Evaluation of the Diagnostic Performance of the Xpert *Clostridium difficile* Assay and Its Comparison With the Toxin A/B Enzyme-Linked Fluorescent Assay and In-House Real-Time PCR Assay Used for the Detection of Toxigenic *C. difficile*

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Background: *Clostridium difficile* genes or toxin can be detected using several laboratory techniques. In this study, we compared the performance of the Xpert *C. difficile* assay with that of a toxin A/B enzyme-linked fluorescent immunoassay (ELFA) and an in-house real-time PCR assay for the *tcdB* gene. **Methods:** From April 2011 through January 2012, 138 soft or liquid stool samples from 138 adult patients at Paik Hospital were tested using the toxin A/B ELFA, in-house real-time PCR assay, and Xpert *C. difficile* assay to detect toxigenic *C. difficile*. Specimens were considered true positives if results were positive in both the in-house real-time PCR for *tcdB* gene and Xpert *C. difficile* assays. **Results:** Sensitivity of the toxin A/B ELFA, in-house *tcdB* gene real-time PCR, and Xpert *C. difficile*

assay were 67.6%, 97.3%, and 100.0%, respectively. The specificity of the in-house *tcdB* gene real-time PCR assay was 100%, while the specificity was 98.0% for the other two methods. The turnaround time (TAT) was 50 min for the Xpert *C. difficile* assay, 75 min for the toxin A/B ELFA, and 160 min for the in-house real-time PCR assay. **Conclusion:** The Xpert *C. difficile* assay and the in-house real-time PCR assay had higher sensitivity than the toxin A/B ELFA; however, the specificities of the three assays were similar. Considering its rapid TAT and high sensitivity, use of the Xpert *C. difficile* assay is highly recommended for rapid and accurate diagnosis of *C. difficile* infection. J. Clin. Lab. Anal. 28:124–129, 2014. © 2014 Wiley Periodicals, Inc.

Key words: *Clostridium difficile*; Xpert; toxin; VIDAS

INTRODUCTION

Clostridium difficile, an anaerobic, spore-forming, gram-positive bacillus, is responsible for the majority of cases of infectious antibiotic diarrhea and pseudomembranous colitis (1, 2). It is a nosocomial pathogen, and its infection is a major medical and infection control problem in many hospitals, long-term care facilities, and nursing homes (3). Over the past decade, the incidence of *C. difficile* infection (CDI) with a more severe course has increased in Europe and North America (4–6). In Korea, the incidence of CDI has also been trending higher, although the diagnostic methods in use yield more variability (7–9). Because clinical symptoms of CDI can be similar to those of diarrhea caused by a change in diet, antibiotic treatment, or infection with another pathogen like Salmonella, it is essential to have accurate and rapid

laboratory diagnosis of CDI in order to treat patients properly and establish appropriate nosocomial infection control measures.

To diagnose CDI, toxin assay using the enzyme immunoassay (EIA) method, *C. difficile* culture, and cell culture cytotoxicity assay have been performed (10, 11). Among these assays, the cell cytotoxicity method is the “gold standard” for detecting toxigenic *C. difficile*, but

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it is technically complex and requires a turnaround time (TAT) of 48–72 h. Most laboratories now use a commercial EIA to detect TcdA and/or TcdB, with a benefit of rapid TAT and ease of use. However, EIA is associated with widely varying sensitivity (50–99%) and specificity (70–100%), with performance largely dependent on the reference method used for comparison, making its reliability questionable for an accurate CDI diagnosis (5, 12). Recently, molecular tests, including several commercial nucleic acid amplification tests (NAATs), have been introduced (13, 14). In this study, we evaluated the performance of the Xpert *C. difficile* assay (Cepheid, Sunnyvale, CA) and compared it with the VIDAS toxin A/B enzyme-linked fluorescent immunoassay (ELFA; VIDAS CDAB assay; bioMérieux SA, Marcy-l'Etoile, France) and an in-house real-time PCR assay that detect the presence of the *tcdB* gene and *C. difficile* to enable rapid, sensitive, and specific detection of toxigenic *C. difficile* in stool specimens from a university hospital in Korea.

MATERIALS AND METHODS

Specimens

A total of 138 stool specimens from 138 adult patients (age > 18 years) were tested in the present study. The specimens tested were unformed stools submitted to the Department of Laboratory Medicine, Inje University Seoul Paik Hospital, Korea, for routine CDI diagnosis between April 2011 and January 2012. Duplicate specimens from the same patients were excluded. All specimens were tested for CDI using the VIDAS toxin A/B ELFA, Xpert *C. difficile* assay, and in-house real-time PCR assays for the *tcdB* gene and *C. difficile*.

For the VIDAS toxin A/B ELFA and Xpert *C. difficile* assays, the specimens were tested daily or stored at 4°C and tested within 48 h. For the in-house real-time PCR for *tcdB* gene and *C. difficile* assay, DNA was extracted daily and frozen at –70°C until testing.

VIDAS Toxin A/B ELFA

Stool specimens were examined for TcdA and TcdB via a VIDAS toxin A/B ELFA. An aliquot (200 µl) of well-mixed liquid stool was added to 1 ml diluents and centrifuged for 5 min at 12,000 × g. Supernatant fluid (300 µl) was added to the sample well of the CDAB kit, according to the manufacturer's instructions. Results were reported as positive, equivocal, or negative, according to the intensity of the fluorescence. Specimens with test values ≥ 0.37 were considered positive; those with test values < 0.13 were considered negative. Specimens with test values between 0.13 and 0.37 were considered equivocal.

Xpert *C. difficile* Assay

The Xpert *C. difficile* assay is a multiplex real-time PCR that detects *tcdB*, the binary toxin gene (*cdt*), and the *tcdC* gene deletion at nt 117. The assay was performed according to the manufacturer's instructions. Briefly, a stool sample was collected on a swab from the container and transferred into the sample reagent vial. The vial was vortexed for 10 s, and the solution was pipetted into the "S" chamber of the cartridge using a transfer pipette. The cartridge was then inserted on the Xpert instrument, and the test was performed using the GeneXpert *C. difficile* assay program. Potential results included the following: toxigenic *C. difficile* positive/027-NAP1-BI presumptive negative, toxigenic *C. difficile* positive/027-NAP1-BI presumptive positive, toxigenic *C. difficile* negative/027-NAP1-BI presumptive negative, invalid, error, no results. In case results as "invalid," "error," or "no results" occur, the test was repeated within 3 h according to the manufacturer's instructions.

In-House Real-Time PCR Assays for the *tcdB* Gene and *C. difficile*

Primers NK104 (5'-GTGTAGCAATGAAAGTCC AAGTTTACGC-3') and NK105 (5'-CACTTAGCT CTTTGATTGCTGCACCT-3') described by Kato et al. were used in the Kato real-time PCR for the *tcdB* gene to amplify 204 bp of the nonrepeating sequence of *tcdB* (15). For the real-time PCR of *C. difficile*, forward primer (5'-TTG AGC GAT TTA CTT CGG TAA AGA-3') and reverse primer (5'-CCA TCC TGT ACT GGCTCA CCT-3') described by Rinttila et al. were used to amplify 157 bp of the species-specific region of the 16S rDNA of *C. difficile* (16). For DNA preparation, approximately 200 mg of fecal matter was added to 2 ml sterile tubes containing 1.4 ml of a stool lysis (ASL) buffer from the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA), and DNA was extracted according to the manufacturer's instructions. The DNA was eluted in a final volume of 200 µl and stored at –70°C until assay. The amplification reaction was performed in a 20 µl final volume, containing 10 µl 2× Quan QuantiTect SYBR green master mix (Qiagen), 1 µl each of 10 µM forward and reverse primers, 6 µl of H₂O, and 2 µl of DNA. The thermal cycling conditions for *C. difficile* and the *tcdB* gene started with an initial activation step at 95°C for 15 min, followed by 45 cycles of 15 s at 95°C, 30 s at 61°C, and 30 s at 72°C, which was finally followed by a 30-s step at 72°C before a stepwise (1°C/5 s) temperature increase to 90°C. For all specimens, separate detection of the 16S rDNA sequence present in all bacteria was also performed as an internal control for PCR inhibition (17). The ATCC (American Type Culture Collection) strain 9689 was used as a *tcdB*-positive

TABLE 1. Comparison of the Results of Xpert *C. difficile* Assay, In-House Real-Time PCR for *tcdB* Gene, In-House Real-Time PCR for *C. difficile* PCR, and VIDAS Toxin A/B ELFA

Specimen group	No. of specimen	Test results				Final decision ^a
		Xpert <i>C. difficile</i> assay	Real-time PCR for <i>tcdB</i> gene (Kato)	Real-time PCR for <i>C. difficile</i> (Rinttila)	VIDAS toxin A/B ELFA	
1	91	Negative	Negative	Negative	Negative	Negative
2	22	Positive	Positive	Positive	Positive	Positive
3	7	Positive	Positive	Positive	Negative	Positive
4	6	Negative	Negative	Negative	Equivocal	Negative
5	5	Positive	Positive	Positive	Equivocal	Positive
6	2	Negative	Negative	Negative	Positive	Negative
7	2	Positive	Positive	Negative	Positive	Positive
8	2	Positive	Negative	Positive	Negative	Negative
9	1	Positive	Negative	Positive	Positive	Positive

^aSpecimens were considered true positive when both the Xpert *C. difficile* assay and in-house real-time PCR (Kato) detected the presence of the *tcdB* gene; the specimens were considered true negative when both results were negative for the *tcdB* gene. In cases where discrepancies existed between the two molecular gene detection methods, specimens were considered true positive when the results of the VIDAS toxin A/B ELFA and Rinttila in-house real-time PCR for *C. difficile* were both positive. ELFA, enzyme-linked fluorescent assay; No., number.

C. difficile control. The Rotor-Gene Q thermocycler (Qiagen) was used for amplification and analysis.

Data Interpretation

Specimens were considered true positive when both the Xpert *C. difficile* assay and Kato real-time PCR detected the presence of the *tcdB* gene; the specimens were considered true negative when both results were negative for the *tcdB* gene. In cases where discrepancies existed between the two molecular gene detection methods, specimens were considered true positive when both the results of the VIDAS toxin A/B ELFA and the Rinttila real-time PCR for *C. difficile* were positive. The concordance rate, sensitivity, and specificity of each assay were based on these final results.

Statistics

All statistical analyses were performed using MedCalc version 12.3.0 (MedCalc Software, Mariakerke, Belgium). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and 95% confidence intervals were calculated for each assay. Fisher's exact test was used to calculate the statistical significance of differences in sensitivity, specificity, PPV, and NPV. The null hypothesis was rejected at $P < 0.05$. The Youden index, a single characteristic that captures the performance of a test, was applied to each method to determine comparability, as previously described (18). The Youden index was calculated as follows: (sensitivity + specificity) – 1. The method with the calculated Youden index closest to 1 exhibited comparatively superior performance.

RESULTS

The performance of the Xpert *C. difficile* assay was assessed in a total of 138 specimens. The results of the Xpert *C. difficile* assay were compared to that of the VIDAS toxin A/B ELFA, Kato in-house real-time PCR for *tcdB* gene, and Rinttila in-house real-time PCR for *C. difficile*. The results of each assay are summarized in Table 1.

Of the 138 specimens, 37 (26.8%) were determined to be true positive and 101 (73.2%) were determined to be true negative. Of the 138 specimens tested, 135 (97.8%) were concordant between the Xpert *C. difficile* assay and Kato in-house real-time PCR for *tcdB* gene, while only 3 were considered to be discordant (see specimen groups 8 and 9 in Table 1).

All three discrepant samples were considered positive by the Xpert *C. difficile* assay and negative by the Kato in-house real-time PCR assay. As described in Materials and Methods, the results of discrepant samples were determined according to the result of the Rinttila in-house real-time PCR for *C. difficile* and VIDAS toxin A/B ELFA. One of the three discrepant samples was positive by the Rinttila in-house real-time PCR for *C. difficile* and VIDAS toxin A/B ELFA and was, therefore, considered to be true positive. Further, two of the three discrepant samples were positive by the Rinttila in-house real-time PCR for *C. difficile* and negative by VIDAS toxin A/B ELFA and were, therefore, considered to be true negative. Of the 138 specimens, based on the VIDAS toxin A/B ELFA, 100 were determined to be negative, 27 were determined to be positive, and 11 were determined to be equivocal. Of the 11 equivocal samples, 6 were

TABLE 2. Sensitivity, Specificity, PPV, NPV, TAT, and Material Cost(\$)/Test for *C. difficile* Assays

Assay	% sensitivity (95% CI)	% specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)	Youden index (%)	TAT ^c (min)	Material cost(\$)/test
Xpert <i>C. difficile</i> assay	100.0 (90.5–100) ^a	98.0 (93.0–99.8)	94.9 (82.7–99.4)	100.0 (96.3–100.0) ^a	98.0	50	54
Real-time PCR for <i>tcdB</i> gene (Kato)	97.3 (85.8–99.9) ^b	100.0 (96.4–100.0)	100.0 (90.3–100.0)	99.0 (94.7–100.0) ^b	97.3	160	11
VIDAS toxin A/B ELFA	67.6 (50.2–82.0)	98.0 (93.0–99.8)	92.6 (75.7–99.1)	89.2 (81.9–94.3)	65.6	75	5

^a $P < 0.001$ for Xpert *C. difficile* assay versus VIDAS toxin A/B ELFA.

^b $P < 0.01$ for in-house real-time PCR for *tcdB* gene (Kato) versus VIDAS toxin A/B ELFA.

^cTAT is based on testing of a single specimen.

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; ELFA, enzyme-linked fluorescent assay.

determined to be true positive and 5 were determined to be true negative.

To determine the final results, the sensitivity, specificity, PPV, NPV, and 95% confidence interval were calculated for each assay and are shown in Table 2. The Xpert *C. difficile* assay showed the highest sensitivity (100%), followed by the Kato in-house real-time PCR for *tcdB* gene (97.3%) and the VIDAS toxin A/B ELFA (67.6%).

The Kato in-house real-time PCR for *tcdB* gene showed the highest specificity (100%), followed by the Xpert *C. difficile* assay (98%) and VIDAS toxin A/B ELFA (98.0%). The differences in sensitivity and NPV between the Xpert *C. difficile* assay and the VIDAS toxin A/B ELFA were significant ($P < 0.001$ for both, Fisher's exact test), and those between the Kato in-house real-time PCR assay for *tcdB* gene and the VIDAS toxin A/B ELFA were also significant ($P < 0.01$ for both, Fisher's exact test).

Of the 138 stool specimens, Xpert *C. difficile* assay yielded 3 positive specimens for *tcdB*, *tcdC* deletion, and *cdt*. These three were reported as having presumptive 027/NAP/BI (binary toxin) strains, which constitute 2.2% of all specimens and 8.1% of *tcdB* gene-positive specimens.

In this study, the time to result and the material cost per test of each assay were also compared (Table 2). The VIDAS toxin A/B ELFA currently in use takes approximately 75 min to yield the initial result. The Xpert *C. difficile* assay takes approximately 50 min to yield the initial result. The Xpert *C. difficile* assay has just five working steps including one vortexing, three pipetting, and one inserting steps and can be completed in less than 5 min per sample. The GeneXpert instrument used in this study was a four-module unit capable of processing up to four samples at once. In this study, because less than four samples were being tested at one time on most occasions, extra time was not required in order to obtain results. In contrast to the Xpert *C. difficile* assay, the Kato in-house real-time PCR for *tcdB* gene required at least 160 min, the longest time to result among all assays, because many manual steps were required for processing. The material costs per

test were highest for the Xpert *C. difficile* assay (\$54), five times that of the home-brew real-time PCR (\$11) and ten times that of the VIDAS ELFA (\$5).

The Xpert *C. difficile* assay had a 2.9% total error (postanalysis error) rate. Further, 100% of specimens that showed an error were resolved, and definitive results were provided after one repeat.

DISCUSSION

The major methods used to detect *C. difficile* in many hospital laboratories in Korea are an EIA that detects toxins A and B, or a culture of *C. difficile*. However, EIA is associated with widely varying sensitivity (50–99%) and specificity (70–100%) in many studies, indicating that this test is suboptimal for the diagnosis of CDI (12). Moreover, the variability in performance among these EIAs may be due to the type of strain circulating in a particular geographic location (19). Toxigenic culture is another option for CDI diagnosis; however, it is not standardized, is impractical for routine diagnosis, and is expensive in terms of technologist time. Furthermore, it can cause a delay of 2–5 days, which requires isolation for patients with suspected CDI until the testing is complete.

At this time, as an alternative to EIA and toxigenic culture for CDI testing, NAATs are being adopted and increasingly applied in many countries. Several commercial NAATs, including the Xpert *C. difficile* assay, are available to clinical laboratories in the United States and Europe and several have been evaluated in the literature. Recently in Korea, the Committee for New Health Technology Assessment, part of the Korean Ministry of Health and Welfare, evaluated the efficacy and safety of NAATs for *C. difficile* testing and approved their use.

In this study, we evaluated the diagnostic performance of the *C. difficile* assay using 138 stool specimens from 138 Korean patients in a university hospital setting. We used in-house real-time PCR assay for *tcdB* gene and *C. difficile* as a comparative method for evaluating the diagnostic

performance of the Xpert *C. difficile* assay and also compared them to the toxin EIA in current use. The Xpert *C. difficile* assays and toxin EIAs were performed on a daily basis to eliminate potential bias related to testing timeliness.

Compared with the VIDAS toxin A/B ELFA, both molecular methods for *tcdB* genes detected approximately 25% more positive specimens for *C. difficile*. These results are consistent with other previous studies, suggesting that single independent molecular methods are 8.5–51.3% more sensitive than nonmolecular methods (20–25). The low sensitivity of ELFA indicates that institutions that provide ELFAs only should warn clinical physicians about performance characteristics and the continued need to consider treatment for patients when there is high suspicion of CDI despite negative ELFA results.

When comparing the three assays using their calculated Youden index values, which include sensitivity and specificity, the Xpert *C. difficile* assay was most efficient (98.1%), followed by the in-house real-time PCR for *tcdB* gene (97.5%) and VIDAS toxin A/B ELFA (65.2%). Therefore, if molecular methods for diagnosing CDI are adopted and applied in Korea, more CDIs would be correctly diagnosed.

In this study, we used the VIDAS toxin A/B ELFA for CDI as a comparative method that is in current use. The results of a VIDAS toxin A/B ELFA could be positive, negative, or equivocal. Interpretation of equivocal VIDAS toxin A/B ELFA results is not part of the manufacturer's instructions. Equivocal results of a toxin assay literally mean not positive and not negative, that is, the intermediate or gray zone between positive and negative. Therefore, in clinical situations, equivocal VIDAS toxin A/B ELFA assay results yield only uncertainty. In this study, 11 of 138 cases (8.0%) showed equivocal test results and 6 of 11 were considered true positive while the remaining 5 of 11 were considered true negative. The results of this study validate equivocal results as uncertain diagnoses of CDI; adopting a molecular test for the *C. difficile* toxin gene can resolve these uncertainties.

Both the Xpert *C. difficile* assay and the Kato in-house real-time PCR assay for *tcdB* gene evaluated in this study target a conserved region of the *tcdB* gene, in accordance with most PCR-based *C. difficile* assays. A possible concern would be that our method only detected the *tcdB* gene, and therefore might have missed the TcdA⁺, TcdB⁻ strain CDI. However, reports of TcdA⁺, TcdB⁻ strain infections are rare worldwide, and Korea is no exception. Thus, the *tcdB* gene is a preferable target to *tcdA* in most PCR-based *C. difficile* assays, because it allows the detection of the increasingly prevalent TcdA⁻, TcdB⁺ strain. In addition, we used real-time PCR for *C. difficile* as a complementary test, and most results were concordant with those of the real-time PCR for *tcdB* gene, indicating

that our CDI diagnoses were considered to be accurate. Another limitation of our study is that the accepted gold standards, being cytotoxicity assay or toxigenic culture, were not used. True positives were defined based only on molecular results. This approach could potentially affect the overall sensitivity and specificity of each assay, with particular risk of overestimating the sensitivity of molecular methods.

The latest guidelines from the Society for Healthcare Epidemiology of America and the Infectious Disease Society of America emphasize the need to consider two-step algorithms that use a glutamate dehydrogenase (GDH) assay to screen for *C. difficile* in stool specimens, followed by either cell culture neutralization assay, toxigenic culture, or NAAT to identify toxin-producing *C. difficile* isolates (4). On the other hand, the European Society of Clinical Microbiology and Infectious Diseases advocated another two-step diagnostic algorithm based on NAAT or GDH for initial toxigenic *C. difficile* screening, with a follow-up toxin EIA as a confirmatory test. They suggested that this algorithm, which uses a PCR test for toxin genes as a first-line screening test, has the advantage of being able to identify symptomatic potential *C. difficile* excretor (26). Furthermore, some laboratories consider NAAT-only testing to be suitable for the diagnosis of CDI (27). Given that NAATs detect the toxin gene and not the toxin, and thus cannot discriminate between CDI and asymptomatic *C. difficile* colonization, the diagnostic accuracy of NAATs can be enhanced by careful selection of patients with true diarrhea and a strong suspicion of CDI (28).

In this study, the sensitivity and specificity of the Xpert *C. difficile* assay and the in-house real-time PCR assay for *tcdB* gene were comparable. However, in terms of workflow and time to results, the Xpert *C. difficile* assay was superior, with a shorter TAT (50 min, including 5 min of sample preparation) than the in-house real-time PCR for *tcdB* gene (160 min). Additionally, the Xpert *C. difficile* assay has the convenience of single batch testing on arrival without reagent or cartridge loss. The material cost for reagents, which was five times that for the in-house real-time PCR assay for the *tcdB* gene and ten times that for the VIDAS ELFA, are the only limitations of this molecular test.

In conclusion, the Xpert *C. difficile* assay and the in-house real-time PCR assay were more sensitive than the VIDAS toxin A/B ELFA; however, the specificities of all three assays were similar. The TAT for the Xpert *C. difficile* assay was much shorter than that of the VIDAS toxin A/B ELFA as well as that for the in-house real-time PCR for *tcdB* gene. Based on high sensitivity, specificity, and rapid TAT, the Xpert *C. difficile* assay is highly recommended for rapid and appropriate diagnosis of CDI.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to the publication of this article.

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