Evaluation of a Gastrointestinal Pathogen Panel Immunoassay in Stool Testing of Patients with Suspected *Clostridioides* (*Clostridium*) *difficile* Infection

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ABSTRACT *Clostridioides* (*Clostridium*) *difficile* infection (CDI) is the most common causative pathogen of health care-associated gastrointestinal infections; however, due to the overlap of clinical symptoms with those of other causes of acute gastroenteritis, the selection of the most appropriate laboratory test is difficult. From April to October 2018, 640 stool samples requested for CDI testing were examined using the mariPOC CDI and Gastro test (ArcDia), which allows the detection of *C. difficile* glutamate dehydrogenase (GDH) and toxin A/B, norovirus genogroups GI and GII.4, rotavirus, adenovirus, and *Campylobacter* spp. In parallel, the *C. Diff* Quik Chek Complete test (Alere) was used as a routine diagnostic assay, and *C. difficile* toxigenic culture was used as a reference method. The sensitivity of the mariPOC CDI and Gastro test was comparable to that of *C. Diff* Quik Chek Complete for the detection of GDH (96.40% [95% confidence interval {CI}, 91.81% to 98.82%] versus 95.68% [95% CI, 90.84 to 98.40%]; $P = 1.00$) and was higher for the detection of toxin A/B (66.67% [95% CI, 57.36 to 75.11%] versus 55.56% [95% CI, 46.08 to 64.74%]; $P = 0.00$). The specificity of the mariPOC CDI and Gastro test was lower than that of *C. Diff* Quik Chek Complete for GDH detection (95.21% [95% CI, 92.96% to 96.91%] versus 97.60% [95% CI, 95.85% to 98.76%]; $P = 0.04$) and comparable to that of *C. Diff* Quik Chek Complete for toxin A/B detection (99.24 [95% CI, 98.05% to 99.79%] versus 99.81% [95% CI, 98.94% to 100.0%]; $P = 0.37$). In 29 cases (4.53%), other causative agents of diarrhea were detected (*Campylobacter* spp. [$n = 17$], rotavirus [$n = 7$], and norovirus genogroup GII.4 [$n = 5$]).

KEYWORDS *Clostridium difficile*, gastrointestinal infection, glutamate dehydrogenase, toxin A/B

Clostridium difficile, recently reclassified as *Clostridioides difficile* (1), represents a major cause of acute gastroenteritis in hospital settings (*Clostridium difficile* infection [CDI]) (2). However, other pathogens can produce the same clinical symptoms, and this should be taken into account when selecting the correct laboratory test. In order not to miss any relevant pathogen associated with diarrhea, an optimal diagnostic assay should be able to detect multiple agents with a high-enough sensitivity.

Recently, an automatic fluorescence immunoassay, the mariPOC CDI and Gastro test, was released and can be considered one of the platforms that have the potential to meet the criteria for the reliable detection of multiple gastrointestinal pathogens. The test allows the simultaneous detection of *C. difficile* glutamate dehydrogenase (GDH) and toxins A and B and of the specific antigens of norovirus genogroups GI and GII.4, rotavirus, adenovirus, and *Campylobacter* spp.

In our study, we aimed to evaluate the benefit of implementing the new multiplex antigen assay mariPOC CDI and Gastro for stool samples originally requested by a physician for only *C. difficile* GDH and toxin A/B testing. In addition, the sensitivity and

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specificity of GDH and toxin A/B detection compared to a *C. difficile* toxigenic culture were determined for the mariPOC CDI and Gastro test and for the widely used lateral-flow immunoassay C. Diff Quik Chek Complete.

(Preliminary data were presented at the 29th European Congress of Clinical Microbiology and Infectious Diseases, Amsterdam, the Netherlands.)

MATERIALS AND METHODS

From April to October 2018, there were 640 requests for CDI testing of diarrheal stool samples in the Department of Medical Microbiology at Motol University Hospital, Prague, Czech Republic. The samples were all processed regardless of time and place of diarrhea onset. The average age of hospitalized patients was 49.6 years (ranging from 83 days to 94 years), and the median age was 59 years; 52.5% ($n = 336$) of the patients were females. Fifty-seven patients (8.91%) were younger than 3 years of age.

Stool samples were tested in parallel using the mariPOC CDI and Gastro test (ArcDia, Finland), a fluorescence immunoassay which allows the detection of *C. difficile* GDH and toxin A/B, norovirus genogroups GI and GII.4, rotavirus, adenovirus, and *Campylobacter* spp. (including *C. jejuni*, *C. coli*, and *C. upsaliensis*), and C. Diff Quik Chek Complete (Alere, USA), a rapid membrane enzyme immunoassay for GDH and toxin A/B detection.

A *C. difficile* toxigenic culture was used as a reference method. Briefly, all stool samples were cultured anaerobically on Brazier's selective medium for *C. difficile* (Oxoid) after alcohol shock treatment. *C. difficile* colonies were confirmed by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Bruker) with a score of >2.0 . After DNA extraction by using Chelex particles (Bio-Rad), the presence of the toxin genes (*tcdA* [toxin A] and *tcdB* [toxin B]) was investigated by multiplex PCR (3). In *C. difficile* isolates with a negative PCR result for the presence of toxin genes, the primers Lok1 (*cdd1*) and Lok3 (*cdu1*) were used to confirm the absence of the pathogenicity locus (4).

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of GDH and toxin A/B detection were calculated based on the true-positive, true-negative, false-positive, and false-negative results compared to *C. difficile* toxigenic culture (5). To compare the sensitivities and specificities of two CDI diagnostic assays, McNemar's test was used.

In cases where stool samples were shown to be positive using the mariPOC CDI and Gastro test, with the exception of GDH and/or toxin A/B detection, these positive results were confirmed, depending on the pathogen detected, using culture on selective medium for *Campylobacter* spp. (Karmali; Oxoid) or the immunochromatographic assays Rapid-Viditest Rota-Adeno Card (Vidia) and/or Rida Quick norovirus (R-Biopharm). When the mariPOC CDI and Gastro test proved positive and the above-mentioned confirmatory tests proved negative, a stool sample was examined by a multiplex nucleic acid amplification test (NAAT) (Faecal Pathogens M; AusDiagnostics) that targeted all pathogens investigated in our study.

Of note, the mariPOC test was not part of the diagnostic laboratory algorithm at the time of the study. Thus, only the results from C. Diff Quik Chek Complete and positive confirmatory tests for other pathogens were reported to physicians.

RESULTS

***Clostridium difficile*.** In our study, we cultured 640 stool samples for *C. difficile*; of these samples, 501 were shown to be *C. difficile* negative and 139 were shown to be *C. difficile* positive, and of the latter samples, 116 *C. difficile* isolates were toxigenic and 23 isolates were nontoxigenic.

GDH was detected in 158 samples by the mariPOC CDI and Gastro test and in 145 samples by C. Diff Quik Chek Complete. Compared to the *C. difficile* cultures, false positivity was found in 24 and 12 samples (other *Clostridia*; *C. clostridioforme*, *C. ramosum*, *C. bolteae*, or *Flavonifractor plautii* was cultured), and false negativity (the *C. difficile* culture proved positive) was found in 5 and 6 samples, respectively (Fig. 1).

Toxins A and B were detected in 82 samples by the mariPOC CDI and Gastro test and in 66 samples by C. Diff Quik Chek Complete. Compared to *C. difficile* toxigenic culture, false positivity (the *C. difficile* toxigenic culture result was negative) was detected in 4 samples and 1 sample, respectively, and false negativity (the result of *C. difficile* toxigenic culture was positive) was detected in 39 and 52 samples, respectively (Fig. 1). Interestingly, two toxin A/B true-positive samples (one sample was positive by the mariPOC CDI and Gastro test as well as by C. Diff Quik Chek Complete, while one sample was positive by C. Diff Quik Chek Complete only) were GDH negative.

Comparing the two immunoassays used in the study, the sensitivity of the mariPOC CDI and Gastro test was comparable to that of C. Diff Quik Chek Complete for the detection of GDH (96.40% [95% confidence interval {CI}, 91.81% to 98.82%] versus 95.68% [95% CI, 90.84 to 98.40%]; $P = 1.00$) and was higher for the detection of toxin A/B (66.67% [95% CI, 57.36 to 75.11%] versus 55.56% [95% CI, 46.08 to 64.74%];

FIG 1 Results for *Clostridioides (Clostridium)* glutamate dehydrogenase and *Clostridioides (Clostridium)* toxin A/B detection by the mariPOC CDI and Gastro test and the C. Diff Quik Chek Complete assay. *Clostridioides (Clostridium)* toxigenic culture was used as a reference method. The numbers of samples that were concluded to have true-positive (TP), true-negative (TN), false-positive (FP), or false-negative (FN) results by both assays are shown in the intersection of circles. Unique true-positive, true-negative, false-positive, and false-negative values for the Quik Chek Complete test only and the mariPOC CDI test only are indicated on the left and right, respectively.

$P = 0.00$). (Table 1). The specificity for GDH was lower for the mariPOC CDI and Gastro test (95.21% [95% CI, 92.96% to 96.91%]) versus 97.60% [95% CI, 95.85% to 98.76%]; $P = 0.04$) yet at the same level for both tests for the detection of toxin A/B (99.24% [95% CI, 98.05% to 99.79%] versus 99.81% [95% CI, 98.94% to 100.0%]; $P = 0.37$).

Other pathogens. From the 640 stool samples originally requested for GDH and toxin A/B testing, 41 were positive by the mariPOC CDI and Gastro test for a pathogen other than *C. difficile*, and 29 of them were confirmed by another confirmatory test.

The presence of *Campylobacter* spp. was detected in 19 samples. For 14 of these samples, the positivity of the *Campylobacter* species test was confirmed by culture on selective medium (*C. jejuni* [$n = 12$] and *C. coli* [$n = 2$]), and for 3 other samples, positivity was confirmed by a NAAT. The mean age of patients was 39 years and ranged from 83 days to 84 years. All patients were admitted with diarrhea from the community: three patients reported contact with a health care environment in the previous 4 weeks (organ transplant [$n = 2$] and oncology patient [$n = 1$]). The remaining two mariPOC CDI and Gastro test-positive results for *Campylobacter* spp. were not confirmed.

Eleven samples proved to be positive for rotavirus antigen, and for 7 of these samples, positivity was confirmed by another confirmatory method. In confirmed cases of rotavirus, only one patient was pediatric (4 years old), and the remaining 6 samples were from adults (ages ranging from 55 to 87 years). Interestingly, all adult cases were linked to the same hospital department.

For norovirus genogroup GI, three samples gave a positive result, but none of these positive cases were confirmed by any other method. Of the eight norovirus genogroup GII.4-positive samples, five were confirmed by another confirmatory test. Four of these patients were 1 year old, and one patient was 60 years old. One child developed

TABLE 1 Specificities and sensitivities of two immunoassays for detection of *Clostridioides (Clostridium) difficile* glutamate dehydrogenase and toxins A and B^a

Assay	Specificity (%)	95% CI for specificity (%)	Sensitivity (%)	95% CI for sensitivity (%)	NPV (%)	95% CI for NPV (%)	PPV (%)	95% CI for PPV (%)
mariPOC CDI test, GDH	95.21	92.96–96.91	96.40	91.81–98.82	98.96	97.58–99.56	84.81	79.06–89.20
Quik Chek Complete, GDH	97.60	95.85–98.76	95.68	90.84–98.40	98.79	97.39–99.44	91.72	86.36–95.10
mariPOC CDI test, toxin A/B	99.24	98.05–99.79	66.67	57.36–75.11	93.01	91.15–94.50	95.12	87.93–98.12
Quik Chek Complete, toxin A/B	99.81	98.94–100.00	55.56	46.08–64.74	90.94	89.13–92.48	98.48	90.25–99.79

^a*Clostridioides (Clostridium)* toxigenic culture was used as a reference method. There were a total of 640 samples in the study. CDI, *Clostridioides (Clostridium) difficile* infection; GDH, glutamate dehydrogenase; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

FIG 2 Overall results for negative samples and samples given a positive result with the mariPOC CDI and Gastro test that were confirmed by *C. difficile* toxigenic culture, *Campylobacter* species culture or a nucleic acid amplification test (NAAT), or rotavirus or norovirus immunochromatographic antigen detection. There was no confirmed concurrent positivity, except for five samples with positive glutamate dehydrogenase (GDH) and *Campylobacter* species ($n = 3$) or GDH and norovirus genogroup GII.4 ($n = 2$) results.

diarrhea on day 10 of hospitalization, and the rest of the patients were admitted with community-acquired diarrhea.

During our study period, no sample proved positive for the presence of the adenovirus antigen.

In summary, out of 640 patients suspected of having CDI, the laboratory diagnosis was confirmed by the detection of GDH and toxin A/B in 77 patients (12.03%) using the mariPOC CDI and Gastro test and in 63 patients (9.84%) by *C. Diff* Quik Chek Complete. For another 29 patients (4.53%), the mariPOC CDI and Gastro test detected *Campylobacter* spp. ($n = 17$), rotavirus ($n = 7$), and norovirus genogroup GII.4 ($n = 5$). Importantly, five samples revealed concurrent positivity for GDH and *Campylobacter* spp. ($n = 3$) and for GDH and norovirus genogroup GII.4 ($n = 2$). No concurrent positivity was confirmed, except for the five samples mentioned above.

The results for true-negative samples and samples giving a true-positive result with the mariPOC CDI and Gastro test that were confirmed by *C. difficile* toxigenic culture, *Campylobacter* species culture or NAAT, or rotavirus or norovirus immunochromatographic antigen detection are shown in Fig. 2.

DISCUSSION

Two immunoassays that detect GDH and toxins A and B from the same sample and that are based on different principles of detection were compared in this study. The first, *C. Diff* Quik Chek Complete, is a manual lateral-flow membrane-type test, and its sensitivity has been evaluated in several studies (6). In our study, the sensitivities for GDH (95.68%) and toxins A and B (55.56%) observed for the lateral-flow immunoassay are in line with those reported in a previous study by Bruins et al. (7).

The second immunoassay, the mariPOC CDI and Gastro test, which launched in 2017, is a new product based on automated two-photon excitation technology (8), and this study is the first to evaluate the sensitivity and specificity of *C. difficile* GDH and toxin A/B detection. For the detection of GDH, the sensitivity of the mariPOC CDI and Gastro test was found to be equivalent to that of the *C. Diff* Quik Chek Complete test and to the pooled sensitivities of the commercial diagnostic test (96.40%, 95.68%, and 94.0%, respectively). On the other hand, the specificity for GDH with the mariPOC CDI and Gastro test was slightly lower than that of the *C. Diff* Quik Chek Complete and/or the pooled specificities of commercial tests (95.21, 97.6, and 96.0%, respectively) (6). In our experience, the lower specificity was probably caused by cross-reactivity with other cultured *Clostridia*, but we also cannot exclude the failure of *C. difficile* culture. Other studies evaluating GDH specificity in commercial tests reported samples with a false-positive GDH result due to a discrepancy with the *C. difficile* culture-negative result

(6, 9). The cross-reactivity of GDH detection with other cultured *Clostridia* was reported for one sample in a previous study by Alfa et al. that evaluated the Triage *C. difficile* test (10).

For toxin A/B detection, the mariPOC CDI and Gastro test had a higher sensitivity than that of the C. Diff Quik Chek Complete and/or the pooled sensitivities of the commercial diagnostic tests (66.40%, 55.56%, and 57.0%, respectively). The specificities for toxin A/B were identical (exceeding 99.0%) for both tests evaluated, as were the pooled specificities of commercial assays (6).

Both immunoassays used in our study allow for the detection of GDH and toxin A/B simultaneously from the same sample and, as such, are approved as an alternative algorithm for CDI diagnostics (6). Two samples in our study gave a negative GDH result but a positive result for toxin A/B, which suggests that this is an invalid result according to current guidelines (6). The first sample gave this invalid result in both assays, whereas the second sample gave this result only in the C. Diff Quik Chek Complete assay; *C. difficile* toxigenic culture was positive for both samples, and both patients received CDI-specific treatment. These two clinical cases support the previous observation of the need to confirm toxin A/B positivity without the detection of GDH (11).

When a screening test for CDI is positive, the detection of toxins A and/or B in the stool sample is crucial for a CDI laboratory diagnosis. The mariPOC CDI and Gastro test showed a higher sensitivity for toxin A/B detection by identifying 14 more cases than the C. Diff Quik Chek Complete assay. For 11 of these patients, specific treatment for CDI was initiated. Two patients died, and CDI contributed definitely to their deaths. Unfortunately, in one other fatality, specific CDI treatment was not initiated, and the false negativity of toxin A/B detection with the C. Diff Quik Chek Complete assay could have influenced the decision not to initiate CDI-specific treatment.

The patients who screened positive for GDH but negative for toxin A/B represent a group of potential carriers who play an important role in the epidemiology of CDI (12). Compared to toxigenic culture, the mariPOC CDI and Gastro test provided a false-negative result for the detection of toxin A/B in 39 cases, and of these 39 patients, CDI-specific treatment was initiated for 22 patients, although 6 of them died.

In 23 samples with a GDH-positive and toxin A/B-negative result, nontoxigenic *C. difficile* was cultured. These results support the need for a multiple-step laboratory algorithm for CDI (6).

Importantly, in 5 patients with a GDH-positive and toxin A/B-negative result, another gastrointestinal pathogen was identified; toxigenic *C. difficile* was cultured from two samples with concurrent *Campylobacter* species infection and from one sample with parallel norovirus genogroup GII.4 positivity. These patients would have remained undiagnosed if a multiplex antigen test had not been used, and moreover, GDH positivity, confirmed by the presence of toxigenic *C. difficile* by toxigenic culture, may have led to the initiation of treatment for CDI instead of symptomatic and/or specific treatment for norovirus or *Campylobacter* species infection.

According to the latest CDI laboratory testing recommendations, testing of children under 3 years of age was suggested to be limited to a physician's request only (6), due to the high rate of asymptomatic colonization in infants and a high probability of a viral etiology of diarrhea (13). When the results of samples from children under 3 years of age ($n = 57$) were excluded from the analysis, the sensitivity and specificity did not change significantly.

Campylobacteriosis is the most commonly reported bacterial gastrointestinal disease in Europe, with the majority of cases being reported in the Czech Republic, Germany, Spain, and the United Kingdom, between 2012 to 2016 (14). *Campylobacter* species is a frequent etiological agent in patients hospitalized with acute gastroenteritis (15, 16), which is in line with our study results, where all patients with confirmed campylobacteriosis suffered from community-acquired diarrhea. In our study, using the mariPOC CDI and Gastro test, three samples proved positive for *Campylobacter* spp. yet proved negative when cultured on selective medium for *Campylobacter* spp; the presence of *Campylobacter* spp. was confirmed by NAAT. The higher sensitivity of NAAT

than of cultures was also found in a previous study by Liang et al. (17). Overall, the diagnosis of campylobacteriosis through the detection of *Campylobacter* species antigen in a stool sample dramatically sped up the diagnostics of our laboratory from 48 h by culture to 2 h by antigen detection. Moreover, cultures proved to be less sensitive than antigen detection.

Worldwide, rotavirus is the most common causative agent of diarrhea in infants and children younger than 5 years of age (18), but due to the introduction of the rotavirus vaccine, a decrease in the incidence of rotavirus-associated hospitalizations of children has been recorded (19). Recent studies that focused on the etiology of acute gastroenteritis in adults showed that rotavirus was a frequently found pathogen (15, 16, 20). In our study, possible nosocomial transmission of rotavirus was detected because five of six patients were hospitalized in the same ward, and the remaining adult sample was derived from health care personnel from the same ward.

Norovirus is the second most common cause of severe childhood gastroenteritis (21), and an increased incidence of norovirus genogroup GII.4 has been reported in some European countries (22) and the United States (23). In our study, four of five patients diagnosed with norovirus genogroup GII.4 gastroenteritis were pediatric patients; an adult patient infected with norovirus underwent a subtotal colectomy.

Conclusions. The results of the study showed that the use of the marioPOC CDI and Gastro test improved microbiology diagnostics for patients with suspected CDI. A higher sensitivity of toxin A/B detection than with the lateral-flow assay used in routine diagnostics contributed to the diagnosis of patients with CDI. Moreover, in a cohort of 640 hospitalized patients, in 29 cases (4.53%), another causative pathogen was detected. These patients would have remained undiagnosed if a multiplex antigen test had not been used.

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We declare that we have no conflict of interest.

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