Evaluation of the performance of C. DIFF QUIK CHEK COMPLETE and its usefulness in a hospital setting with a high prevalence of *Clostridium difficile* infection

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ABSTRACT

Rapid and accurate diagnosis of *Clostridium difficile* infection (CDI) is crucial for patient care, infection control, and efficient surveillance. We evaluated C. DIFF QUIK CHEK COMPLETE (QCC; TechLab), which detects glutamate dehydrogenase (GDH) antigen (QCC-Ag) and toxin A/B (QCC-Tox) simultaneously, and compared it to the laboratory diagnostics for CDI currently in use in a tertiary hospital setting with a high prevalence of CDI. QCC, RIDASCREEN *C. difficile* toxin A/B assay (Toxin EIA; R-Biopharm AG), chromID *C. difficile* agar (bioMérieux) culture (ChromID culture), and Xpert *C. difficile* PCR assay (Xpert PCR; Cepheid) were performed according to the manufacturers' instructions. Performances of the assays were compared against that of Xpert PCR as a reference. Of the 231 loose stool specimens, 83 (35.9%) were positive by Xpert PCR. The sensitivity, specificity, and positive and negative predictive values were 97.6%, 93.9%, 90.0%, and 98.6%, respectively, for QCC-Ag and 55.4%, 100%, 100%, and 80.0%, respectively, for QCC-Tox. The median threshold cycle values of the QCC-Tox(+) specimens were lower than those of the QCC-Tox(−) specimens. Results of QCC as an initial screening test were confirmed in 81.0% (187/231) of samples; these specimens did not require further testing. QCC is a rapid, easy, and cost-effective method that would be a useful first-line screening assay for laboratory diagnosis of CDI in a tertiary hospital with a high prevalence of CDI. A two-step algorithm using QCC as an initial screening tool, followed by Xpert PCR as a confirmatory test, is a practical and cost-effective approach.

BACKGROUND

Clostridium difficile is the leading cause of healthcare-associated diarrhea, particularly in hospitalized patients receiving antimicrobial therapy. The incidence of *C. difficile* infection (CDI) among hospitalized patients varies widely from year to year and in different locations but has generally been increasing.[1 2] The reasons for this increase are manifold, but include an aging population, increased use of fluoroquinolones, emergence of at least two

Significance of this study

What is already known about this subject?

- *Clostridium difficile* is the leading cause of healthcare-associated diarrhea. Rapid and accurate laboratory diagnosis of *C. difficile* infection is crucial for patient care, infection control, and efficient surveillance.
- The laboratory diagnostics for *C. difficile* infection currently in use have several limitations.
- Recently, a combination assay, C. DIFF QUIK CHEK COMPLETE (TechLab, USA), was introduced into our region; this assay detects both glutamate dehydrogenase (GDH) and toxin A/B simultaneously with one easy-to-use cartridge. However, there are little data on the performance of the assay, particularly in regard to the hospitals with a high prevalence of *C. difficile* infection.

What are the new findings?

- The sensitivity, specificity, positive predictive value, and negative predictive value of C. DIFF QUIK CHEK COMPLETE were 97.6%, 93.9%, 90.0%, and 98.6%, respectively, for the GDH assay and 55.4%, 100%, 100%, and 80.0%, respectively, for the toxin A/B assay.
- The median threshold cycle values of the toxin-positive specimens were lower than those of the toxin-negative specimens.
- Results of C. DIFF QUIK CHEK COMPLETE as an initial screening test were confirmed in 81.0% (187/231) of samples; therefore, these specimens did not require further testing such as Xpert *C. difficile* PCR assay.

How might these results change the focus of research or clinical practice?

- It provides useful information about laboratory diagnosis of *C. difficile* infection and would be helpful for treatment and infection control of *C. difficile*.

highly virulent ribotypes (027 and 078), and improved diagnostic testing.[3]

A case definition of CDI should include the presence of symptoms (usually diarrhea) and either a stool test that is positive for *C. difficile* toxins or toxigenic *C. difficile*, or colonoscopic findings of pseudomembranous colitis. Clinical manifestations of CDI range from asymptomatic carriage, to mild or moderate diarrhea, to fulminant and sometimes fatal pseudomembranous colitis.[4] The clinical differentiation of CDI from other causes of diarrhea is not possible with any certainty, especially since those at greatest risk for CDI are also more likely to have other causes—medical, surgical, or iatrogenic—of diarrhea.[5] Thus, rapid and accurate laboratory diagnosis of CDI is crucial for patient care, infection control, and efficient surveillance.

Several laboratory tests are available for the diagnosis of CDI, including the cell culture cytotoxicity neutralization assay (CCCNA), toxigenic culture, toxin immunoassay, glutamate dehydrogenase (GDH) assay, combination testing of GDH detection and toxin assay, and molecular assays.[3] Until very recently, enzyme immunoassays for *C. difficile* toxin detection (toxin EIAs) were the most frequently used assays for *C. difficile* detection in clinical laboratories.[3 4 6] It is fast, convenient, and inexpensive. However, the poor sensitivity of toxin EIAs is well established, so toxin EIAs should no longer be considered adequate stand-alone tests for the diagnosis of CDI.[4 6 7] GDH, a common antigen (a metabolic enzyme) in the *C. difficile* cell wall, is a sensitive marker for the detection of *C. difficile*. GDH assays detect toxigenic and non-toxigenic *C. difficile* strains, as well as other clostridial species; therefore, these assays have a low specificity and positive predictive value (PPV). GDH-positive results should be followed up with a test to confirm toxin production.[4 6 7] Several EIAs that combine GDH detection and a toxin EIA in one test are now available in the market. These combination assays are relatively rapid, and the cost per test is less than that of a molecular assay.[3] Recently, a combination assay, C. DIFF QUIK CHEK COMPLETE (QCC; TechLab, USA), was introduced into our region; this assay detects both GDH and toxin A/B simultaneously with one easy-to-use cartridge. However, there are little data on the performance of QCC,[8–12] particularly in regard to the hospitals with a high prevalence of CDI.[13 14]

We evaluated the performance of QCC in comparison with methods currently applied for the diagnosis of CDI, including RIDASCREEN *C. difficile* toxin A/B assay (R-Biopharm AG), chromID *C. difficile* agar (bioMérieux) culture (ChromID culture), and Xpert *C. difficile* PCR assay (Xpert PCR; Cepheid), in a clinical laboratory of a tertiary hospital with a high prevalence of CDI.

MATERIAL AND METHODS

Clinical specimens

A total of 231 loose stool specimens were collected from the patients with suspected CDI at a tertiary care hospital; 83 (35.9%) and 148 (64.1%) were positive and negative, respectively, for CDI by Xpert PCR. These specimens were submitted to the clinical laboratory for *C. difficile* toxin EIA, *C. difficile* culture using chromogenic agar, and *C. difficile* toxin gene PCR assay. The stool specimens were stored at −4°C for processing within 72 hours, and frozen at −20°C for processing after 72 hours. This study was approved by the institutional review board of our institution.

Laboratory tests for *C. difficile*

All assays, *C. difficile* toxin EIA, ChromID *C. difficile* agar culture, Xpert PCR, and QCC (TechLab), were performed for all of the 231 specimens. All assays except QCC were performed on the same day as the specimen reception according to the manufacturers' instructions.

A toxin EIA was performed directly on stool samples by the RIDASCREEN *C. difficile* toxin A/B assay (Toxin EIA; R-Biopharm AG), which is a qualitative 96-well microplate assay that detects toxins A and B of *C. difficile*. In the clinical laboratory, Toxin EIA was performed in a batch once a day. The assay required ~2 hours to complete.

The ChromID culture can detect and identify β-glucosidase-producing *C. difficile* strains within 24 hours of incubation based on the presence of grey-to-black colonies with irregular or smooth borders. Alcohol shock-treated stool specimens were inoculated onto chromID *C. difficile* agar and incubated in an anaerobic jar at 35°C and examined at 48 hours for grey-to-black colonies (ChromID culture).

Xpert PCR (Cepheid, USA), a random-access, rapid, real-time multiplex PCR assay was performed using the GeneXpert System (Cepheid), which automates and integrates sample purification, nucleic acid amplification, and detection. Xpert PCR detects the toxin B gene (*tcdB*), binary toxin genes (*cdtA* and *cdtB*), and *tcdC* 117-nucleotide deletion (epidemic 027 ribotype); as a result, it can detect toxigenic *C. difficile* strains and differentiate *C. difficile* presumptive 027/NAP1/BI. A threshold cycle (Ct) of 37 was used as the cut-off for *tcdB* detection.

C. difficile GDH antigen, toxin A, and toxin B are simultaneously detected in a single cartridge using QCC. The assay detects GDH to screen for the presence of *C. difficile* (QCC-Ag) and then confirms the presence of toxigenic *C. difficile* by detecting toxins A and B in stool specimens (QCC-Tox). In brief, a stool specimen is added to a tube containing the diluent and conjugate, and the mixture is transferred to the device sample well. After incubation for 15 min at room temperature, a wash buffer and then the substrate are added to the reaction window. Results are read after 10 min. QCC-Ag and QCC-Tox are considered positive if a visible band is visible in the device display window.

Algorithmic approach using QCC

We simulated a two-step algorithmic approach with Toxin EIA or QCC as an initial test followed by Xpert PCR. Algorithmic approaches were simulated with the known results. Specimens that were positive by Toxin EIA were considered CDI positive and not followed up with a confirmatory testing. Specimens that were negative by Toxin EIA were tested by Xpert PCR. If the results of QCC-Ag and QCC-Tox testing were positive (QCC-Ag(+)/Tox(+)), the specimen was considered CDI positive; if both tests were negative (QCC-Ag(−)/Tox(−)), the specimen was considered CDI negative. No confirmatory testing was needed in these cases with concordant results. If the result was indeterminate, such as a positive result by QCC-Ag but a negative result by QCC-Tox testing (QCC-Ag(+)/Tox(−)), the sample was tested by Xpert PCR.

Statistical analysis

The performances of the assays were compared, using Xpert PCR as a reference. The sensitivities, specificities, and PPV and negative predictive value (NPV) were determined, and the Ct values of the QCC-Tox(+) and QCC-Tox(−) specimens were compared. A statistical analysis was performed using MedCalc V.10.0 (MedCalc Software bvba, Belgium). A p value of <0.05 were considered statistically significant.

RESULTS

The sensitivity, specificity, PPV, and NPV of the assays are shown in table 1. QCC-Ag was more sensitive than ChromID culture (97.6 vs 69.9%; p<0.0001) in detecting CDI. The specificities of QCC-Ag and ChromID culture were not significantly different (93.9 vs 94.6%; p=0.993). QCC-Tox was more sensitive than Toxin EIA, but this difference was not statistically significant (55.4 vs 48.2%; p=0.440). The specificities of the two assays for *C. difficile* toxins detection were excellent: 100% and 99.3% for QCC-Tox and Toxin EIA, respectively. Discrepancies between results of QCC with Xpert PCR testing among all results of the 231 specimens are indicated in table 2. The QCC-Ag screen resulted in nine false-positives; among these, five specimens were positive by ChromID culture and negative by Toxin EIA, which strongly suggests infection with non-toxigenic *C. difficile*. The agreement between QCC-Tox and Toxin EIA results was 93.5%. The median Ct values of the QCC-Tox(−) specimens were significantly higher than those of the QCC-Tox(+) specimens (p=0.001), with Ct values of 24.7 (95% CI 24.1 to 27.3) and 29.3 (27.3 to 31.6), respectively (figure 1).

Simulated results of the two-step algorithmic approaches are shown in figure 2. Using Toxin EIA as an initial test, 190 specimens (82.3%) were required confirmation by Xpert PCR; among these specimens, 43 (22.6%) were confirmed positive by Xpert PCR. There was one false-positive result among the 41 specimens with a positive Toxin EIA result. QCC-Ag(+)/Tox(+) and QCC-Ag(−)/Tox(−) results were observed in 46 and 141 specimens, respectively. Therefore, QCC as an initial test provide definitive results in 82.6% (176/213) of cases, and no further testing was needed. All 46 QCC-Ag(+)/Tox(+) results were true-positives (Xpert PCR positive), and all but 1 of the 141 QCC-Ag(−)/Tox(−) results were true-negatives (Xpert PCR negative). The QCC-Ag(+)/Tox(−) results were confirmed by Xpert PCR; of 44 specimens with QCC-Ag(+)/Tox(−) results, 35 (79.5%) were positive by Xpert PCR.

DISCUSSION

In this study, the performance of QCC was evaluated and compared with that of assays currently conducted for the diagnosis of CDI, in a clinical laboratory of a tertiary hospital setting with a high prevalence of CDI. The monthly

Table 1 The performance of the assays for detection of *Clostridium difficile* infection

Assays	Result (N)	Xpert PCR +	Xpert PCR −	Sensitivity	Specificity	PPV	NPV
				% (95% CI)			
ChromID culture	+	58	8	69.9 (58.8 to 79.5)	94.6 (89.6 to 97.6)	87.9 (77.5 to 94.6)	84.8 (78.4 to 89.9)
	−	25	140				
Toxin EIA	+	40	1	48.2 (37.1 to 59.4)	99.3 (96.3 to 99.9)	97.6 (87.1 to 99.6)	77.4 (70.8 to 83.1)
	−	43	147				
QCC-Ag	+	81	9	97.6 (91.6 to 99.6)	93.9 (88.8 to 97.2)	90.0 (81.9 to 95.3)	98.6 (95.0 to 99.8)
	−	2	139				
QCC-Tox	+	46	0	55.4 (44.1 to 66.3)	100 (97.5 to 100)	100 (92.2 to 100)	80.0 (73.5 to 85.5)
	−	37	148				

ChromID culture, chromID *C. difficile* agar (bioMérieux) culture; NPV, negative predictive value; PPV, positive predictive value; QCC-Ag, C. DIFF QUIK CHEK COMPLETE glutamate dehydrogenase antigen (TechLab); QCC-Tox, C. DIFF QUIK CHEK COMPLETE toxin A/B (TechLab); Toxin EIA, RIDASCREEN *C. difficile* toxin A/B assay (R-Biopharm AG); Xpert PCR, Xpert *C. difficile* PCR assay (Cepheid).

Table 2 Discrepant results of QCC (TechLab) with Xpert PCR

N	Result of the assays: Xpert PCR	ChromID	QCC-Ag	QCC-Tox	Toxin EIA	Result interpretation
3	+	+	+	−	+	QCC-Tox FN
15	+	+	+	−	−	QCC-Tox/Toxin EIA FN
17	+	−	+	−	−	QCC-Tox/Toxin EIA/ChromID FN
1	+	+	−	−	−	QCC, Toxin EIA FN
1	+	−	−	−	−	Only Xpert positive
1	+	+	−	−	+	QCC FN
5	−	+	+	−	−	Non-toxigenic *C. difficile*
4	−	−	+	−	−	Possible non-toxigenic *C. difficile* or QCC-Ag FP

C. difficile, *Clostridium difficile*; ChromID, chromID *C. difficile* agar (bioMérieux); FN, false negative; FP, false positive; QCC, C. DIFF QUIK CHEK COMPLETE; QCC-Ag, QCC glutamate dehydrogenase antigen (TechLab); QCC-Tox, QCC toxin A/B (TechLab); Toxin EIA, RIDASCREEN *C. difficile* toxin A/B assay (R-Biopharm AG); Xpert PCR, Xpert *C. difficile* PCR assay (Cepheid).

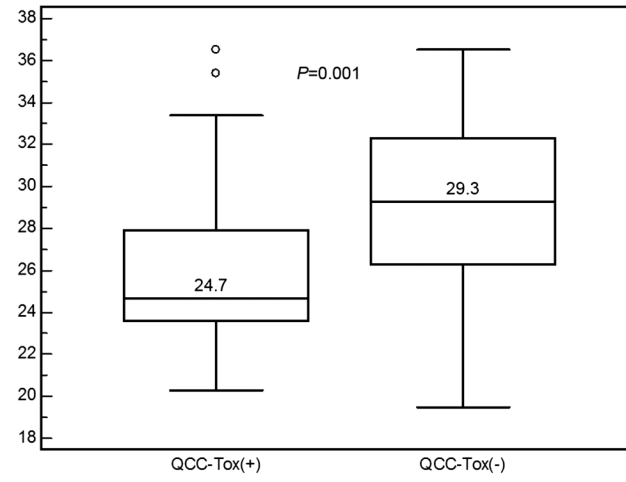

Figure 1 Comparison of the Ct values of QCC-Tox(+) and QCC-Tox(–) specimens. Ct, threshold cycle; QCC-Tox, C. DIFF QUIK CHEK COMPLETE toxin A/B (TechLab).

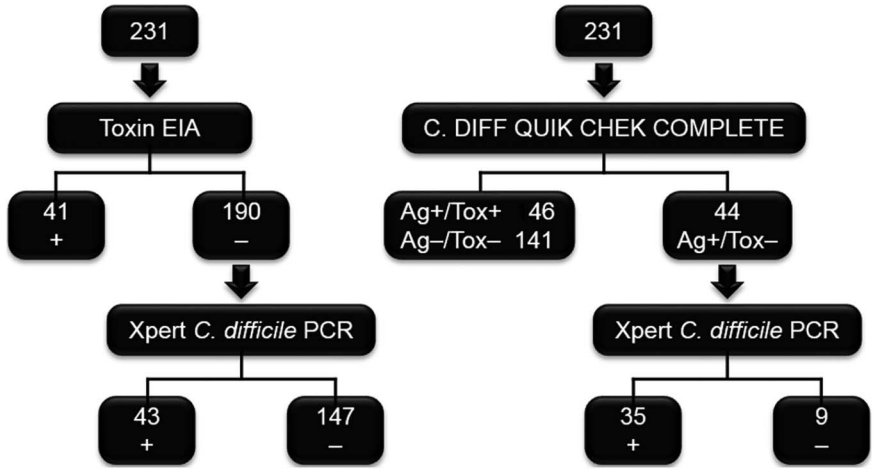

Figure 2 The two-step testing algorithmic approaches using Toxin EIA and C. DIFF QUIK CHEK COMPLETE (TechLab) as initial tests followed by Xpert PCR (simulation with the known results). Ag, C. DIFF QUIK CHEK COMPLETE glutamate dehydrogenase antigen (TechLab); Tox, C. DIFF QUIK CHEK COMPLETE toxin A/B (TechLab); Toxin EIA, RIDASCREEN *Clostridium difficile* toxin A/B assay (R-Biopharm AG); Xpert PCR, Xpert *C. difficile* PCR assay (Cepheid).

CDI-positive rates, determined by Xpert PCR in our laboratory, were about 30% in 2015 (data not shown).

When the results of QCC were considered positive if both QCC-Ag and QCC-Tox were positive and considered negative if both QCC-Ag and QCC-Tox were negative, the sensitivity and specificity of QCC largely reflected those of QCC-Tox: the assay showed 55.4% sensitivity and 100% specificity. This is comparable with the findings of previous studies, which showed sensitivities of 42.3–68.4% and specificities of 98.0–100%.[8–13]

QCC-Ag showed excellent sensitivity (97.6%) and relatively low specificity (93.9%). This result is compatible with those of earlier studies on GDH assays, showing sensitivities of 87.6–100% and specificities of 76.4–98%.[3] Nine QCC-Ag-positive specimens were negative by Xpert PCR; all of these specimens were also negative by QCC-Tox. Since QCC-Ag(+)/Tox(–) was followed by Xpert PCR testing, none of these specimens were misclassified as positive for CDI. Therefore, the low specificity of QCC-Ag is complemented by QCC-Tox testing, demonstrating the main advantage of combination assays such as QCC that perform GDH detection and Toxin EIA.

ChromID culture detects the presence of *C. difficile*, similar to the GDH assay; however, the sensitivity of this assay was considerably lower than that of QCC-Ag. Moreover, ChromID culture requires at least 24 hours before results can be reported. Therefore, the usefulness of ChromID culture as an initial screening test is limited.

There were five specimens of positive by QCC-Ag and ChromID culture, but negative by Xpert PCR, QCC-Tox, and Toxin EIA, which strongly indicated non-toxigenic *C. difficile*. Non-toxigenic *C. difficile* could not be ruled out in an additional four specimens that were positive only by QCC-Ag and three specimens positive only by ChromID culture (data not shown). The proportion of non-toxigenic *C. difficile* can vary considerably, depending on the patient population and clinical setting; overall, populations of symptomatic hospitalized adult patients showed lower proportions of non-toxigenic *C. difficile* than those of healthy volunteers or asymptomatic hospitalized patients.[15] In a population with a low proportion of non-toxigenic *C. difficile*, GDH assays could be more useful because false-positives due to non-toxigenic *C. difficile* would be reduced.

QCC-Tox showed sensitivity (55.4%) and specificity (100%) similar to that of *C. difficile* toxin EIA currently in use in our laboratory. The low sensitivities of *C. difficile* toxin EIAs are recognized, and most current guidelines for management of CDI recommend that these assays should not be used as stand-alone tests.[4 6 7] Rather, toxin EIAs should be used as a part of a two-step or three-step testing algorithm. The QCC assay combines a GDH assay and toxin EIA, and saves time and workload by one step. Unlike toxin, which is currently performed in a batch once a day, QCC can be used for individual patients at any time, and the result can be obtained in <30 min. This format makes QCC very useful for making rapid decisions in an urgent situation.

In general, the Ct value is inversely related to the number of targets. As expected, the median Ct values of the QCC-Tox(−) specimens were higher than those of the QCC-Tox(+) specimens. Even though the results were expected, it was important to confirm the hypothesis based on a statistical significance. This finding suggests that specimens with negative results by QCC-Tox but positive results by Xpert PCR have lower numbers of toxigenic *C. difficile* and, in at least some patients, likely to represent asymptomatic carriage.[16]

Recently, the American College of Gastroenterology announced the clinical practice guideline for the diagnosis, treatment, and prevention of CDI.[7] This guideline recommends the use of the nucleic acid amplification tests (NAATs) as the definitive test for CDI either as a stand-alone test or part of a multistep algorithm. The American Society for Microbiology also recommends that laboratories can also use a NAAT to detect *C. difficile* toxin genes as a stand-alone diagnostic test.[6] Therefore, an NAAT was performed as the reference method for the comparison of assays in this study. NAATs for CDI diagnosis have improved through the years, and several commercial NAATs are approved by the US Food and Drug Administration.[3] These tests usually detect toxin genes (*tcdB* and/or *tcdA*); therefore, positive NAAT results indicate toxigenic *C. difficile*.

However, costs of most NAATs are more than those of other assays for CDI such as Toxin EIAs and chromogenic media culture assay. Xpert PCR is particularly expensive; the price per test is ~10-fold more than that of toxin EIAs in our region. In addition, an initial capital expenditure is necessary for the testing instrument and analysis software. Although many NAATs for a broad spectrum of human diseases are available in this era of molecular genetic tests, many clinical laboratories, especially those in small hospitals, still do not routinely perform NAATs. Therefore, adopting NAATs for CDI such as Xpert PCR as a stand-alone test in all laboratories is not feasible. The laboratories that perform NAATs also suffer from the cost issue.

Multistep (2 or 3) testing algorithmic approaches for the diagnosis of CDI have been developed to improve the diagnostic performance; in addition, some of these approaches save the testing costs. In this study, a two-step testing algorithmic approach, with QCC as an initial test and Xpert PCR for confirmation, detected 55.4% (46/83) of Xpert PCR-positive results and 94.6% (140/148) of Xpert PCR-negative results in the first step. QCC as the initial test can save cost and time by more than 80% by reducing the number of specimens that must be tested by Xpert PCR. If Xpert PCR can be performed on a smaller subset of QCC-Ag(+)/Tox(−) specimens (<20% in this study), costs will be significantly reduced. Toxin EIA as an initial test can also reduce the number of specimens that must be tested by Xpert PCR, but more than 80% of all the specimens needed to be confirmed by Xpert PCR. Therefore, QCC is more useful than Toxin EIA as an initial test in a multistep algorithmic approach for the diagnosis of CDI.

In conclusion, QCC is a rapid, easy, and cost-effective test method that is a useful first-line screening assay for laboratory diagnosis of CDI in tertiary hospitals with a high prevalence of CDI. A two-step algorithm with QCC as an initial screening test, followed by Xpert PCR as a confirmatory test for specimens with indeterminate results, offers a practical and cost-effective approach to rapidly and accurately diagnose CDI.

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