Evaluation of the Xpert *Clostridium difficile* Assay for the Diagnosis of *Clostridium difficile* Infection

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Infection with *Clostridium difficile* is a growing concern because of the increasing prevalence and spread of nosocomial infections. Emergence of the hypervirulent 027/NAP1/BI strain is also notable. Existing diagnostic methods have low sensitivity or are time-consuming. Therefore, establishing a rapid and accurate microbiological diagnostic assay is needed. We evaluated the Xpert *C. difficile* assay (Xpert CD assay; Cepheid, USA) to detect toxigenic *C. difficile*. This assay is a real-time multiplex PCR assay that can be used to detect toxigenic *C. difficile* strains and differentiate the *C. difficile* presumptive 027/NAP1/BI strain. A total of 253 loose stool specimens were collected and toxigenic cultures, VIDAS *C. difficile* A & B assays (VIDAS CDAB assay; bioMérieux, France), and the Xpert CD assay were performed. In comparison to toxigenic cultures, the sensitivity, specificity, and positive and negative predictive values were 100%, 94.6%, 83.1%, and 100%, respectively, for the Xpert CD assay and 40.8%, 98.0%, 100%, and 88.9%, respectively, for VIDAS CDAB assay. Because of the low prevalence of the PCR ribotype 027 in Korea, the evaluation of the usefulness of the Xpert CD assay for screening for the 027 strain was limited. The Xpert CD assay provides great sensitivity in diagnosing toxigenic *C. difficile* infection. In addition, this method has excellent usability because it is simple and fast.

Key Words: *Clostridium difficile*, Real-time PCR, Enzyme immunoassay
C. difficile strains and differentiate C. difficile presumptive 027/NAP1/BI. We evaluated the Xpert CD assay for rapidity and accuracy in diagnosing CDI.

A total of 253 consecutive loose stool specimens were collected in a stool specimen container from suspected CDI patients from April to June 2011, in a tertiary hospital. For toxigenic cultures, alcohol-shocked stool specimens were inoculated on C. difficile selective agar (CDSA; Becton, Dickinson and Company, Sparks, MD, USA) and incubated at 37°C in an anaerobic chamber (Forma scientific, Marietta, OH, USA) for 48 hr. Suspected C. difficile colonies were used to make a gram-stained smear to observe typical morphology. The species were identified by using the ATB 32A system (bioMérieux, Marcy l’Etoile, France). The identified C. difficile isolates were used to detect tcdA repetitive regions, tcdB as well as cdtA and cdtB genes, following the previously described PCR method [7] and using the PCR primers listed in Table 1.

Xpert CD assays were performed according to the manufacturer’s instructions. A stool specimen was transferred to a vial containing a buffer solution by using a sterile swab. The vial was vortexed, and the solution was then transferred to a cartridge. The test was run on the GeneXpert DX module. The results were reported as C. difficile-positive 027/NAP1/BI presumptive negative, C. difficile-positive 027/NAP1/BI presumptive positive, C. difficile-negative, invalid, error, or no result. The test was repeated if the result was “invalid,” “error,” or “no result.” Sequencing of the tcdC gene was performed on isolates that were positive for the presumptive 027/NAP1/BI strain. PCR ribotyping and tcdC sequencing were performed in accordance with previously described methods [8, 9] for isolates that tested positive for binary toxin genes in the Xpert CD assay in order to confirm the results.

VIDAS C. difficile A & B assays (VIDAS CDAB assay; bioMérieux) were performed according to the manufacturer’s instructions. Test results are presented as positive, negative, or equivocal for toxins A and/or B. Specimens with equivocal results were retested once.

By anaerobic culture, 55 of 253 (21.7%) specimens yielded C. difficile isolates. Of these, 49 (19.4%) isolates were confirmed to be tcdB-positive (Table 2).

### Table 1. Sequences of the PCR primers used in this study

| Test       | Target   | Primer | Sequence (5’→3’) | Reference   |
|------------|----------|--------|-----------------|-------------|
| Toxin gene | tcdArep  | NK9    | CCA CCA GCT GCA GCC ATA | [7]         |
|           | detection|        | TGA TGA TAA TAA CTA TAA ATG GGT AAC |            |
| tcdB       |          | NK104  | GTC TAG CAG TAA AAG TCG AAG TTGTACGC |            |
| cdtA       | cdtApos  | TGA ACC TGG AAA AGG TGA CGG |            |
|            | cdtArev  | AGG ATT ATT TGG AAATT CGG |            |
| cdtB       | cdtBpos  | CTT AAT GCA AGT AAA TAC TGA G |            |
|            | cdtBrev  | AAG GGA TCT CGT TGG ACC ATTT GC |            |
| Ribotyping | 16S-23S  | CD1    | GCG CCC TTT GGA GAG TCA TGA TGG | [8]         |
|            | rRNA     | CD1445 | CTG GGG TGA TGG CGT ACG AAC AGG |            |
| tcdC       | tcdC     | PaL15  | TCT CTA CAG CTA TCC CTG GT | [9]         |
| sequencing | tcdC     | PaL16  | AAA AAT GAG GGT AAC TAA GTT |            |

Abbreviations: tcdA rep, toxin A gene repetitive region; tcdB, toxin B gene; cdtA and cdtB, binary toxin genes.

### Table 2. Evaluation of Xpert Clostridium difficile and VIDAS Clostridium difficile A & B assays for the detection of toxigenic Clostridium difficile isolates

| Toxigenic culture (N. of isolates) | Xpert CD | VIDAS-CDAB |
|-----------------------------------|----------|------------|
|                                   | B+, CDT, 027 | B+, CDT, 027 | B+, CDT, 027 | B+, CDT, 027 | Error | A and/or B Positive | A and/or B Negative | Equivocal |
| Growth (55)                       | 44       | 1          | 0         | 0         | 0 | 19 | 23 | 3 |
|                                   | A’B’, A’, B+, CDT (45) |
|                                   | 0       | 3          | 1*        | 0         | 0 | 1 | 2 | 1 |
|                                   | A’, CDT (4) |
|                                   | 2       | 0          | 0         | 4         | 0 | 0 | 5 | 1 |
| No growth (198)                   | 8†       | 0          | 0         | 189       | 1† | 0 | 195 | 3 |
| Total (253)                       | 54       | 4          | 1         | 193       | 1 | 20 | 225 | 8 |

*One presumptive 027/NAP1/BI strain identified as ribotype 078 on PCR ribotyping as well as a 39-base pair deletion and a point mutation at position 184 in tcdC; †Four specimens showed positive results by enrichment culture; ‡One “error” in the Xpert CD assay: no growth on anaerobic culture and negative on VIDAS-CDAB.

Abbreviations: Xpert CD, Cepheid Xpert Clostridium difficile assay; VIDAS-CDAB, VIDAS Clostridium difficile Toxin A&B assay; A, toxin A; B, toxin B; CDT, C. difficile binary toxin; 027, presumptive 027/NAP1/BI strain.
The Xpert CD assay detected tcdB in all 49 isolates identified as tcdB-positive by toxigenic culture (sensitivity 100%, Table 3). For 8 specimens that tested positive in the Xpert CD assay but were negative upon toxigenic culture, an enrichment culture was performed using cycloserine-cefoxitin fructose broth supplemented with 0.1% sodium taurocholate (TCCFB). Four of these eight specimens yielded a positive result for toxigenic C. difficile (Table 4). On the basis of analyses of other samples from the same patients, we suspect that at least 2 samples were contaminated with residual DNA [10]. In 3 undetermined cases, possible explanations for the discrepant results are residual DNA from prior CDI, false-positive PCR result, or true-positive PCR result.

Compared to the toxigenic culture, the sensitivity, specificity, and positive and negative predictive values were 100%, 94.6%, 83.1%, and 100%, respectively, for the Xpert CD assay, and 40.8%, 98.0%, 100%, and 88.9%, respectively, for the VIDAS CDAB assay (Table 3). The overall agreement between the Xpert CD assay and toxigenic culture was 95.7%. Data from the enrichment culture were not included in the calculation of sensitivity, specificity, and positive and negative predictive values. One “error” case of the Xpert CD assay and 8 “equivocal” cases of the VIDAS CDAB assay were included in the calculation of assay performance (Table 2).

Binary toxin genes (cdtA and cdtB) were detected in 5 specimens by the Xpert CD assay, and 1 of them showed a 027/NAP1/BI presumptive positive result. The binary toxin genes were confirmed by toxin gene-specific PCR, PCR ribotyping, and tcdC sequencing. Four (including one 027/NAP1/BI presumptive positive isolate) of the 5 isolates revealed positive results for binary toxin genes. In addition, all 4 isolates showed an identical pattern to that of ribotype 078 and no deletion at position 117 of the tcdC gene. All ribotype 078 strains showed a 39-base pair deletion and a point mutation at position 184 in the tcdC gene [11].

Similar to a previous study, the evaluation of the usefulness of the Xpert CD assay for screening for the 027 strain was limited in this study due to the low prevalence of binary toxin-producing

**Table 3.** Assay performance of Xpert Clostridium difficile and VIDAS Clostridium difficile A & B assays for the detection of toxigenic Clostridium difficile isolates compared with toxigenic culture

| Assay       | Sensitivity (%) | Specificity (%) | PPV (%)  | NPV (%) |
|-------------|-----------------|-----------------|----------|---------|
| Xpert CD    | 100             | 94.6 (91.5-97.7)| 83.1 (73.5-92.7)| 100     |
| VIDAS-CDAB  | 40.8 (27.0-54.6)| 98.0 (96.1-99.9)| 100      | 88.9 (84.8-93.0) |

*Sensitivity, specificity, PPV, and NPV are calculated as follows (×100): sensitivity, (number of true-positive assay results)/(sum of toxigenic culture-positive results); specificity, (number of true-negative assay results)/(sum of toxigenic culture-negative results); PPV, (number of true-positive assay results)/(sum of true-positive and false-positive assay results); NPV, (number of true-negative assay results)/(sum of true-negative and false-negative assay results).

**Table 4.** Discordant results and further analysis of Xpert Clostridium difficile and toxigenic culture

| Sample No. | Results Enrichment culture | tcdB PCR | VIDAS -CDAB | Comment | Possible explanation |
|------------|-----------------------------|----------|-------------|---------|---------------------|
| 1          | Growth                      | Negative | Negative    | Previous C. difficile positive (toxigenic culture) | Residual DNA |
| 2          | Growth                      | Negative | Negative    | Only one sample submitted | Undetermined |
| 3          | Growth                      | Negative | Equivocal   | Only one sample submitted | Undetermined |
| 4          | Growth                      | Positive | Negative    | Enrichment culture C. difficile positive (toxigenic culture) | True-positive PCR |
| 5          | Growth                      | Positive | Negative    | Enrichment culture C. difficile positive (toxigenic culture) | True-positive PCR |
| 6          | Growth                      | Positive | Negative    | Enrichment culture C. difficile positive (toxigenic culture) | True-positive PCR |
| 7          | No growth                   | Not done | Negative    | Previous C. difficile positive (toxigenic culture) | Residual DNA |
| 8          | No growth                   | Not done | Negative    | Only one sample submitted | Undetermined |

*All samples with initially no growth on anaerobic culture and Clostridium difficile-positive 027/NAP1/BI presumptive negative on Xpert CD assay; *False-positive PCR, residual DNA, or true-positive PCR.

Abbreviations: Xpert CD, Cepheid Xpert Clostridium difficile assay; VIDAS-CDAB, VIDAS Clostridium difficile A & B assay; PPV, positive predictive value; NPV, negative predictive value.
C. difficile strains (3.8% to 7.1%) and PCR ribotype 027 (0.6%) in Korea [12, 13]. A previously published study reported that the agreement between the Xpert CD assay and PCR-ribotyping was 93% [10]. Other studies reported discordant results for the presumptive 027/NAP1/BI strain between the Xpert CD assay and conventional typing and sequencing [14, 15]. These authors reported 1 ribotype 053 strain [15], 1 strain similar to the 078 strain [14], and 6 strains of unknown type. Ribotype 078 is the most frequent type present as a binary toxin-positive strain in Korea [12, 13]. Therefore, results of the presumptive 027/NAP1/BI strain must be interpreted with caution, particularly in Korea, where the prevalence of ribotype 027 is low.

The most significant advantage of the Xpert CD assay is its rapidity and simplicity. A loose stool specimen can be directly used, and the assay takes only 45 min.

In conclusion, the Xpert CD assay is a reliable method for detecting toxigenic C. difficile directly from stool specimens and provides greater sensitivity than an enzyme immunoassay.

Authors’ Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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