RESEARCH ARTICLE

Laboratory diagnosis of *Clostridium difficile* infection: Comparison of Techlab C. diff Quik Chek Complete, Xpert C. difficile, and multistep algorithmic approach

Ja Young Seo | Ji Hun Jeong | Kyung Hee Kim | Jeong-Yeal Ahn | Pil-Whan Park | Yiel-Hea Seo

Department of Laboratory Medicine, Gachon University Gil Medical Center, Incheon, Korea

Correspondence

Yiel-Hea Seo, Department of Laboratory Medicine, Gil University Gachon Medical Center, Incheon, Korea.
Email: seoyh@gilhospital.com

Background: *Clostridium difficile* is a major pathogen responsible for nosocomial infectious diarrhea. We explored optimal laboratory strategies for diagnosis of *C. difficile* infection (CDI) in our clinical settings, a 1400-bed tertiary care hospital.

Methods: Using 191 fresh stool samples from adult patients, we evaluated the performance of Xpert C. difficile (Xpert CD), C. diff Quik Chek Complete (which simultaneously detects glutamate dehydrogenase [GDH] and *C. difficile* toxins [CDT]), toxigenic culture, and a two-step algorithm composed of GDH/CDT as a screening test and Xpert CD as a confirmatory test.

Results: *Clostridium difficile* was detected in 35 samples (18.3%), and all isolates were toxigenic strains. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value of each assay for detecting CDI were as follows: Quik Chek Complete CDT (45.7%, 100%, 100%, 89.1%), Quik Chek Complete GDH (97.1%, 99.4%, 97.1%, 99.4%), Xpert CD (94.3%, 100%, 100%, 98.7%), and toxigenic culture (91.4%, 100%, 100%, 98.1%). A two-step algorithm performed identically with Xpert CD assay.

Conclusion: Our data showed that most *C. difficile* isolates from adult patients were toxigenic. We demonstrated that a two-step algorithm based on GDH/CDT assay followed by Xpert CD assay as a confirmatory test was rapid, reliable, and cost effective for diagnosis of CDI in an adult patient setting with high prevalence of toxigenic *C. difficile*.

KEYWORDS

adult, *Clostridium difficile*, diagnosis, glutamate dehydrogenase, multistep algorithm, tertiary care hospital

1 | INTRODUCTION

Clostridium difficile is a major pathogen responsible for infectious diarrhea in health care settings. Toxin A (tcdA, enterotoxin) and toxin B (tcdB, cytotoxin) are primary virulence factors of *C. difficile* and *C. difficile* infection (CDI) is associated with considerable morbidity and risk of mortality.^{1,2} During the past decade, the incidence of CDI has markedly increased in most countries.³ In United States, the health care costs attributable to hospital-acquired CDI have been estimated at \$500

million to \$1.5 billion per year.⁴⁻⁶ To reduce hospital stays and health care costs associated with CDI, laboratories have been challenged to provide rapid, accurate, and cost-effective results. The reference method for the detection of *C. difficile* is toxigenic culture or cell culture cytotoxin neutralization assay.^{7,8} Given the test's labor-intensive and time-consuming nature, however, its use in clinical laboratories has been limited.⁹ In recent years, rapid diagnostic tests, performed directly on the stool samples, have been introduced and are beginning to replace the traditional culture-based methods. Enzyme immune assay

(EIA) targeting *C. difficile* toxin A or B is fast and inexpensive, but its low sensitivity limits its use as a stand-alone diagnostic test. The current Infectious Diseases Society of America guidelines recommend two-step testing that uses EIA detection of glutamate dehydrogenase (GDH), a *C. difficile* common antigen, as initial screening and then confirmatory test based on culture method.⁹ Nucleic acid amplification methods detect toxin B (*tcdB*) and/or toxin A (*tcdA*) genes within the pathogenicity locus (PaLoc) of *C. difficile*.¹⁰ They are rapid, sensitive, and specific, and fully automated on-demand PCR assays, such as Xpert *C. difficile* (Xpert CD; Cepheid, Sunnyvale, CA, USA) or BD MAX Cdiff Assay (BD Diagnostics, Franklin Lakes, NJ, USA) became available in clinical laboratories. However, molecular methods usually can be quite costly and the clinical significance of detecting low-level, non-viable *C. difficile* needs to be elucidated.¹¹

Two- or three-step approaches for the laboratory diagnosis of CDI, which are composed of one screening test followed by a confirmatory test, have been suggested to overcome the limitation of single rapid test.^{9,12-15} At an institutional level, diagnostic algorithms that optimize test combinations should reflect the clinical setting of the institution. The prevalence of toxin-producing *C. difficile* is one of the most important factors to be considered, because this will inevitably have an impact upon the positive and negative predictive values of any diagnostic tests.^{16,17} The goal of this study was to investigate the prevalence of CDI in our institution, a 1400-bed tertiary care hospital, and to select the optimal testing strategy based on our clinical setting. Two different rapid tests, a lateral-flow membrane immunoassay and one real-time PCR assay, were compared with the toxigenic culture method.

2 | MATERIALS AND METHODS

2.1 | Samples

This prospective study was conducted between November 2013 and March 2014 at a 1400-bed tertiary care hospital in Korea. During the study period, we examined 191 consecutive diarrheal stool samples from adult patients (median 65 years) for diagnosis of CDI. Samples from pediatric patients were excluded because of insufficient sample volume. Following routine testing, residual stool samples were used for additional molecular studies and GDH assay. The samples were tested daily or stored at 4°C and tested within 24-48 h of collection. This study protocol was approved by the Institutional Review Board of Gachon University Gil Medical Center.

2.2 | Toxigenic culture

All stool samples were inoculated on *C. difficile* selective chromogenic agar plate (ChromID *C. difficile* agar; bioMerieux SA, Craponne, France) and incubated anaerobically at 37°C for 48 h. Gray-to-black colonies suspicious for *C. difficile* were identified using Gram stain and VITEK 2 ANC card (bioMerieux). The identified *C. difficile* isolates were used to detect *tcdA*, *tcdB*, and triose phosphate isomerase (*tpi*; a *C. difficile*-specific internal fragment of a housekeeping gene) genes by multiplex PCR using published primer pairs.¹⁸

2.3 | C. diff Quik Chek Complete assay

C. diff Quik Chek Complete assay (GDH/CDT; TechLab, Blacksburg, VA, USA), a lateral-flow membrane immunoassay which detect GDH antigen and toxin A and B concurrently, was performed according to the manufacturer's instructions. GDH antigen and/or toxins were reported as positive, if a corresponding band was seen on the device display window.

2.4 | Xpert *C. difficile* assay

Xpert CD assay (Cepheid), the fully automated real-time PCR platform, was performed on the GeneXpert DX system to detect *tcdB*, binary toxin genes (*cdtA* and *cdtB*), and *tcdCΔ117* (deletion in the negative regulator of toxin production). Binary toxin gene specific PCR and *tcdC* sequencing were performed in accordance with published methods for isolates that tested positive for binary toxin genes.^{19,20}

2.5 | Interpretation of results and statistical analysis

Given the reported recovery rate on ChromID *C. difficile* agar (about 98%),^{21,22} true positive was defined as positivity by toxigenic culture or positivity by both Xpert CD and GDH or CDT. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for the GDH/CDT, Xpert CD and toxigenic culture, and by using an algorithm in which GDH/CDT followed by Xpert CD to resolve discrepant samples (GDH+/CDT-). Xpert CD invalid results were excluded from the performance characterization.

3 | RESULTS

One hundred and ninety-one fresh stool samples were tested for CDI and 35 samples (18.3%) were determined to be positive by our interpretation criteria (Table 1). Thirty-five samples (18.3%) were positive for GDH with 34 samples (97.1%) giving a true positive result. Among 35 GDH positive samples, 16 (45.7%) were also positive for CDT, and

TABLE 1 Results of three laboratory assays for 191 stool samples

Result				
GDH	CDT	Xpert CD	Toxigenic culture	No. of samples
P	P	P	P	15
P	P	P	N	1
P	N	P	P	15
P	N	P	N	2
P	N	N	P	1
P	N	N	N	1
N	N	N	P	1
N	N	N	N	154
N	N	I	N	1

GDH, glutamate dehydrogenase; CDT, *C. difficile* toxin; P, positive; N, negative; I, invalid.

TABLE 2 Performance characteristics of individual assays and two step algorithms for detecting toxigenic *C. difficile* compared to toxigenic culture as a reference method

	% sensitivity (95% CI)	% specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)
GDH	97.1 (85.1-99.9)	99.4 (96.5-100)	97.1 (85.1-99.9)	99.4 (96.5-100)
CDT	45.7 (28.8-63.4)	100 (97.7-100)	100 (79.4-100)	89.1 (83.6-93.3)
Xpert CD ^a	94.3 (80.8-99.3)	100 (97.7-100)	100 (89.4-100)	98.7 (95.5-99.9)
Toxigenic culture	91.4 (76.9-99.2)	100 (97.7-100)	100 (89.1-100)	98.1 (94.6-99.6)
GDH/CDT + Xpert CD	94.3 (80.8-99.3)	100 (97.7-100)	100 (89.4-100)	98.7 (95.5-99.9)

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; GDH, glutamate dehydrogenase; CDT, *C. difficile* toxin.

^aInvalid result was excluded from analysis.

subsequently confirmed by toxigenic culture except for one sample. There was no false positive result for CDT according to our criteria. Thirty-two (16.8%) samples were positive by toxigenic culture including one sample positive by toxigenic culture only. Three CDI positive samples were missed by toxigenic culture. Xpert CD assay was positive for 33 samples (17.3%). There were three invalid results by Xpert CD assay. Two of them were resolved after repeated tests, however, the remaining one was invalid repeatedly. We found no *C. difficile* ribotype 027 in our study. The sensitivity, specificity, PPV, and NPV for each method are described in Table 2. Overall, the performances of GDH/CDT assay and Xpert CD assay were comparable with toxigenic culture for diagnosis of CDI.

We simulated the two-step testing algorithm by employing *C. diff* Quik Chek Complete assay as a first line test, followed by Xpert CD assay (Figure 1). About half of CDI positive samples (45.7%; 16/35) with GDH+/CDT+ needed no further test for diagnosis of CDI if *C. diff* Quik Chek Complete assay has been used as a screening test. Overall, 171(89.5%; 16 GDH+/CDT+ and 155 GDH-/CDT-) of samples were correctly classified using GDH/CDT assay only. *C. diff* Quik Chek Complete/Xpert CD algorithm identified three CDI+ samples which were negative by toxigenic culture, and 2 CDI+ samples were missed

by this algorithm (Table 1, Figure 1). In conclusion, our two-step algorithm for diagnosis of CDI showed performance equal to that of Xpert CD assay alone, and only 19 GDH+/CDT- samples (9.9%) needed additional Xpert CD assay as a confirmatory test (Table 2, Figure 1).

4 | DISCUSSION

In this study, we found that most *C. difficile* isolates from adult patients in our institution were toxin-producing strains. GDH assay and Xpert CD assay performed comparably with toxigenic culture for the diagnosis of CDI. GDH assay is a screening method that is preferable in two- or three-step algorithms over previous studies for CDI diagnosis, because of its high sensitivity and excellent NPV.²³⁻²⁵ Two meta-analyses on the performance of GDH assay for detection of CDI, however, showed that PPV of GDH varied from ~30% to ~100% in a range of CDI prevalence from 5% to 50%.^{26,27} Such wide-range variability in the performance of GDH assay arises from the target detected. A positive GDH assay indicates the presence of the *C. difficile* in stool samples and is not indicative of CDI itself. Such a limitation mandates its use to a screening test rather than stand-alone

FIGURE 1 Results of two step algorithm testing composed of *C. diff* Quik Chek Complete assay followed by Xpert CD assay for diagnosis of CDI

test. Usually, GDH assay is coupled with a confirmatory test indicating the presence of toxin, such as toxigenic culture, cytotoxin assay, toxin PCR, or toxin EIA. In our study, the prevalence of CDI was 18.3% (35/191) and PPV (97.1%) of GDH assay was higher than the findings of others who worked under similar CDI prevalence.^{17,25,28–30} Such a high PPV may result from the finding that most *C. difficile* isolates from adult patients in our institution were toxigenic strains. The frequency was quite high compared to that of pediatric patients during the same period (32%, 16 of 50 *C. difficile* isolates confirmed by toxigenic culture, data not shown in this article).

A variety of nucleic acid amplification methods for detection of CDI have been extensively studied and, compared to other non-culture-based methods, are reported to be the most sensitive methods.³¹ Xpert CD assay is the first on-demand molecular diagnostic test to detect CDI and its use as a stand-alone test or a component of two-step algorithms has been evaluated well.³² In this study, the diagnostic performance of Xpert CD assay was comparable to toxigenic culture. Xpert CD assay identified two positive samples, in agreement with the findings of GDH assay, but negative by toxigenic culture. These discrepancies might result from the presence of low-level or non-cultivable organisms in the stool samples. The clinical significance of detecting low-level or non-cultivable *C. difficile* by molecular methods, however, needs to be clarified.¹¹ Definitive results were not obtained upon first analysis for three samples tested with Xpert CD assay, most probably due to PCR inhibition or a technical error. After repeated testing as recommended by the manufacturers, only one sample remained invalid.

Toxin detection by *C. diff* Quik Chek Complete assay showed poor diagnostic sensitivity compared to other methods, and all CDT positive samples were concurrently GDH positive. Combined with GDH, CDT results showed 100% PPV for diagnosis of CDI. Toxin EIAs are usually highly specific; however, our data shows that a more sensitive assay is preferable for CDI diagnosis in our clinical settings where toxigenic *C. difficile* accounts for majority of *C. difficile* isolates.

In our virtual simulation of a two-step testing algorithm, *C. diff* Quik Chek Complete assay alone detected a half of CDI positive samples without further tests (Figure 1). When Xpert CD assay was used as a confirmatory test, two false negative results were observed. Novak-Weekley et al.³² argued that Xpert CD assay alone was superior to other individual tests and multistep testing algorithms in terms of sensitivity and NPV, while decreasing turnaround time significantly. Our data showed similar results. However, the two-step algorithm with *C. diff* Quik Chek Complete assay followed by Xpert CD assay seemed to be more cost effective because only 19 Xpert CD assays (9.9%; 19/191) were needed by this algorithm while maintaining the clinical performance similar to toxigenic culture or Xpert CD assay alone. Delay in reporting time remains a concern under this two step algorithm, but Xpert CD assay needs an additional hour to obtain final results, and it means that the reporting of the results would not be delayed significantly.

A limitation to our study is the small sample size (191 samples), and the exclusion of pediatric stool samples due to limited sample volume. The low sensitivity and NPV of GDH assay in pediatric population has

been reported.^{33,34} It means that more confirmatory tests would be needed for pediatric samples under *C. diff* Quik Chek Complete/Xpert CD algorithm. Epidemiologic characteristics of CDI in pediatric population from our clinical setting should be delineated to optimize the diagnostic algorithm.

In summary, we demonstrated that a two-step algorithm based on *C. diff* Quik Chek Complete assay followed by Xpert CD assay as a confirmatory test was rapid, reliable and cost effective for diagnosis of CDI in adult patient setting with high prevalence of toxigenic *C. difficile*. As a screening test, *C. diff* Quik Chek Complete assay showed superior sensitivity to culture method in detection of *C. difficile*.

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