



# Laboratory Diagnostic Methods for *Clostridioides difficile* Infection: the First Systematic Review and Meta-analysis in Korea

Hae-Sun Chung , M.D., Ph.D.<sup>1</sup>, Jeong Su Park , M.D., Ph.D.<sup>2</sup>, and Bo-Moon Shin , M.D., Ph.D.<sup>3</sup>

<sup>1</sup>Department of Laboratory Medicine, Ewha Womans University College of Medicine, Seoul, Korea; <sup>2</sup>Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam, Korea; <sup>3</sup>Department of Laboratory Medicine, Sanggye Paik Hospital, School of Medicine, Inje University, Seoul, Korea

**Background:** Various methods are used for the diagnosis of *Clostridioides difficile* infection (CDI). We systematically analyzed and investigated the performance of current laboratory diagnostic methods for CDI.

**Methods:** We performed systematic review and meta-analysis of studies in PubMed, Web of Science, Cochrane Library, and KoreaMed. The following methods were evaluated: glutamate dehydrogenase (GDH) enzyme immunoassays (GDH EIAs), toxin A and B detection by enzyme immunoassays (toxin AB EIAs), and nucleic acid amplification tests (NAATs) for *C. difficile* toxin genes. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each method were calculated.

**Results:** Based on 39 studies, the pooled sensitivities/specificities were 92.7%/94.6%, 57.9%/97.0%, and 90.0%/95.8% for GDH EIAs, toxin AB EIAs, and NAATs, respectively, compared with those of toxigenic culture. The pooled sensitivities of automated EIAs were significantly higher than those of non-automated EIAs for both GDH and toxins A and B. The pooled sensitivity of Xpert *C. difficile* was significantly higher than those of other NAATs. PPVs increased as CDI prevalence increased, and NPVs were excellent when CDI prevalence was low; at CDI prevalence of 5%, PPV=37%–65% and NPV=97%–100%; at CDI prevalence of 50%, PPV=92%–97% and NPV=65%–98%.

**Conclusions:** Toxin AB EIAs still show unsatisfactory sensitivity, whereas GDH EIAs and NAATs show relatively high sensitivity. However, toxin AB EIAs are the most specific tests. This study may provide useful information for CDI diagnosis.

**Key Words:** *Clostridioides difficile*, *Clostridioides difficile* infection, Laboratory diagnostic methods, Systematic review, Meta-analysis

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**Corresponding author:**

Bo-Moon Shin, M.D., Ph.D.  
Department of Laboratory Medicine,  
Sanggye Paik Hospital, School of Medicine,  
Inje University, 1342 Dongil-ro, Nowon-gu,  
Seoul 01757, Korea  
Tel: +82-2-950-1227  
Fax: +82-2-950-1244  
E-mail: bmshin@unitel.co.kr



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## INTRODUCTION

*Clostridioides difficile* (formerly known as *Clostridium difficile*) infection (CDI) is one of the most common healthcare-associated infections. *C. difficile* is an anaerobic gram-positive, spore-forming, toxin-producing bacillus that is transmitted among humans through the fecal-oral route, as a result of ingestion of spores. Colonization of *C. difficile* is prevented by barrier proper-

ties of the fecal microbiota; weakening of this resistance by antibiotics is a major risk factor for disease. Toxin production is the key to pathogenesis, which leads to colonocyte death, loss of intestinal barrier function, and neutrophilic colitis. CDI can cause a variety of clinical manifestations, including asymptomatic colonization, mild diarrhea, toxic megacolon, and death [1, 2].

Various methods are used to diagnose CDI [1, 2], including detection of glutamate dehydrogenase (GDH)—an antigen se-

creted by *C. difficile*—through enzyme immunoassays (GDH EIAs), detection of toxins A or B of *C. difficile* strains through enzyme immunoassays (toxin AB EIAs), or nucleic acid amplification tests (NAATs) for *C. difficile* toxin genes. Each assay has advantages and disadvantages and exhibits performance differences. When toxin production cannot be confirmed, GDH EIAs may be used to determine the presence of *C. difficile*. GDH EIAs are convenient and inexpensive tests with a rapid turnaround time and can be used as screening tests. GDH EIAs are very sensitive but not specific, because GDH is present in both toxigenic and nontoxigenic strains of *C. difficile* and therefore, to confirm the presence of a toxigenic strain, should be accompanied by an additional test, such as a toxin AB EIA or NAAT. Toxin AB EIAs are also rapid, inexpensive, and easy-to-perform tests that were widely adopted by many laboratories. Toxin AB EIAs have high specificity but unacceptably low sensitivity and are no longer recommended as stand-alone tests for CDI diagnosis [1, 2].

Many commercialized NAATs, including PCR of toxin A and B genes, have been used. Most assays detect the toxin B gene alone or both toxin A and toxin B genes. A multiplex PCR test that detects genes of multiple pathogens that cause diarrhea, including *C. difficile* toxin genes, is also available. NAATs are rapid tests with high specificity and sensitivity for *C. difficile* detection. However, NAATs can detect clinically insignificant infections such as asymptomatic carriage. Additionally, NAATs require trained personnel and are associated with high costs for clinical application. The use of multiple assays for CDI diagnosis suggests that no single test is optimal. In fact, several guidelines recommend combining test methods or using a multi-step algorithm [3, 4].

We systematically analyzed the current laboratory diagnostic methods for CDI. A literature review and meta-analysis were performed to investigate the usefulness of the diagnostic methods by calculating the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each method. This study is an update of the previous meta-analysis [3] and analyzed the latest Korean data. In addition, it represents the first systematic review and meta-analysis of laboratory diagnostic methods for CDI in Korea.

## MATERIALS AND METHODS

### Search strategy

In 2016, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) study group published CDI diag-

nostic methods evaluation based on studies evaluating CDI diagnostic methods published between January 2009 and June 2014 [3]. We applied the same search criteria as those used in that report. We searched PubMed, Web of Science, and Cochrane Library for studies written in English language and published from June 2014 to July 2018, and KoreaMed for Korean studies published from January 2009 to July 2018, with an additional criterion that the study should have been conducted in Korea.

### Index test

We reviewed data from three diagnostic methods commonly used in clinical laboratories: GDH EIAs, toxin AB EIAs, and NAATs. EIAs are available in a well-type format (results are displayed as a color change that can be detected visually or spectrophotometrically) or a membrane-type format (results can be visually read from a membrane). Some EIAs are performed through an automated process that minimizes the manual process during the test. Among NAATs, Xpert *C. difficile* (Cepheid, Sunnyvale, CA, USA), BD Max Cdiff (Becton Dickinson, Sparks, MD, USA), and AdvanSure CD (LG Chem., Seoul, Korea) have been used with a relatively high frequency in Korea [5]. The information for each diagnostic method is shown in Table 1.

### Reference test

Reference tests against which index tests were compared were toxigenic culture (TC) and the cell cytotoxicity neutralization assay (CCNA). Except for studies that used *C. difficile* culture, TC was used as a reference test in Korean studies. *C. difficile* culture was accepted as a reference test for comparison with GDH assays.

### Eligibility criteria and selection process

Studies eligible for inclusion had to: (1) describe original research, (2) compare a commercially available index test with a reference test (CCNA or TC), (3) perform the tests on *C. difficile*-negative and-positive clinical human stool samples and (4) provide sufficient information to recalculate sensitivity and specificity and their confidence intervals (CIs).

Studies were excluded if: (1) the reference test was not clearly stated, (2) the reference test was performed only on positive, negative, or discordant samples (to exclude partial verification bias), (3) not all samples were tested by the same reference test, (4) the reference method was a composite of more than one test, (5) the reference method included clinical data for interpretation, (6) the index test was partly used as reference test, (7) the manufacturers' instructions were not followed for index

**Table 1.** Pooled sensitivities and specificities of index tests

Index test	Studies (N)	Sensitivity (%)	95% CI	Specificity (%)	95% CI
Compared with TC					
Well-type GDH EIA					
C. diff Chek-60	3	93.1	88.0–96.5	99.1	98.5–99.5
RIDA QUICK	1	81.7	69.6–90.5	91.1	88.1–93.6
RIDASCREEN GDH	1	88.3	77.4–95.2	91.1	88.1–93.6
Membrane-type GDH EIA					
C. Diff Quik Chek Complete	6	92.5	88.8–95.3	91.8	90.1–93.4
Automated GDH EIA					
LIAISON <i>C. difficile</i> GDH	2	99.1	95.2–100.0	96.0	94.4–97.3
VIDAS <i>C. difficile</i> GDH	1	96.4	87.5–99.6	85.2	79.7–89.6
Well-type toxin AB EIA					
<i>C. difficile</i> Tox A/B II	3	67.4	59.9–74.3	91.0	88.0–93.5
Remel ProSpecT <i>C. difficile</i> Toxin A/B	1	60.9	40.8–77.8	91.3	82.3–96.0
RIDASCREEN <i>C. difficile</i> Toxin A/B	3	42.4	34.8–50.2	99.4	98.5–99.8
Membrane-type toxin AB EIA					
C. Diff Quik Chek Complete	5	48.5	41.5–55.6	98.4	97.4–99.0
ImmunoCard Toxins A & B	1	40.0	22.7–59.4	99.1	95.1–100.0
RIDA QUICK	1	55.0	41.6–67.9	99.1	97.7–99.8
X/Pect Toxin A/B	1	27.3	13.3–45.5	100.0	95.8–100.0
Automated toxin AB EIA					
LIAISON <i>C. difficile</i> Toxins A&B	2	63.0	52.8–72.4	95.2	92.1–97.3
VIDAS <i>C. difficile</i> Toxin A & B	9	63.2	59.6–66.6	96.7	96.0–97.3
NAAT					
AdvanSure CD	2	89.2	74.6–97.0	98.3	96.1–99.4
AmpliVue <i>C. difficile</i>	3	91.9	88.3–94.8	91.7	89.9–93.3
artus <i>C. difficile</i> QS-RGQ	2	98.8	93.5–100.0	91.7	88.4–94.3
BD GeneOhm Cdiff	2	96.6	91.4–99.1	97.3	95.4–98.5
BD MAX Cdiff	6	90.3	87.0–93.0	97.2	96.2–98.0
Cobas Cdiff	1	92.9	87.4–96.1	98.7	97.4–99.4
GenomEra <i>C. difficile</i>	1	91.8	84.5–96.4	99.1	95.1–99.9
GenoType Cdiff	1	86.7	69.3–96.2	88.3	80.8–93.6
llumigene <i>C. difficile</i>	6	89.7	86.6–92.3	94.1	92.9–95.1
IMDx <i>C. difficile</i>	2	73.6	66.4–79.9	96.4	94.2–98.0
Lyra Direct <i>C. difficile</i>	2	85.9	81.9–89.3	98.3	97.6–98.9
PCRFast <i>C. difficile</i>	1	76.3	59.8–88.5	98.1	95.6–99.7
Seeplex Diarrhea-B1 ACE detection	1	90.0	80.5–95.9	97.1	93.4–99.0
Simplexa <i>C. difficile</i> Universal Direct*	2	88.4	81.3–93.5	99.4	97.0–100.0
Verigene CDF	2	95.2	86.7–99.0	96.8	91.9–99.1
Xpert <i>C. difficile</i>	11	93.6	91.3–95.4	95.2	94.2–96.1

(Continued to the next page)

Table 1. Continued

Index test	Studies (N)	Sensitivity (%)	95% CI	Specificity (%)	95% CI
Compared with <i>C. difficile</i> culture					
Well-type GDH EIA					
C. diff Chek-60	1	88.1	73.6–95.5	99.6	97.5–100.0
RIDASCREEN GDH	1	87.8	78.2–94.3	93.7	90.9–95.8
Membrane-type GDH EIA					
C. Diff Quik Chek Complete	1	94.1	85.6–98.3	94.6	91.7–96.7
C. Diff Quik Chek-60	2	94.3	92.3–95.9	96.0	95.3–96.6
Automated GDH EIA					
LIAISON <i>C. difficile</i> GDH	1	100.0	89.6–100.0	96.5	93.3–98.3
VIDAS <i>C. difficile</i> GDH	4	95.4	93.9–96.7	90.9	89.9–91.7

\*Multiplex PCR test that detects the genes of multiple pathogens that cause multiple diarrhea, including *C. difficile* toxin genes. Abbreviations: CI, confidence interval; TC, toxigenic culture; GDH, glutamate dehydrogenase; EIA, enzyme immunoassay; NAAT, nucleic acid amplification test.

testing or sample collection, (8) only selected samples were included, (9) insufficient information was provided, (10) clinical human stool samples were not included, (11) no commercial diagnostic test was investigated, or (12) only culture methods were evaluated.

Study eligibility was assessed in a two-step selection process by two independent investigators. Initially, one author identified the potentially relevant studies by screening titles and abstracts, and then two authors evaluated their eligibility for being included in this study through a full text assessment. Inconsistencies were resolved by consensus and by consulting a third investigator.

The following data were extracted from each study by two independent investigators: the numbers of true and false positive and negative results, year of publication, information on the index and reference test methods, and information on the study population and country.

We collected 31 studies from international databases (Fig. 1A) and 15 Korean studies from the international and Korean databases (Fig. 1B). After excluding seven Korean studies from the 31 international ones, we assessed a total of 39 studies (24 foreign and 15 Korean) (Table 2). The details of each study are summarized in Supplemental Data Table S1 [6-44].

### Statistical analysis

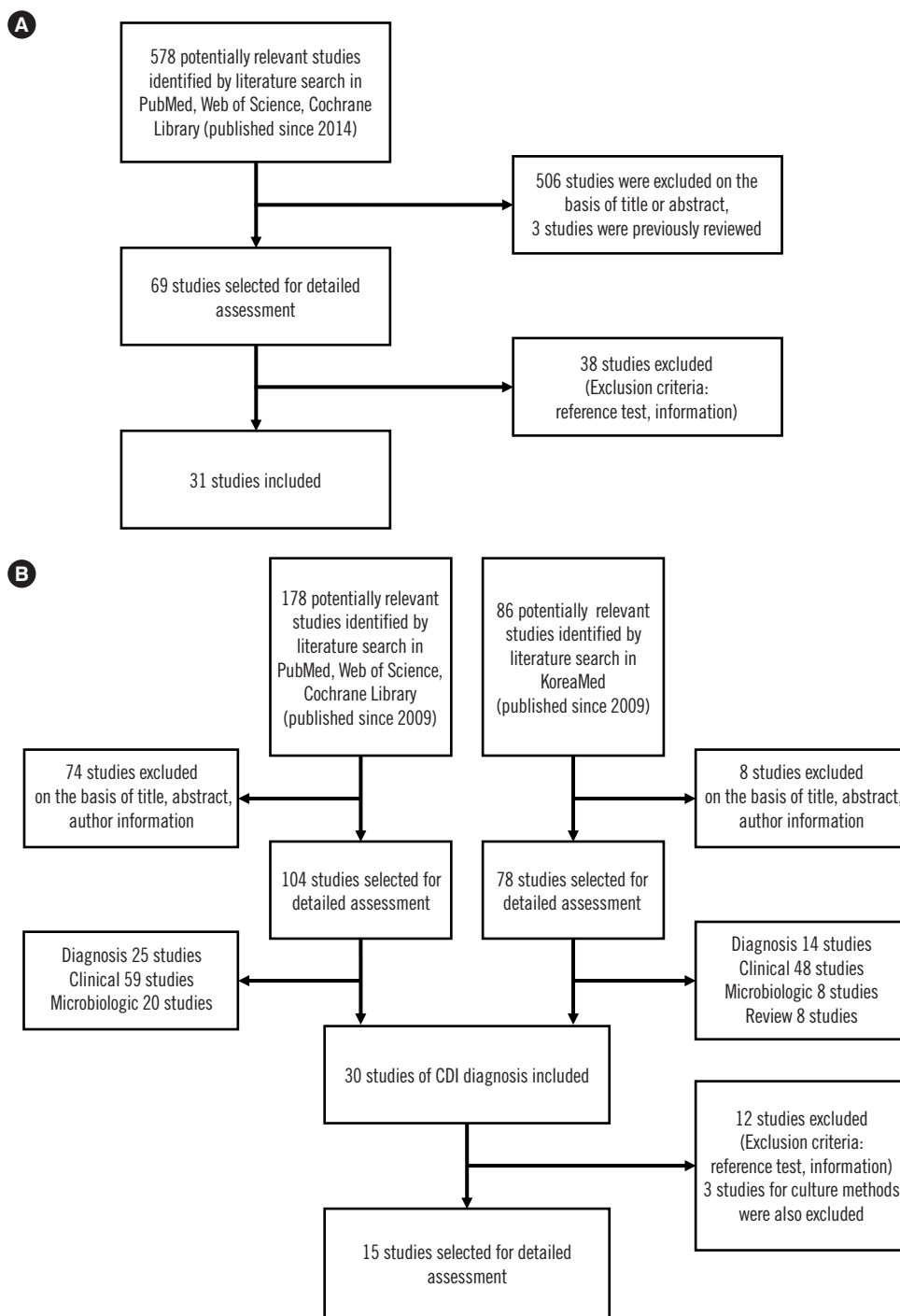
For all index tests in all studies, the sensitivity and specificity and their CIs were calculated from the number of true and false positive and negative results reported in these studies. Meta-DiSc version 1.4 (Hospital Universitario Ramon Y Cajal, Madrid, Spain) was used to calculate pooled sensitivities and specificities. We compared and evaluated the foreign and Korean stud-

ies to assess differences in the diagnostic performance. Pooled sensitivity and specificity for the three test types in the Korean study group were calculated and compared with those of the foreign studies. Among NAATs, sensitivities and specificities were compared separately for Xpert *C. difficile*, BD Max Cdiff, and AdvanSure CD. Further, automated and non-automated EIAs were compared. In addition, hypothetical PPVs and NPVs were calculated using a prevalence of CDI of 5%, 10%, 20%, and 50% in the population tested according to the predictive value theory (Bayes theorem) [45]. The pooled sensitivities and specificities compared with TC in 39 studies searched in international and Korean database were used to compute hypothetical PPVs and NPVs.

Pooled sensitivity and specificity were compared using Fisher's exact test or chi-square test, as appropriate. The data were analyzed using Microsoft Excel 2016 (Microsoft, Redmond, WA, USA). Statistical analysis was performed using MetaDiSc version 1.4 and MedCalc version 10.0 (MedCalc Software Bvba, Ostend, Belgium).

## RESULTS

Pooled sensitivities/specificities of index tests and the sensitivity and specificity of the index test in each study are shown in Table 1 and Supplemental Data Table S2, respectively. GDH EIA and NAAT showed sensitivity and specificity of 90% or higher, and toxin AB EIA had the highest specificity but low sensitivity (Table 2). The pooled sensitivity of toxin AB EIAs in Korean studies was significantly higher than that in foreign studies. Meanwhile, the pooled specificities of NAATs in foreign studies were significantly higher than those in Korean studies (Table 3).



**Fig. 1.** Summary of the selection process for studies in international databases (A) and Korean studies in international and Korean databases (B).

As the sensitivity of NAAT was significantly lower in foreign studies than that in Korean studies, the methods commonly used in Korea were analyzed separately. The pooled sensitivity of Xpert *C. difficile*, which is the most commonly used NAAT in Korea, was significantly higher than that of all NAATs combined

( $P=0.01$ ), and no statistically significant difference in sensitivity was found for BD MAX Cdiff and AdvanSure CD ( $P=0.89$  and  $0.78$ , respectively). The pooled specificities of BD MAX Cdiff and AdvanSure CD were significantly higher than that of all NAATs combined ( $P=0.01$  and  $0.05$ , respectively).

**Table 2.** Pooled sensitivities and specificities of the different types of index tests in the 39 studies

Type	Test	Compared with CCNA			Compared with TC			Compared with <i>C. difficile</i> culture		
		Studies (N)	Sensitivity (%)	95% CI	Studies (N)	Sensitivity (%)	95% CI	Studies (N)	Sensitivity (%)	95% CI
GDH EIA	Total	4	93.4	89.8–96.1	11	92.7	90.6–94.5	7	94.6	93.5–95.6
	Well-type	1	100.0	86.2–100.0	4	89.6	85.4–92.9	2	87.9	80.6–93.2
	Membrane-type	2	92.0	87.6–95.2	6	92.5	88.8–95.3	3	94.3	92.4–95.8
	Automated	1	100.0	86.2–100.0	3	98.2	94.9–99.6	5	95.6	94.1–96.8
Toxin AB EIA	Total	5	60.3	56.0–64.6	18	57.9	55.4–60.3			
	Well-type	4	62.6	56.6–68.4	5	55.4	50.2–60.6			
	Membrane-type	2	54.5	47.7–61.1	7	46.8	41.3–52.4			
	Automated	1	88.0	68.8–97.3	11	63.2	59.8–66.4			
NAAT	Total	3	90.1	86.5–93.0	24	90.0	88.9–91.0			

Abbreviations: CCNA, cell cytotoxicity neutralization assay; CI, confidence interval; NAAT, nucleic acid amplification test; TC, toxigenic culture.

**Table 3.** Comparison of pooled sensitivities and specificities of categories of tests compared with TC between foreign and Korean studies

Type	Test	Foreign		Korean		P	Foreign		Korean		P	
		Studies (N)	Sensitivity (%)	95% CI	Studies (N)		Sensitivity (%)	95% CI	Studies (N)	Sensitivity (%)		95% CI
GDH EIA	Total	8	92.4	89.9–94.5	93.6	89.1–96.6	0.72	95.1	94.3–95.8	91.5	89.5–93.3	<0.01
	Well-type	4	89.6	85.4–92.9				96.0	95.1–96.7			
	Membrane-type	4	92.6	87.2–96.3	92.4	86.5–96.3	0.87	89.4	86.3–92.0	93.6	91.5–95.4	0.01
	Automated	2	99.1	95.2–100.0	96.4	87.5–99.6	0.25	96.0	94.4–97.3	85.2	79.7–89.6	<0.01
Toxin AB EIA	Total	8	51.3	47.0–55.6	61.3	58.3–64.3	<0.01	98.2	97.6–98.7	96.3	95.7–96.8	<0.01
	Well-type	3	56.7	48.8–64.4	54.4	47.3–61.4	0.74	98.4	97.3–99.1	87.4	82.9–91.1	<0.01
	Membrane-type	5	43.0	36.7–49.5	57.5	46.4–68.0	0.03	98.9	98.1–99.4	98.3	97.1–99.1	0.41
	Automated	3	60.0	51.0–68.5	63.7	60.1–67.2	0.48	96.3	93.9–97.9	96.6	95.9–97.2	0.83
NAAT	Total	14	91.6	90.3–92.8	87.0	84.9–88.9	<0.01	95.9	95.5–96.4	95.5	94.8–96.2	0.37

P was calculated using Fisher's exact test or the chi-square test, as appropriate. Abbreviations: CI, confidence interval; GDH, glutamate dehydrogenase; EIA, enzyme immunoassay; NAAT, nucleic acid amplification test; TC, toxigenic culture.

**Table 4.** Comparison of pooled sensitivities and specificities of the different types of index tests compared with TC between automated and non-automated methods

Type	Test	Studies (N)	Sensitivity (%)	95% CI	P	Specificity (%)	95% CI	P
GDH EIA	Automated	3	98.2	94.9–99.6	<0.01	93.6	91.8–95.0	0.23
	Non-automated	10	91.1	88.4–93.3		94.6	93.7–95.3	
Toxin AB EIA	Automated	11	63.2	59.8–66.4	<0.01	96.6	95.9–97.2	0.03
	Non-automated	12	51.4	47.6–55.1		97.5	96.9–98.0	

P was calculated using by Fisher's exact test or the chi-square test, as appropriate.

Abbreviations: TC, toxigenic culture; GDH, glutamate dehydrogenase; EIA, enzyme immunoassay.

**Table 5.** Hypothetical PPV and NPV for different types of index tests in 39 studies at CDI prevalence of 5%, 10%, 20%, and 50%

Type	Test	CDI prevalence							
		5%		10%		20%		50%	
		PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV
GDH EIA	Total	47.5	99.6	65.6	99.1	81.1	98.1	94.5	92.8
	Well-type	54.1	99.4	71.3	98.8	84.8	97.4	95.7	90.2
	Membrane-type	37.3	99.6	55.6	99.1	73.8	98.0	91.9	92.4
	Automated	44.7	99.9	63.0	99.8	79.3	99.5	93.9	98.1
Toxin AB EIA	Total	50.4	97.8	68.2	95.4	82.8	90.2	95.1	69.7
	Well-type	41.0	97.6	59.4	95.1	76.7	89.6	93.0	68.2
	Membrane-type	65.5	97.2	80.0	94.3	90.0	88.1	97.3	65.0
	Automated	49.5	98.0	67.4	95.9	82.3	91.3	94.9	72.4
NAAT	Total	53.0	99.5	70.4	98.9	84.3	97.5	95.5	90.5

Abbreviations: CDI, *Clostridioides difficile* infection; GDH, glutamate dehydrogenase; EIA, enzyme immunoassay; NAAT, nucleic acid amplification test; PPV, positive predictive value; NPV, negative predictive value.

LIAISON *C. difficile* GDH/Toxins A&B and VIDAS *C. difficile* GDH/toxin A&B were included in automated methods, and well-type and membrane-type EIAs served as non-automated methods. Automated methods had a significantly higher sensitivity than the non-automated methods (Table 4).

Regarding hypothetical PPVs and NPVs of the categories of index tests at different CDI prevalences, PPVs were low at low CDI prevalence and increased as CDI prevalence increased. In contrast, NPVs were excellent when the CDI prevalence was low and decreased as the prevalence increased. Comparison of PPVs and NPVs of assay types revealed no considerable difference among GDH EIAs, toxin AB EIAs, and NAATs. Among the toxin AB EIAs, the membrane-type toxin AB EIAs had relatively higher PPVs at CDI prevalence of 5%–20% than well-type and automated toxin AB EIAs. NPVs of GDH EIAs, toxin AB EIAs, and NAATs did not differ when the CDI prevalence was 10%–20%. However, at a CDI prevalence of 50%, NPVs of all types of toxin AB EIAs were relatively low (Table 5).

## DISCUSSION

The analysis of 39 studies available in international and Korean databases revealed that the pooled sensitivities were 92.7%, 57.9%, and 90.0% for GDH EIAs, toxin AB EIAs, and NAATs, respectively (Table 2). The pooled sensitivities of GDH EIAs and NAATs were somewhat lower than those reported by the ESCMID group (96% and 95%, respectively) [3]. However, there was no difference between the pooled sensitivities of toxin AB EIAs in our study and that by the ESCMID group (57%) [5]. The pooled specificities were 94.6%, 97.0%, and 95.8% for GDH EIAs, toxin AB EIAs, and NAATs, respectively (Table 2), which were similar to those reported by the ESCMID group (96%, 99%, 98%, respectively) [3].

Our meta-analysis revealed that GDH EIAs had higher sensitivities and narrower sensitivity ranges than toxin AB EIAs. Toxin AB EIAs had both low sensitivities and the widest sensitivity range. However, toxin AB EIAs were the most specific tests. NAATs had a stable sensitivity of approximately 90% and the

narrowest sensitivity ranges (Supplemental Data Table S2).

In the 2016 study on CDI by ESCMID [3], the sensitivities of toxin AB EIAs were improved when compared with those reported in 2009 [46]. It is assumed that the main reason for this improvement is that toxin A EIAs were replaced with toxin AB EIAs during 2008-2009. In the present study, no particular improvement was observed, although approximately four years had passed since the last ESCMID study. Despite product development and yearly technological advances, the sensitivity of toxin AB EIAs remains unsatisfactory.

We found that the pooled sensitivity of toxin AB EIAs in Korean studies was significantly higher than that in foreign studies. Among three test methods of toxin AB EIAs, membrane-type methods showed most significant *P* value when compared with well-type and automated methods. However, this might be explained by the higher proportion of automated EIAs in Korean studies (8/10) than in foreign studies (3/8). The pooled specificities of GDH EIAs and toxin AB EIAs in foreign studies were significantly higher than those in Korean studies (Table 3). Although the specificity of NAATs did not differ significantly between foreign and Korean studies, their sensitivity was significantly lower in Korean studies. However, the pooled sensitivity of Xpert *C. difficile*, which is the most common NAAT in Korea, was significantly higher than that of all NAATs, and no statistically significant difference in sensitivity was found for BD MAX Cdiff and AdvanSure CD.

TC and CCNA, which were used as reference tests, have different targets. TC detects the presence of toxigenic *C. difficile* strains, whereas CCNA detects *in-vivo* toxin production [2]. The sensitivity and specificity of an index test may differ depending on which reference test was used. This means that toxin AB EIAs may be less sensitive when compared with TC than when compared with CCNA. As NAATs cannot differentiate the presence of *in-vivo* toxin from *in-vitro* toxin, they will be less specific when evaluated using CCNA as the reference test. As the CCNA test method is difficult to standardize and maintain, TC was used as the reference test in most foreign studies and all Korean studies.

We found that the pooled sensitivities of automated EIAs were significantly higher than those of non-automated EIAs (well-type and membrane-type) for both GDH and toxin A and B. It is believed that automated tests are less prone to errors than non-automated tests and therefore have higher sensitivity.

Hypothetical PPV and NPV analysis revealed that PPVs were low at low CDI prevalence and increased as CDI prevalence increased. This result is in line with the findings reported in the

2016 ESCMID study (prevalence 5%, PPV 34%–81%; prevalence 50%, PPV 81%–99%) [3]. At a CDI prevalence of 5%–10%, PPVs of toxin AB EIAs were 69%–90%, which were higher than those found in the present study, whereas PPVs of GDH EIAs and NAATs were 34%–54% and 46%–64%, respectively, which were lower than those found in the present study. In contrast, NPVs were 95%–100% for most tests, but decreased to 80% for toxin AB EIAs at a CDI prevalence of 50%, which is higher than the NPVs of toxin AB EIAs in the present study. According to the hypothetical PPVs and NPVs in our meta-analysis, in an epidemic outbreak with a CDI prevalence of 50%, GDH EIAs and NAATs would have PPVs  $\geq 95\%$  and NPVs  $\geq 90\%$ , and toxin EIAs, which have low sensitivity, would have PPVs  $\geq 95\%$  and NPVs around 70%. PPVs of NAATs were the highest, followed by toxin AB EIAs, and GDH EIAs, whereas NPVs remained  $\geq 90\%$  for all test methods except membrane-type toxin AB EIAs at CDI prevalence  $< 20\%$ .

There are several limitations in this study. Although 39 studies were reviewed, some test types or index tests were analyzed with a small number of studies. Additionally, some results of the analysis showed inconsistency. Furthermore, as our study was restricted to studies published in English or Korean, there may be language bias.

The data from this study may be useful for CDI diagnosis in clinical microbiology laboratories and for clinicians diagnosing and treating CDI. Furthermore, this study provided basic data for establishing a standard CDI diagnosis guideline, which will greatly help in the development of national guidelines in Korea.

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## AUTHOR CONTRIBUTIONS

HSC, JSP, and BMS designed the study and analyzed the data. HSC and BMS wrote the draft. All authors reviewed and approved the manuscript.

## CONFLICTS OF INTEREST

None declared.

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## ORCID

Hae-Sun Chung <https://orcid.org/0000-0001-6382-4099>

Jeong Su Park <https://orcid.org/0000-0001-5149-1362>

Bo-Moon Shin <https://orcid.org/0000-0001-8432-9556>

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