An Evaluation of Diagnostic Tests for *Clostridium difficile* Infection

Jonathan Swindells, Nigel Brenwald, Nathan Reading, Beryl Oppenheim*

Department of Medical Microbiology, City Hospital, Birmingham, United Kingdom.

*Corresponding author. Mailing address: Department of Microbiology, City Hospital, Dudley Road, Birmingham, United Kingdom. Phone: (44) 0121-507-4078. Fax: (44) 0121-507-5521. Email: beryl.oppenheim@swbh.nhs.uk.
We evaluated toxigenic *Clostridium difficile* detection by lateral flow assay for antigen and toxin, enzyme immunoassay, and two commercial PCR methods. Compared to cell cytotoxicity neutralization assay and toxigenic culture, both toxin detection methods lacked sensitivity. PCR following combined antigen and toxin detection provided the most useful diagnostic information.

Since the advent of enzyme immunoassay tests for the *Clostridium difficile* toxins (CDT), the promise of a rapid diagnosis of *Clostridium difficile* infection (CDI) has led to their adoption for routine testing by many laboratories (1,14,16). However, recently concerns have been raised regarding the reliability of many of the rapid CDT detection methods (8,15), leading to debate over the optimal testing strategy (2,3,5,9,10,13). We therefore sought to compare the diagnostic accuracy of four rapid tests, including two commercially available PCR methods, against the reference standards of cell cytotoxicity neutralization assay (CCNA) performed on stool samples, and toxigenic culture.

During December 2008 and January 2009, 150 consecutive liquid stool specimens were evaluated from patients aged over 65 who developed diarrhoea at least 48 hours after admission. Each specimen was subjected to the following four tests: VIDAS® *Clostridium difficile* A & B (VIDAS; bioMerieux, Marcy l’Etoile, France) enzyme immunoassay for CDT; Gene Ohm™ PCR (BD Diagnostics, San Diego, CA, USA) and Xpert™ *C. difficile* PCR (Cepheid, Sunnyvale, CA, USA), both of which detect the toxin B gene but the latter also detects the gene for binary toxin and the *tdcC* deletion, which are features of PCR ribotype 027 (7,9); and *C. DIFF QUIK CHEK COMPLETE™* (Techlab, Blacksburg, VA, USA), which independently detects both the constitutive antigen glutamate dehydrogenase (GDH) and CDT in a lateral flow device. Each test was performed according to the manufacturer’s instructions by a different operator, with blinding to the other results.

The stool samples were also subjected to alcohol shock and cultured on Brazier’s medium (Oxoid, Cambridge, UK) for growth of *C. difficile*, with colony identification based on morphology and cell wall antigen latex agglutination according to standard methods (6). CCNA was performed directly on...
each sample and positive culture isolates using Monkey African Green Kidney ‘Vero’ cells (ECACC number 84113001). After incubation at 33°C for 24 hours, diluted stool or culture isolates producing a cell cytopathogenic effect that was inhibited by the presence of *C. sordellii* antitoxin (Pro-Lab Diagnostics, Richmond Hill, ON, Canada) were inferred to contain CDT.

All the commercial tests were performed in accordance with the instructions issued by the manufacturer. The VIDAS, *C. DIFF QUIK CHEK COMPLETE*™, and stool CCNA tests were all undertaken within 48 hours of receipt of the samples in the laboratory and, where not tested immediately, the specimens were stored at 2-8°C to prevent degradation of their targets. Testing was performed to simulate in-use conditions and therefore none of the tests were repeated.

Ribotyping was performed on positive culture isolates 48 hours after anaerobic subculture onto Columbia blood agar, as previously described (12). The gel electrophoresis pattern of the PCR products was then compared for an identical match with that of a known PCR ribotype 027 *C. difficile* strain (NCTC 13366).

*C. difficile* was cultured from 19 specimens, and the GDH component of the *C. DIFF QUIK CHEK COMPLETE*™ test was positive for all these samples. Three samples were GDH-positive but negative by all other tests, one of which contained a CCNA-negative isolate of *C. difficile*. The overall toxigenic *C. difficile* carriage rate was therefore determined to be 12%, as 18 of the isolates were CCNA-positive.

The Xpert™ *C. difficile* PCR was positive in each of these cases, whilst the Gene Ohm™ PCR produced one false negative result (table 1).

The stool CCNA was positive for 15 specimens (10%) and using this as the reference standard, the sensitivities were 73.3% for the CDT component of the *C. DIFF QUIK CHEK COMPLETE*™ test and 53.3% for the VIDAS (table 2). All CCNA-positive stool specimens were positive by both of the PCR tests but there were four stool CCNA-negative samples that were positive in the Xpert™ *C. difficile* PCR, three of which also produced a positive Gene Ohm™ PCR result. Toxigenic isolates were
recovered from three of these specimens, whilst the final sample yielded no organism on culture despite
the detection of GDH and positive results by both PCR tests (table 1).

Eight samples were identified by the Xpert™ C. difficile PCR as containing all three genetic targets.
One of these samples was culture-negative but an isolate belonging to PCR ribotype 027 was recovered
from the remaining seven. The 11 specimens lacking these molecular features all yielded C. difficile
isolates belonging to other PCR ribotypes. In addition, 7/8 of the samples with all three Xpert™ C.
difficile PCR targets produced positive toxin results in the C. DIFF QUIK CHEK COMPLETE™ test
compared to only 4/11 specimens lacking these molecular features (P = 0.037, Fisher’s exact test).

We found that the GDH component of the C. DIFF QUIK CHEK COMPLETE™ test and both PCR
methods were highly sensitive for the detection of toxigenic C. difficile organisms in stool specimens.
These tests can therefore be relied upon for the exclusion of C. difficile carriage or infection and are
ideally suited to screening large numbers of specimens as the results are quickly available. The PCR
methods offer greater specificity, although their cost is greater and there is a risk that mutations in the
toxin B gene may reduce their sensitivity in the future, which may go undetected if PCR is used alone.

The stool CCNA remains a suitable reference standard for detection of CDT itself in specimens
(3,5,9,13,14), and by comparison both rapid tests for CDT lacked the required sensitivity to warrant
their use in isolation. Previous studies have reached similar conclusions for different enzyme
immunoassays (3,5,13,17), whilst concerns regarding poor positive predictive values of these tests have
also been raised (8,15).

As in the present study, other authors have observed good agreement between PCR methods and
stool CCNA testing (4,9,11,14). We also found that there was even better concordance between PCR
and toxigenic culture, which has been suggested as a more sensitive method for diagnosing CDI (1).
Additionally, our data suggest that the Xpert™ C. difficile PCR was able to indicate the presence of
PCR ribotype 027, which could aid in the epidemiological assessment of clusters and has been
proposed as a potential risk factor for metronidazole treatment failure (7). Our observation that toxin
detection by the C. DIFF QUIK CHEK COMPLETE™ test was significantly more likely in these samples is consistent with the fact that the tcdC deletion leads to loss of suppression of CDT production (7,9), and suggests that the relative burden of this ribotype may be overestimated if CDT detection is relied upon without utilising the enhanced sensitivity of PCR.

We therefore propose a two-step testing algorithm using the C. DIFF QUIK CHEK COMPLETE™ test to screen all diarrhoeal samples, followed by Xpert™ C. difficile PCR testing of any that are GDH-positive. Our strategy has several advantageous features: results can be reported rapidly for samples if they are GDH-negative (85.3% in our study), or positive for both GDH and CDT (7.3% in our study). For samples with discordant results, PCR testing can then exclude the presence of toxigenic C. difficile strains in approximately one additional hour. Figure 1 shows how this algorithm would have performed in the present study. For samples with positive CDT or PCR results, the diagnosis of CDI will always require clinical correlation with the laboratory findings. However, it is desirable that all such patients be isolated, and even where the results are thought to represent toxigenic carriage a higher treatment threshold may be adopted when starting antibiotic therapy for other indications.

In conclusion, to provide an optimal laboratory service that incorporates rapid turnaround times and reliable diagnostic accuracy, the rapid methods for C. difficile detection must all be combined. The most useful information can be obtained by screening all samples for the presence of GDH, with toxin detection performed either concurrently or subsequently on GDH-positive specimens, followed by PCR to distinguish between toxigenic and non-toxigenic strains in those samples with discordant GDH and CDT results. Our observation that CDT detection rates may vary between PCR ribotypes deserves further study.

References


TABLE 1. Comparison of results obtained for *C. DIFF QUIK CHEK COMPLETE*™, VIDAS®

*Clostridium difficile* A & B enzyme immunoassay, Xpert™ *C. difficile* PCR, Gene Ohm™ PCR, stool CCNA, *C. difficile* culture and CCNA performed on culture isolates.

<table>
<thead>
<tr>
<th>C. DIFF QUIK CHEK COMPLETE™</th>
<th>VIDAS</th>
<th>Xpert™ <em>C. difficile</em> PCR</th>
<th>Gene Ohm™ PCR</th>
<th>Stool CCNA</th>
<th><em>C. difficile</em> Culture</th>
<th>Culture CCNA</th>
<th>Total</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDH</td>
<td>CDT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>8</td>
<td><em>C. difficile</em> infection</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>2</td>
<td>Toxigenic <em>C. difficile</em> carriage</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>N/A</td>
<td>1</td>
<td>Non-toxigenic carriage</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>N/A</td>
<td>2</td>
<td>False positive GDH result</td>
</tr>
<tr>
<td>22</td>
<td>11</td>
<td>8</td>
<td>19</td>
<td>18</td>
<td>15</td>
<td>19</td>
<td>18</td>
<td>150</td>
</tr>
</tbody>
</table>

*Interpreted as a false negative result; +, Positive result; -, Negative result; N/A, Not applicable.*

TABLE 2. Performance of *C. DIFF QUIK CHEK COMPLETE*™, VIDAS® *Clostridium difficile* A & B enzyme immunoassay, Xpert™ *C. difficile* PCR and Gene Ohm™ PCR compared to stool CCNA and toxigenic culture.

<table>
<thead>
<tr>
<th>C. DIFF QUIK CHEK COMPLETE™</th>
<th>VIDAS</th>
<th>Xpert™ <em>C. difficile</em> PCR</th>
<th>Gene Ohm™ PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDH</td>
<td>CDT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Performance Compared to Stool CCNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Sensitivity (95% CI)</td>
<td>100 (79.4-100)</td>
<td>73.3 (47.6-89.0)</td>
<td>53.3 (29.9-75.3)</td>
</tr>
<tr>
<td>% Specificity (95% CI)</td>
<td>94.8 (89.6-97.4)</td>
<td>100 (97.3-100)</td>
<td>100 (97.3-100)</td>
</tr>
<tr>
<td>% PPV (95% CI)</td>
<td>68.2 (47.1-83.6)</td>
<td>100 (73.5-100)</td>
<td>100 (66.4-100)</td>
</tr>
<tr>
<td>% NPV (95% CI)</td>
<td>100 (97.2-100)</td>
<td>97.1 (92.8-98.8)</td>
<td>95.1 (90.2-97.6)</td>
</tr>
<tr>
<td>Performance Compared to Toxigenic Culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Sensitivity (95% CI)</td>
<td>100 (82.4-100)</td>
<td>61.1 (38.4-79.7)</td>
<td>44.4 (24.4-66.5)</td>
</tr>
<tr>
<td>% Specificity (95% CI)</td>
<td>97.0 (92.5-98.8)</td>
<td>100 (97.3-100)</td>
<td>100 (97.3-100)</td>
</tr>
<tr>
<td>% PPV (95% CI)</td>
<td>81.8 (61.2-92.5)</td>
<td>100 (73.5-100)</td>
<td>100 (66.4-100)</td>
</tr>
<tr>
<td>% NPV (95% CI)</td>
<td>100 (97.2-100)</td>
<td>95.0 (90.0-97.5)</td>
<td>93.0 (87.5-96.1)</td>
</tr>
</tbody>
</table>

CI, Confidence interval; PPV, Positive predictive value; NPV, Negative predictive value.
FIGURE 1. Illustration of the proposed testing algorithm for detection of *C. difficile* in stool samples for our study population.

Diarrhoeal stool specimen (N=150)

**C. DIFF QUIK CHEK COMPLETE™**

GDH positive (N = 22)

Toxin positive (N = 11)

Toxin negative (N = 11)

**Presumed CDI**

GDH negative (N = 114)

**C. difficile negative**

**Xpert™ C. difficile PCR**

PCR positive (N = 8)

CDI (N = 4) or toxigenic carriage (N = 4)

Clinical assessment essential

PCR negative (N = 3)

Non-toxigenic carriage (N = 2) or false positive GDH (N = 1)

No CDI

Clinical assessment essential