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Clostridium difficile

Evaluation of the VIDAS glutamate dehydrogenase assay for the detection of *Clostridium difficile*

Bo-Moon Shin [a, *], Eun Joo Lee [a], Jung Wha Moon [a], Seon Yeong Lee [b]

[a] Department of Laboratory Medicine, Sanggye Paik Hospital, Seoul, South Korea
[b] Department of Family Medicine, Sanggye Paik Hospital, Seoul, South Korea

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ABSTRACT

We evaluated the performance of the VIDAS GDH assay for the detection of *Clostridium difficile*. In total, 350 fecal specimens collected from patients clinically suspected of having CDI were analyzed by *C. difficile* culture and enzyme-linked fluorescent immunoassay (VIDAS GDH); the results were compared with those of toxigenic *C. difficile* culture (TC), PCR (Xpert *C. difficile* assay), and toxin AB EIA (VIDAS CDAB). The numbers of culture-positive and culture-negative samples were 108 and 242, respectively. The concordance between the GDH assay and *C. difficile* culture was 90.3%. With PCR, 12 more samples were found to be positive in GDH-positive/*C. difficile* culture-negative specimens. Thus, the concordance between GDH assay and *C. difficile* culture/PCR was 93.7%. The sensitivity, specificity, positive predictive value, and negative predictive value of the VIDAS GDH assay were 97.2%, 87.2%, 77.2%, and 98.6%, respectively, based on the *C. difficile* culture, and 97.5%, 91.7%, 86.0%, and 98.6%, respectively, based on *C. difficile* culture/PCR. Positivity rates of the GDH assay were partially associated with those of semi-quantitative *C. difficile* cultures, which were maximized in grade 3 (>100 colony-forming unit [CFU]) compared with grade 1 (<10 CFU).

We evaluated the two-step or three-step algorithm using GDH assay as a first step. No toxin EIA-positive case was found among GDH-negative samples, and 60.8% (48/79) were TC- and/or PCR-positive among the GDH-positive/toxin EIA-negative samples. Thus, approximately 25% of the 350 samples required a confirmatory test (TC or PCR) in the GDH-toxin EIA algorithm, whereas only 2.3% of the total samples in GDH-PCR algorithm was discrepant and required another confirmatory test like TC.

1. Introduction

Clostridium difficile is the major cause of antibiotic-associated diarrhea-causing *C. difficile* infection (CDI), which is the most common identifiable cause of health care-associated diarrhea and pseudomembranous colitis (PMC) [1,2]. Community-associated CDI cases are now increasing and are an emerging threat in populations without a history of hospitalization or antibiotic therapy [3–5]. The high recurrence rate of CDI is an additional important concern [6–8]. Therefore, an accurate diagnosis is very important for the treatment of CDI, because timely infection control interventions can improve treatment outcomes with a reduction in healthcare cost [8–10]. False-positive results may lead to unnecessary treatment and isolation of patients, whereas false-negative results might lead to cross-infection to other patients and overtreatment with empirical antibiotics.

The latest guidelines from the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) recommended a two-step algorithm for screening *C. difficile* in stool specimens, using glutamate dehydrogenase (GDH) assay followed by cell culture neutralization assay (CCNA), toxigenic culture (TC), or PCR for identifying toxin-producing *C. difficile* isolates [11]. In contrast to the SHEA/IDSA, the European Society of Clinical Microbiology and Infectious Disease (ESCMID) recommends a combination of two positive test results (enzyme immunoassay [EIA], GDH, CCNA, and/or PCR) for the diagnosis of CDI, but accepts any negative test results [12]. However, specimens with a first positive test result and a second negative result (GDH-positive/toxin EIA -negative) require further testing with a reference method (CCNA, PCR, or TC) as a third step.

* Corresponding author. Department of Laboratory Medicine, Sanggye Paik Hospital, School of Medicine, Inje University, Sanggye-7 dong, Nowon-gu, Seoul, 139-707, South Korea.
E-mail address: bmshin@unitel.co.kr (B.-M. Shin).

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Therefore, a quick and accurate method for the diagnosis of CDI is highly desirable for providing optimal patient care. Continuing controversy still exists regarding the best option for the diagnosis of CDI [13–16]. Nevertheless, the value of the GDH assay cannot be neglected, and it is generally accepted as a first screening test, regardless of the difference between SHEA/IDSA and ESCMID guidelines.

Recently, a new GDH assay, VIDAS *C. difficile* GDH, has been introduced, which is an *in vitro* diagnostic test for the detection of GDH Ag of *C. difficile* from samples of patients suspected of having CDI. The assay is performed with a VIDAS analyzer approved by the Committee European (CE) and the United States Food and Drug Administration (US FDA). In this study, we evaluated the performance of the VIDAS GDH (bioMérieux, Marcy-l'Etoile, France) kit for CDI.

2. Materials and methods

2.1. Specimens

In total, 350 stool specimens were collected between January 2014 and June 2014 from patients admitted to a teaching hospital in Seoul with clinical signs compatible with CDI. The average age of the patients was 61 years (range: 20–91 years); 56.3% (197) were males and 43.7% (153) were females. These patients had the following underlying diseases: malignancy (15.8%), gastro-enteric disease including ulcerative colitis and gastroenteritis (12.3%), hepatic diseases including liver cirrhosis and hepatitis (10.3%), renal disease including chronic renal failure (8.9%), orthopedic disorders including fracture (8.4%), neurosurgical problems including cerebral infarction (6.4%), infectious disease including pneumonia (3%), and other miscellaneous diseases (34.9%). The Institutional Review Board of the hospital approved the study protocol.

2.2. Culture and identification of C. difficile

Semi-quantitative culture for *C. difficile* was performed as previously described [17]. The extent of growth was rated as follows: grade 1, <10 colonies; grade 2, 10–100 colonies; and grade 3, >100 colonies. Briefly, a stool specimen (1.0 mL) was mixed with an equal volume of 70% isopropanol and incubated for 30 min at room temperature. One drop (~100 μL) was inoculated onto pre-reduced *C. difficile* selective agar (CDSA; Becton Dickinson, MD, USA); the plate was incubated at 37 °C under anaerobic conditions (GasPak EZ Anaerobe Pouch; Becton Dickinson) for 72 h. *C. difficile* colonies were identified on the basis of typical morphological features, spore staining, and odor, using a Vitek 2 ANC ID card identification test kit (bioMérieux SA, Marcy-l'Etoile, France). Any *Clostridium* spp. other than *C. difficile* growing on culture media was defined as *Clostridium* spp. other than *C. difficile* (OTCD).

2.3. Multiplex PCR assay for toxin A (tcdA), toxin B (tcdB), and triose phosphate isomerase (tpi)

Multiplex PCR for *tcdA*, *tcdB*, and *tpi* was performed for 108 *C. difficile* isolates as previously described [18]. The PCR product for *tpi* was 230 bp in length if the isolate was *C. difficile*. The PCR product for *tcdA* was 369 bp in length if the gene was intact, and 110 bp in length if the isolate contained the variant gene ($tcdA^{-}tcdB^{+}$). The PCR product for *tcdB* was 160 bp in length if the gene was intact.

2.4. PCR for direct detection of tcdB in stool specimen

Xpert *C. difficile* assay (Xpert) was performed according to the manufacturer's instructions, as previously described [19]. Briefly, a stool sample was collected on a swab from the container and transferred into the reagent vial. The vial was vortexed for 10 s, and the solution was pipetted into the cartridge chamber. The cartridge was then inserted into the Xpert instrument, and the test was performed using the GeneXpert *C. difficile* assay program.

2.5. GDH assay

For VIDAS GDH, an aliquot (200 μL) of well-mixed liquid stool or 200 mg of mixed semi-solid was dispensed in a centrifuge tube. Next, 1000 μL of pretreatment reagent (R1 *C. difficile*) was added to the centrifuge tube, mixed thoroughly, and centrifuged for 10 min at 3000 × *g*. The supernatant (300 μL) was collected and added to the sample well of the GDH kit to perform the assay.

2.6. EIA for toxin AB (toxin EIA)

Stool specimens were examined for toxin AB via enzyme-linked fluorescent immunoassay (VIDAS; bioMerieux SA, Marcy-l'Etoile, France) as previously described [20]. Assay results were positive, negative, or equivocal according to the fluorescence intensity as described in the relevant package insert for each assay.

3. Results

Of 350 stool specimens collected, 108 (30.9%) were culture positive for *C. difficile* and 242 (69.1%) were culture-negative (183 cases of no bacteria isolated and 59 cases of OTCD). Of 108 *C. difficile* isolates, 89 were toxigenic, 19 were non-toxigenic, and all were *tpi*-positive via toxigenic culture. The concordance between GDH and *C. difficile* culture was 90.3% (316/350). PCR via Xpert *C. difficile* assay detected 12 additional positive samples among culture-negative/GDH-positive samples. The concordance between GDH and *C. difficile* culture/PCR was 93.7% (328/350). Furthermore, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the VIDAS GDH assay were 97.2% (105/108, 95% CI 92.1–99.4), 87.2% (211/242, 95% CI 82.3–91.1), 77.2% (105/136), and 98.6% (211/214), respectively, based on the *C. difficile* culture results and 97.5% (117/120, 95% CI 92.8–99.5), 91.7% (211/230, 95% CI 87.4–94.9), 86.0% (117/136), and 98.6% (211/214), respectively, based on *C. difficile* culture/PCR results.

For evaluation of the two or three-step algorithm using the GDH assay as a first step, we performed toxin AB EIA, toxigenic culture, and PCR (Fig. 1). Among 136 GDH-positive samples, numbers of EIA-positive, EIA-equivocal and EIA-negative samples were 46 (33.8%) and 11 (8.1%), and 79 (58.1%), respectively. Of 46 EIA-positive samples, 45 (97.8%) were positive in TC and/or PCR (42 samples were TC/PCR co-positive, two samples were only PCR-positive, and one sample was only TC-positive), and one (2.2%) was TC/PCR co-negative. Of 11 EIA-equivocal samples, 10 (90.9%) were TC/PCR co-positive and one was TC/PCR co-negative. Of 79 GDH-positive/toxin EIA-negative samples, 60.8% (48/79) were TC- and/or PCR-positive (29 samples were TC/PCR co-positive, 5 samples were only TC-positive, and 14 samples were only PCR-positive). In 214 GDH negative samples, 204 samples were toxin EIA-negative (95.3%). No toxin EIA-positive samples were observed except for 10 (4.7%) samples of toxin EIA-equivocal results. *C. difficile* was isolated from three samples (one $tcdA^{+}tcdB^{+}$ sample and one $tcdA^{-}tcdB^{-}$ sample in EIA-equivocal samples, and one $tcdA^{+}tcdB^{+}$ sample in EIA-negative samples) in 214 GDH negative samples, but no PCR-positive sample was observed. The positivity rates of GDH were partially associated with those of semi-quantitative cultures (Table 1). Positivity rates of GDH were 91.7% in grade 1, but increased in grade 2 (94.4%), and maximized in grade