrheal stool specimens were tested by the Simplexa, AmpliVue, and Illumigene methods. Specimens with discrepant results among the three assays and a representative number of concordant specimens were further evaluated by toxigenic culture. The sensitivity and specificity were 98 and 100% and 96 and 100% for the Simplexa Universal Direct and AmpliVue assays, respectively. Both assays are easy to perform, with rapid turn-around-times, supporting their utility in the clinical laboratory as routine diagnostic platforms.

The management and control of *Clostridium difficile* infection (CDI) continue to present a formidable challenge in the 21st century for hospitals, long-term-care facilities, and nursing homes (1). Infection rates have increased markedly in the United States over the past decade (2–4), and health care costs associated with CDI are a substantial burden to the health care system (1). The availability of a rapid and accurate laboratory diagnostic test for CDI is essential for patient treatment and prevention of transmission (5). Nucleic acid amplification tests (NAATs) that target the toxin A and/or B genes of *C. difficile* have gained popularity among laboratories in the United States. These tests have better sensitivity than traditional toxin antigen-based assays and culture (6) and have been shown to be a cost-effective alternative to traditional diagnostic methods (7). In recent years, a plethora of *C. difficile*-specific NAATs have been approved or cleared by the U.S. Food and Drug Administration (FDA). This study evaluated the performance of two newly cleared *C. difficile* assays, the Simplexa Universal Direct and AmpliVue *C. difficile* assays, in comparison with that of the Meridian Illumigene assay and toxigenic *C. difficile* culture. The performance characteristics of the Illumigene assay compared to the “gold standard” toxigenic culture (TC) in detecting toxigenic *C. difficile* in clinical stool samples have been established previously. Lalande et al. showed that Illumigene had higher sensitivity (91.8% compared to TC) than the cytotoxicity assay (69.4%) when they looked at 476 stool specimens (8). Studies by Norén et al. showed 98% sensitivity and specificity when they compared Illumigene with TC in their study of 272 consecutive stool samples (9). More recently, studies evaluating multiple molecular platforms against TC as the reference method have shown sensitivities of 86.7 to 93.3% (10, 11). Overall, the Illumigene assay has proven to be as reliable as TC in detecting infection by toxigenic *C. difficile* in routine clinical settings.

The Simplexa *C. difficile* Universal Direct real-time PCR assay uses bifunctional fluorescent probes-primer to amplify a conserved region of the toxin B gene (*tcdB*) in *C. difficile* directly in heat-treated stool samples (12). The AmpliVue *C. difficile* assay uses helicase-dependent amplification technology for isothermal amplification of a highly conserved 83-bp fragment of the 5′ end of the toxin A gene (*tcdA*) and a self-contained disposable amplification detection device that incorporates a lateral-flow strip for visual evaluation of assay results (13). The Illumigene *C. difficile* assay uses loop-mediated isothermal DNA amplification (LAMP) technology to target a partial DNA fragment of *tcdA* (14).

Fresh, unfomed stool samples submitted to the laboratory for *C. difficile* detection between January and March 2013 were tested by the Illumigene *C. difficile* assay in accordance with the manufacturer’s instructions. For each positive specimen, two negative specimens were randomly selected daily, for a total of 50 positive and 150 negative specimens. Duplicate specimens from the same patients were excluded. These remnant stool specimens were assigned study numbers, deidentified, and tested by the Simplexa *C. difficile* Universal Direct assay and the AmpliVue *C. difficile* assay on the same day as Illumigene testing was performed. Two of three specimens with discordant results among the three assays (*n = 2*), along with an equal number of concordant specimens (*n = 2*), were sent to a reference laboratory for further testing by TC (15, 16). One specimen with discrepant results could not be sent because of insufficient volume. This study was approved by the local Institutional Review Board.

All NAATs were performed according to the manufacturer’s specifications. Briefly, for the Simplexa *C. difficile* Universal Direct assay, a flocked swab was dipped into a thoroughly mixed stool specimen, transferred into Tris-EDTA (TE) buffer, and heated at 97°C for 10 min (swab and TE buffer not provided by the manufacturer). A reaction mixture was prepared and added to each sample and control well of a 96-well Universal Disc for the Simplexa Universal Direct assay. The AmpliVue *C. difficile* assay uses the Integrated Cycler. Results were recorded as positive (*Cₚ* value of <40 with or without a valid internal control curve), negative (*Cₚ* value of 0 or ≥40 with a valid internal control curve), or invalid (*Cₚ* value of <40 without a valid internal control curve). The AmpliVue *C. difficile* assay has been shown to have sensitivities of 86.7 to 93.3% and specificities of 98 to 100% (13). The Simplexa *C. difficile* assay has been shown to have sensitivities of 91.8% and specificities of 99.8% (8).
Focus Simplexa Universal Direct assay. Two specimens were discrepant with the Illumigene assay. This specimen had a relatively high cycle threshold ($C_T$) value of 38.3 and was loaded next to a sample with a lower $C_T$ value (30.3); it is therefore possible that the false positivity was due to cross-contamination during sample loading, although this was not resolved at the time of the discrepancy. Meanwhile, 48 samples tested positive and 152 tested negative by the AmpliVue $C. difficile$ assay (Table 1). Two specimens were discrepant with the Illumigene assay. In addition to the false negative shared by the Simplexa $C. difficile$ Universal Direct assay, a second false negative occurred that was positive by both the Simplexa $C. difficile$ Universal Direct assay and TC. Both concordant specimens were confirmed by TC. Overall, the Simplexa $C. difficile$ Universal Direct assay and the AmpliVue $C. difficile$ assay showed 98.7% concordance with the Illumigene assay. The Simplexa $C. difficile$ real-time PCR assay was 98% (95% confidence interval [CI], 88 to 99.9%) sensitive and 100% (95% CI, 96.9 to 100%) specific, and the AmpliVue $C. difficile$ assay was 96% (CI, 95%, 85.1 to 99.3%) sensitive and 100% (CI, 95%, 96.9 to 100%) specific in comparison with the Illumigene assay. Predictive values were not calculated, as sampling was not representative of the true prevalence in the population. TC yielded results that were consistent with results obtained by the Illumigene assay. Of the patients who were positive for $C. difficile$ by the Illumigene assay, 16 had severe disease and 34 had mild disease. Discordant specimens were from patients with mild disease.

### Table 1. Performance of the Focus Simplexa Universal Direct and Quidel AmpliVue assays compared to that of the Meridian Illumigene assay

<table>
<thead>
<tr>
<th>Assay and result</th>
<th>No. of Meridian Illumigene assay results</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Focus Simplexa Universal Direct</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>49</td>
<td>98 (88–99.9)</td>
<td>100 (96.9–100)</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>1,2,3</td>
<td></td>
</tr>
<tr>
<td><strong>Quidel AmpliVue</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>48</td>
<td>96 (85.1–99.3)</td>
<td>100 (96.9–100)</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>2,3</td>
<td></td>
</tr>
</tbody>
</table>

* Positive by TC.

* Sample disregarded because of insufficient specimen amount, inability to confirm by TC.

Of 200 specimens tested, 50 were positive and 150 were negative by the Simplexa $C. difficile$ Universal Direct assay. Two specimens were discrepant with respect to the Illumigene assay, one false positive and one false negative (Table 1). The false-negative specimen was also falsely negative by the AmpliVue $C. difficile$ assay with Illumigene as the reference but was positive by TC (Table 2). The false positive was not tested by TC because of limited specimen availability but was negative by the AmpliVue $C. difficile$ assay. This specimen had a relatively high cycle threshold ($C_T$) value of 38.3 and was loaded next to a sample with a lower $C_T$ value (30.3); it is therefore possible that the false positivity was due to cross-contamination during sample loading, although this was not resolved at the time of the discrepancy. Meanwhile, 48 samples tested positive and 152 tested negative by the AmpliVue $C. difficile$ assay (Table 1). Two specimens were discrepant with the Illumigene assay. In addition to the false negative shared by the Simplexa $C. difficile$ Universal Direct assay, a second false negative occurred that was positive by both the Simplexa $C. difficile$ Universal Direct assay and TC. Both concordant specimens were confirmed by TC. Overall, the Simplexa $C. difficile$ Universal Direct assay and the AmpliVue $C. difficile$ assay showed 98.7% concordance with the Illumigene assay. The Simplexa $C. difficile$ real-time PCR assay was 98% (95% confidence interval [CI], 88 to 99.9%) sensitive and 100% (95% CI, 96.9 to 100%) specific, and the AmpliVue $C. difficile$ assay was 96% (CI, 95%, 85.1 to 99.3%) sensitive and 100% (CI, 95%, 96.9 to 100%) specific in comparison with the Illumigene assay. Predictive values were not calculated, as sampling was not representative of the true prevalence in the population. TC yielded results that were consistent with results obtained by the Illumigene assay. Of the patients who were positive for $C. difficile$ by the Illumigene assay, 16 had severe disease and 34 had mild disease. Discordant specimens were from patients with mild disease.

### Table 2. Method comparison for 10 samples

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Illumigene</th>
<th>AmpliVue</th>
<th>Simplexa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throughput$^a$</td>
<td>1–10</td>
<td>1–24</td>
<td>1–94</td>
</tr>
<tr>
<td>Specimen preparation time (min)</td>
<td>10</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Lysis time (min)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Reaction preparation time (min)</td>
<td>8</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Amplification time (min)</td>
<td>40</td>
<td>60</td>
<td>55</td>
</tr>
<tr>
<td>No. of steps postamplification</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Time postamplification (min)</td>
<td>0</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Total time to completion (min)</td>
<td>68</td>
<td>91</td>
<td>73</td>
</tr>
<tr>
<td>Hands-on time (min)</td>
<td>18</td>
<td>11</td>
<td>8</td>
</tr>
</tbody>
</table>

$^a$ Throughput is the total possible number of samples per run.
The three assays were compared in a timed run of one operator processing 10 samples. Throughput, number of steps, amount of time required for the individual steps, total hands-on time, and time to completion were evaluated (Table 2). The SimplexA C. difficile Universal Direct assay can accommodate a higher capacity of patient specimens per instrument \((n = 1 \text{ to } 94)\) than the Illumigene \((n = 1 \text{ to } 10)\) and AmpliVue \((n = 1 \text{ to } 24)\) platforms can. The total assay time was the shortest for the Illumigene assay, 68 min versus 91 and 73 min for the Simplexa and AmpliVue assays, respectively. The hands-on time per batch of 10 samples was shorter for both the Simplexa (8 min) and AmpliVue (11 min) assays than for Illumigene (18 min). The AmpliVue assay requires an additional step in which the amplification product is transferred to a disposable cartridge containing a lateral-flow strip and incubated for 10 min prior to the final readout.

The continuously expanding market of FDA-cleared NAATs reflects the need for rapid and accurate diagnostic tests for CDI. There are currently several commercially available FDA-cleared NAATs that are highly sensitive and specific for the detection of toxigenic C. difficile directly in stool specimens, all within 2 h. These include the BD Gene-Ohm, Roche LightCycler, Cepheid assays, most of which have been evaluated in the literature (18). In NAATs that are highly sensitive and specific for the detection of C. difficile infection in adults, 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). Infect. Control Hosp. Epidemiol. 34:431–435. http://dx.doi.org/10.1086/651706.

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ACKNOWLEDGMENTS

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REFERENCES


