Rapid detection of *Clostridium difficile* toxins from stool samples using real-time multiplex PCR

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In this study, a total of 650 stool samples were tested to show that our method is capable of detecting four *Clostridium difficile* genes; *tcdA*, *tcdB*, encoding toxin A (TcdA) and toxin B (TcdB), and the binary toxin *C. difficile* transferase genes (*cdtA* and/or *cdtB*) encoding CDT toxin. Besides detecting the targeted *C. difficile* genes, our method can be used to detect the presence of any inhibitory components in the PCR. This assay, combined with a selective culture medium, such as the chromID™ *C. difficile*, can be applied directly for screening *C. difficile*-associated disease. The PCR-based assay developed here is rapid (4 h per 21 stool samples) and accurate in diagnosing *C. difficile* infection, 100 % assay sensitivity and negative predictive value (NPV) were obtained. However, the assay specificity of 99.1 % and positive predictive value (PPV) of 94.9 % were slightly lower than the optimal value of 100 %. The assay protocol outlined here can be used as a rapid screening tool to assist infection control units and in managing infected patients by reducing the number of patients requiring isolation and extended hospitalization. Rapid detection can prevent unnecessary antibiotic therapy and potentially reduce the spread of infection by emerging hypervirulent *C. difficile* strains.

**INTRODUCTION**

*Clostridium difficile* is the most common cause of health care- and antibiotic-associated diarrhoea in Australian hospitals. It causes significant illness and is a leading cause of hospital-acquired infection. Two major toxins produced by *C. difficile*, toxin A (308 kDa) and toxin B (270 kDa), are the main source of *C. difficile*-associated disease (CDAD). It is argued that the level of toxins produced somewhat correlates with the severity of disease (Voth & Ballard, 2005; Drudy et al., 2007). Toxin A is a cytotoxic enterotoxin that causes tissue damage while toxin B is a more potent faecal cytotoxin. Toxin B has a direct cytopathic effect by depolymerizing filamentous actin and causing destruction of the cytoskeleton, which in turn causes damage to the colonic mucosa of the gastrointestinal tract (Guilbault et al., 2002).

*C. difficile* is also capable of producing binary actin-ADP-ribosyltransferase toxin as an additional clinically relevant toxin, which is also shown to be produced by other pathogenic species of the genus *Clostridium* and *Bacillus*. Schwan et al. (2009) reported that 35 % of *C. difficile* clinical isolates produce actin-ADP-ribosylating toxin, known as binary toxin *C. difficile* transferase (CDT). The toxin consists of two unlinked components, CDTa, the enzymic component for translocation into the cell and CDTb, the receptor-binding component. The CDT components are not related to the two large clostridial toxins (toxin A and B) but similarly are able to disrupt the host’s actin cytoskeleton in epithelial cells (Perelle et al., 1997; Geric et al., 2003; McEllistrem et al., 2005; Schwan et al., 2009; Bacci et al., 2011). Schwan et al. (2009) reported that the CDT is also able to induce the formation of unique long microtubule-based protrusions on the surface of epithelial cells *in vitro*, leading to increased adherence of clostridia. This suggests that CDT plays an important role in the host–pathogen interactions due to its effects on adherence and colonization of the *C. difficile* organism (Bacci et al., 2011).

Earlier strategies to detect *C. difficile* infection consisted of (i) anaerobic cultures of the stool samples, grown with or without pre-treatment with alcohol shock, (ii) cell culture cytotoxicity assays (faecal cytotoxin and cytotoxigenic culture assays), (iii) phenotypic tests, especially enzyme

**Abbreviations:** BHIA, brain heart infusion agar; CDAD, *C. difficile*-associated disease; CDT, *C. difficile* transferase; TC, *C. difficile*-BHIA-taurocholate; CYT, cytotoxin; CYTG, cytotoxigenic culture; RT-MPCR, real-time multiplex PCR.
immunoassays, which are less expensive and fast but insensitive, and (iv) nucleic acid detection method of the toxin genes, which is accurate and quick but expensive for routine applications in pathology laboratories.

The cell culture cytotoxicity assays are considered the ‘gold standard’ method and are still used in many diagnostic laboratories for the detection of toxin B. However, the method is time consuming with results often delayed from 24 to 72 h. In addition, it is labour intensive and requires maintenance of particular cell lines (Guilbault et al., 2002; Peterson et al., 2007; Eastwood et al., 2009). Hence, a more rapid and more accurate method of diagnosis of CDAD is required for not only patient management but also the implementation of effective infection control measures in hospitals (Riley, 2004; Eastwood et al., 2009; Carroll & Loeffelholz, 2011).

This study has developed and validated a rapid, more sensitive and toxin-specific single-tube real-time multiplex PCR (RT-MPCR) assay to detect C. difficile infectious genes. The method has a turn-around time of about 4 h for 21 stool samples, including processing, automated extraction, PCR and analysis/reporting. It is also cost-effective ($10.50 for a single PCR tube including extraction and the PCR assays).

**METHODS**

**Faecal specimens.** A total of 650 stool samples were collected from different patients from January to April 2012, de-identified and stored at −80 °C for the purpose of this study. Stool samples were processed for enteric culture on the day of receipt and the *C. difficile* cultures were performed on unformed and liquid stool samples according to the standard practices of Austin Pathology.

**Bacterial cultures.** Stool samples were directly cultured onto pre-reduced chromID™ *C. difficile* (chromID) culture plates and incubated anaerobically at 36 °C for 24 h. Colonies that were grey or black and roughly circular with an irregular edge or round smooth edges were confirmed as *C. difficile* (Oxoid *C. difficile* Agglutination Test Kit). The *C. difficile* black colonies from the chromID culture plates were further confirmed by culturing them onto pre-reduced anaerobic blood agar and *C. difficile*-BHIA-taurocholate (TC) culture plate (Thermo Fisher Scientific) and incubated anaerobically at 36 °C for another 24 to 48 h. All positive *C. difficile* colonies were further confirmed again based on the morphological criteria, Gram-stain results and odour.

**Cell culture cytotoxicity assays.** For the faecal cytotoxin assay (CTY), faecal samples were diluted 1:10 in phosphate-buffered saline (PBS, pH 7.2), centrifuged for 15 min at 3500 g and the supernatant filtered through 0.20 μm membrane filters. For cytotoxicogenic culture (CTYGc), *C. difficile* colonies grown on pre-reduced anaerobic blood agar or TC culture plates were inoculated into a cooked meat broth supplemented with 0.2 ml of 5% l-cysteine hydrochloride mono-hydrate solution. After 48 h of anaerobic incubation at 36 °C, the cultures were centrifuged for 5 min at 16200 g and supernatant filtered through a 0.20 μm membrane filter.

For the CTY and CTYG assays, 25 μl of *C. difficile* toxin suspension (positive control), supernatant filtrates from the stool or isolate filtrates were added to duplicate VERO cell monolayers in the Falcon 24-well trays, one of which was protected with 25 μl of *C. difficile* antitoxin (Alere™, QLD). After incubation for 2 days in a 5% CO2 incubator at 36 °C, the results were compared with the positive control wells. The presence of *C. difficile* toxin B was only considered positive when cells in the unprotected well were rounded whilst the cytopathic effects were prevented in the protected cells.

**DNA extraction.** Liquid stool sample (100 μl) or 1 g of unformed stool was suspended in 500 μl PBS (pH 7.2) without magnesium or calcium (Lonza). The suspension was thoroughly mixed and centrifuged for 10 min at 3500 g. A QiAsymphony Virus/Bacteria Mini kit reagent (Qiagen) was used on the fully automated QiAsymphony SP equipment (Qiagen) to extract DNA from the supernatant.

As for the *C. difficile* isolates, before extraction, they were processed by incubating 500 μl of the supplemented cooked meat broth at 95 °C for 10 min followed by centrifugation at 16200 g for 5 min. Next, 180 μl of tissue lysis buffer, ATL (Qiagen) and 20 μl proteinase K were added to the bacterial pellet, mixed and incubated at 56 °C for 30 min. Then 200 μl of AL buffer (Lysis buffer, Qiagen) was added, mixed and further incubated at 70 °C for 10 min. A DNA blank, 450 μl PBS (pH 7.2), without magnesium or calcium, was included in every extraction batch. During the automated extraction process, an internal DNA control (Sigma-Aldrich) (jalal et al., 2006) and carrier RNA (Qiagen) were also used.

**Real-time multiplex PCR assay.** Unique primer and probe sets were used to target *C. difficile* enterotoxin A gene (*tcdA*), cytotoxin B gene (*tcdB*), CDT genes A and B (*tcdA* and *tcdB*). These were based on a study carried out by Grando *et al.* (Wroblewski *et al.*, 2009; Grando *et al.*, 2012). A BLAST search was performed on these primers and probes to confirm that there was no cross-reactivity with any other organisms. Internal-control primers and probe (jalal *et al.*, 2006) were also included in the reaction mixtures. All primers were purchased from Sigma-Aldrich and probes from Geneworks.

To detect toxins, the RT-MPCR assay was performed on DNA extracted from stool samples or *C. difficile* isolates, in triplicates or duplicates, respectively, using the Rotor-Gene 6000 (Qiagen). The total PCR volume was 25 μl and consisted of 10 μl of DNA template, 12.5 μl (2 x) of the Rotor-Gene Multiplex PCR Master Mix (Qiagen), PCR-grade water, 0.16 μM of toxin-gene-specific probes and 0.1 μM internal-control probe, and sets of primers: 0.6 μM *tcdA*, 0.72 μM *tcdB*, 0.4 μM *cdtA*, 0.1 μM *cdtB* and 0.05 μM internal control. The thermal cycling conditions were as follows: 95 °C for 5 min, followed by 50 cycles at 95 °C for 15 s and at 60 °C for 15 s. The RT-MPCR assay chemistry is based on TaqMan probe chemistry.

**Analytical specificity.** To determine specificity, 10 different strains from the clostridia group were tested using the RT-MPCR. These included ATCC organisms (Abacus ALS): *C. bifurcans* ATCC 638; *C. septicum* ATCC 12464; *C. difficile* ATCC 9869; *C. sordellii* ATCC 9714; *C. histolyticum* ATCC 19401; *C. difficile* ATCC 700057; *C. tetani* ATCC 19406; *C. sporogenes* ATCC 3584; *C. perfringens* ATCC 13124 and *C. difficile* 196 strain (non-epidemic).

**Analytical sensitivity.** Two different methods were used based on procedures as outlined by Luna *et al.* (2011). Method 1: DNA was extracted from the *C. difficile* strain 196, followed by absorbance spectrophotometric quantification (Spectrophotometer Nanodrop 2000, ThermoFisher Scientific) and 10-fold serial dilutions of the extracted DNA. Method 2: 1 ml of a liquid stool sample (negative for *C. difficile* toxins) was spiked with a large inoculum of *C. difficile* strain 196, followed by 10-fold serial dilutions into a liquid stool sample that was negative for *C. difficile* toxins. For each undiluted as well as serially diluted samples, 100 μl was used (i) for DNA
extraction and RT-MPCR protocol and (ii) to plate *C. difficile* cultures onto chromID plates in triplicate and incubated anaerobically at 36 °C for 24 to 48 h and colonies were counted (c.f.u. reaction).

**Reference and verification methods.** To confirm the CDT genes detected using the RT-MPCR, another method of the Xpert *C. difficile* assay (Cepheid) was used. This assay detects the presence of toxin genes, specifically targeting toxin B, as well as the CDT genes. Stool samples that had discrepant results for toxin B detection between CYT and RT-MPCR assays were all tested using this assay to determine the validity of the results.

Stool samples detected for toxin A gene by RT-MPCR were verified using the high-resolution melting (HRM) real-time PCR method on the Rotor-Gene 6000. HRM was performed on extracted DNA in duplicate using *tcdA* primers from the RT-MPCR. The total PCR consisted of 5 μl of DNA template, 10 μl (2 ×) MeltDoctor™ HRM Master Mix (Applied Biosystems), 2.0 μM *tcdA* primers and 4.6 μl PCR-grade water. Thermal cycling conditions for HRM real-time PCR on the Rotor-Gene 6000 were as follows: 95 °C for 10 min, 50 cycles of 95 °C for 15 s and 60 °C for 15 s (initial amplification process) followed by one cycle of HRM from 60 °C up to 85 °C in 0.1 °C increments to generate a melt curve. HRM results were then analysed using the Rotor-Gene 6000 software (Qiagen, GmbH) with the melt temperature peak for *tcdA* ranging from 72.90 to 73.85 °C.

The cell culture cytotoxicity assays have been considered as ‘gold standard’ for detection of *C. difficile* toxins at Austin Pathology and were therefore used as a reference standard against the RT-MPCR assay to determine assay sensitivity and specificity. In this study, CYT was performed on all the collected stool samples and CYTGC was only used to reconfirm non-toxigenic *C. difficile* isolates detected by RT-MPCR and to resolve discrepancies between CYT and RT-MPCR results.

Culture on selective media was repeated for *C. difficile* growth when PCR results did not match culture results. The stool sample was pre-treated with alcohol shock (absolute ethanol for 30 min) prior to culturing onto two different pre-reduced media: TC plates and treated with alcohol shock (absolute ethanol for 30 min) prior to PCR results did not match culture results. The stool sample was pre-

Culture on selective media was repeated for 48 h.

**RESULTS**

**Real-time multiplex PCR**

The results from the RT-MPCR and cell culture cytotoxicity assays showed that of the 650 stool samples tested, 93 (14.3 %) were identified as positive for toxigenic *C. difficile* while 552 (84.9 %) were toxin negative (Table 1). The results from pre-reduced chromID plates followed by other selective culture media showed that of 100 *C. difficile* isolates cultured, 7 % were non-toxigenic and 93 % toxigenic. Of 93 % toxigenic *C. difficile*, 75 stool samples were positive for *tcdA* and *tcdB* while 18 samples were positive for *tcdA*, *tcdB*, *cdtA* and *cdtB* genes (Tables 1 and 2).

The performance characteristics of the RT-MPCR were compared in all the 650 stool samples, using the cell culture cytotoxicity assays (CYT and CYTGC) as the reference standards (Table 3). True positives were defined by the reference standard approach, and true negatives were defined as negative by cell culture cytotoxicity and RT-MPCR methods. There were five false-positive results with low PCR yield for *tcdA* and *tcdB* targets, with the cycle threshold (Ct) close to 40 Ct. *C. difficile* isolates were not available for these samples to test with CYTGC as culture was persistently negative even after several repeats. Cepheid Xpert *C. difficile* assay and HRM real-time PCR were performed using DNA extracted from the direct stool samples to determine the validity of these results. The Cepheid Xpert *C. difficile* assay (*tcdB*) produced negative results but HRM real-time PCR (*tcdA*) results were positive, probably because the maximum number of cycles for the Cepheid Xpert *C. difficile* assay was most likely 30 cycles. The HRM real-time PCR results had low PCR yields and a Ct value from 35 to 40 cycles.

**Analytical specificity and sensitivity of the PCR assay**

Analytical sensitivity results for the RT-MPCR yielded 100 % specificity against all 10 different strains from the genus clostridia. *tcdA* and *tcdB* genes were detected in the *C. difficile* ATCC 9689 strain and *tcdA*, *tcdB*, *cdtA* and *cdtB* in the *C. difficile* 196 strain. The non-toxigenic *C. difficile* ATCC 700057 strain and non-*C. difficile* strains showed no amplification signal or cross-reactivity with *tcdA*, *tcdB*, *cdtA* or *cdtB* targets tested in the RT-MPCR. An absolute lowest limit of genomic DNA concentration and bacterial colony count that was detectable on the RT-MPCR assay was determined (Table 4).

**Verification of the toxin genes detected by real-time multiplex PCR assay**

Toxin genes detected in the RT-MPCR were compared individually against several other assays that were available in Austin Pathology (Table 5). These included: CYT and CYTGC (*tcdB*), HRM real-time PCR (*tcdA*) and Cepheid

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### Table 1. Summary of toxin genes detected using real-time multiplex PCR assay

<table>
<thead>
<tr>
<th>Assay/gene toxin</th>
<th>Results of RT-MPCR (no. of samples)</th>
<th>Detected</th>
<th>Not detected</th>
<th>False positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>tcdA</em> and <em>tcdB</em></td>
<td>75</td>
<td>552</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td><em>tcdA</em>, <em>tcdB</em>, <em>cdtA</em> and <em>cdtB</em></td>
<td>18</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>93</strong></td>
<td><strong>552</strong></td>
<td></td>
<td><strong>5</strong></td>
</tr>
</tbody>
</table>
confirmed positive for toxin B. As for between 72.90 and 73.85
by RT-MPCR, all 93 samples showed a melt temperature
shock to isolate
these samples. Cultures were repeated on selective media
tcdB
performed to confirm the validity of
CYTGC and matched the RT-MPCR results for
C. difficile
tcdB
negative. Of the 39 stool samples, 34 were tested with
detection. However, 5 of the 39 stool samples were culture
the
tcdB
results for CYT, which matched the RT-MPCR results for
Fifty-four of the 93 (58.1 %) stool samples had positive
Figures from tested stool samples

**DISCUSSION**

Nucleic acid amplification tests (NAATs) are gradually
replacing more conventional methods for C. difficile

**Table 2. Summary of toxins produced by C. difficile isolated from tested stool samples**

<table>
<thead>
<tr>
<th>Toxins</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>93</td>
</tr>
<tr>
<td>B</td>
<td>93</td>
</tr>
<tr>
<td>CDT*</td>
<td>18</td>
</tr>
<tr>
<td>None</td>
<td>7</td>
</tr>
</tbody>
</table>

*CDT, Binary toxin C. difficile transferase.

Xpert C. difficile assay (cdtA/B). Altogether, 93 of the 650
stool samples had tcdB and tcdA detected by RT-MPCR.
Fifty-four of the 93 (58.1 %) stool samples had positive
results for CYT, which matched the RT-MPCR results for
the tcdB gene target, but 39 of the 93 (41.9 %) were
negative. Of the 39 stool samples, 34 were tested with
CYTG and matched the RT-MPCR results for tcdB
detection. However, 5 of the 39 stool samples were culture
negative and the Cepheid Xpert C. difficile assay testing was
performed to confirm the validity of tcdB detection on
these samples. Cultures were repeated on selective media
after pre-treating these five stool samples with alcohol
shock to isolate C. difficile to perform CYTG that were
certified positive for toxin B. As for tcdA gene detection
by RT-MPCR, all 93 samples showed a melt temperature
between 72.90 and 73.85 °C by HRM real-time PCR, which
matched the positive controls. CDT genes were detected in
18 of 93 (19.4 %) stool samples. This was verified by the
Cepheid Xpert C. difficile assay, and in addition, a single
nucleotide deletion at position 117 of the tcdC gene was
also detected in 12 stool samples.

**Table 3. Performance characteristics of the real-time multiplex
PCR compared to cell culture cytotoxicity assays as our defined reference standard**

<table>
<thead>
<tr>
<th>Results</th>
<th>RT-MPCR</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>True positive</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>True negative</td>
<td>552</td>
<td></td>
</tr>
<tr>
<td>False positive</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>False negative</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>% Sensitivity</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>% Specificity</td>
<td>99.1</td>
<td></td>
</tr>
<tr>
<td>PPV (%)‡</td>
<td>94.9</td>
<td></td>
</tr>
<tr>
<td>NPV (%)†</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

*PPV, Positive predictive value.
†NPV, Negative predictive value.

In comparison to other real-time PCR assays, our RT-
MPCR assay includes an additional set of primers and
probe in the single-tube assay to detect the internal DNA
control, spiked during the extraction process to monitor
the efficiency of sample preparation and extraction. This is
necessary to overcome inhibition issues encountered using
direct stool samples (Bélinger et al., 2003; Peterson et al.,
2007; de Boer et al., 2010; Luna et al., 2011). The internal
DNA control is detected for all extracted stool samples and
controls except the no template control (NTC), which does
not go through the extraction process. Hence, the presence
of inhibitor within a PCR can be determined using a probe
specific channel on the Rotor-Gene 6000 software. The
assay uses four other internal probe specific channels to
detect the tcdA, tcdB, cdtA and cdtB genes, respectively, and
the detection of each toxin was verified individually.

The importance of toxin A and/or toxin B as exotoxins was
considered an essential criterion in the development of our
assay and as an effective countermeasure against C. difficile
infection (Voth & Ballard, 2005; Lytras et al., 2009;
Wroblewski et al., 2009; Kuehne et al., 2010; Bacci et
al., 2011). The C. difficile strains from our validation results
were mainly toxin A+ B+ and no toxin-variant strains
were detected. However, our assay includes uniquely
designed primers and probes, which are based on
TaqMan probe chemistry and each channel on the Rotor-
Gene 6000 software is specific for the detection of a
particular toxin gene. Hence, the assay is capable of
detecting toxin-variant strains that produce toxins A+ B+,
A+ B− or A− B+ CDT+. The CDT genes were also included
because it has been suggested that there is a correlation
between the severity of CDAD and toxins A, B, and CDT-
positive strains. The increased severity of disease, in C.
difficile strains producing CDT is linked with the
production of large clostridial toxins A and B (Stubbs et
al., 1999; McEllistrem et al., 2005; Geric et al., 2006).
Stool samples that were positive for \( tcdA \), \( tcdB \), \( cdtA \) and \( cdtB \) toxin genes were sent to the Microbiology Diagnostic Unit (MDU) for further confirmation. The MDU also performed PCR ribotyping that was based on the method of Stubbs et al. (1999), which targeted the 16S–23S rRNA gene intergenic spacer region of \( C. difficile \). This was used to detect hypervirulent strains, especially the \( C. difficile \) strain NAP1/027. No hypervirulent strains were detected (data not shown) especially in the \( C. difficile \) strain NAP1/027. However, it is important to consider that there are other non-NAP1 hypervirulent strains that can cause equally severe illness in patients (Carroll & Loeffelholz, 2011; Cheng et al., 2011).

This is supported by Bacci et al. (2011), who reported that patients infected with \( C. difficile \) strains possessing the CDT genes and genes encoding toxins A and B have a higher 30 day case-fatality rate, irrespective of the PCR ribotype. This indicates that the CDT genes are closely linked with genes encoding toxin A and B. Hence, our RT-MPCR can be used as an indicator or a screening tool to determine the virulence of \( C. difficile \) strains and enable the infection control unit to rapidly take steps to implement appropriate patient management procedures to stop the possible spread of an epidemic strain (Perelle et al., 1997; Spigaglia & Mastrantonio, 2002).

Anaerobic stool culture and cell culture cytotoxicity assays have been used diagnostically in pathology to report toxin B detection in \( C. difficile \)-infected patients for years (Bowman & Riley, 1988; Salari et al., 2008; Huang et al., 2009; Tenover et al., 2010). However, both these methods have limitations as alternative testing platforms because they are time consuming, labour intensive, only toxin B is detectable and lack of sensitivity from poor standardization and inexperience in reading cell culture cytotoxicity assays (Bélanger et al., 2003; Eastwood et al., 2009; Cohen et al., 2010; Cheng et al., 2011; Luna et al., 2011). In contrast, when using chromID culture and our RT-MPCR assay as the testing method, results would be available to clinicians within 24 h for \( C. difficile \) growth and toxin gene detection.

### Conclusion

Efficient detection of \( C. difficile \) infection in pathology laboratories together with selecting a testing strategy that

<table>
<thead>
<tr>
<th>Channels/target gene</th>
<th>Genomic DNA</th>
<th>Bacterial</th>
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<tbody>
<tr>
<td></td>
<td>Conc (fg reaction (^{-1}))</td>
<td>Ct value</td>
</tr>
<tr>
<td>Crimson/( tcdB )</td>
<td>12.6</td>
<td>37.66</td>
</tr>
<tr>
<td>Orange/( tcdA )</td>
<td>1.14</td>
<td>38.04</td>
</tr>
<tr>
<td>Green/( cdtA )</td>
<td>12.0</td>
<td>37.01</td>
</tr>
<tr>
<td>Yellow/( cdtB )</td>
<td>1.11</td>
<td>36.54</td>
</tr>
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</table>

### Table 4. Verification of toxins detected by real-time multiplex PCR assay

Toxin genes detected in the RT-MPCR were compared individually against several other assays that were available in Austin Pathology. These included: CYT and CYTGC (\( tcdB \)), HRM real-time PCR (\( tcdA \)) and Cepheid Xpert \( C. difficile \) assay (\( cdtA/B \)). Stool samples that were positive for \( tcdA \), \( tcdB \), \( cdtA \) and \( cdtB \) toxin genes were sent to the Microbiology Diagnostic Unit (MDU) for further confirmation.

<table>
<thead>
<tr>
<th>Verification methods/toxins</th>
<th>No. of samples</th>
<th>*Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Detected</td>
<td>Not detected</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>CYT†</td>
<td>–11</td>
<td>54</td>
</tr>
<tr>
<td>CYTGC‡</td>
<td>39</td>
<td>–</td>
</tr>
<tr>
<td>HRM real-time PCR</td>
<td>93</td>
<td>–</td>
</tr>
<tr>
<td>GenXpert Cepheid</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>MDU§</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

*Number of samples tested.
†CYT, Faecal cytotoxin culture assay.
‡CYTGC, Cytotoxigenic culture assay.
§MDU, In-house multiplex PCR assays used by the Microbiology Diagnostic Unit.
||Not tested.
can address the need for rapid and accurate results has been quite challenging. Our RT-MPCR is a possible solution. The C. difficile detection method outlined here is rapid to complete (turn-around time about 4 h for 21 stool samples, including processing, automated extraction, PCR and analysis/reporting), with shorter turn-around time for diagnosis. It can help detect and identify the source of C. difficile infection within the hospital. The protocol can therefore be used as a screening tool to assist the hospital infection control unit and in managing infected patients by reducing the number of patients requiring isolation and extended hospitalization. For further confirmation of the results, it is however still necessary/recommended, to couple the assay with other testing modalities such as the chromID culture plate.

ACKNOWLEDGEMENTS

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