

Detection of *Clostridium difficile* antigen and toxin in stool specimens: Comparison of the *C. difficile* quik chek complete enzyme immunoassay and GeneXpert *C. difficile* polymerase chain reaction assay

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Abstract

Background/Aims: Accurate and rapid laboratory diagnosis of *Clostridium difficile* infections (CDI) remains a significant challenge. A two-step algorithm for detection of toxigenic *C. difficile* in stool based on initial screening for glutamate dehydrogenase assay followed by confirmation by toxin A+B detection using an enzyme immunoassay (EIA) or molecular assay has been proposed. We aimed to evaluate the *C. difficile* Quik Chek Complete[®] (QCC-EIA) versus the GeneXpert[®] *C. difficile* polymerase chain reaction (PCR) assay in this two-step algorithm.


Materials and Methods: Two hundred and ten liquid stool samples obtained between June 2014 and June 2015 from patients suspected of CDI were tested by the QCC-EIA and GeneXpert PCR assay. The GeneXpert assay was used as the reference standard to calculate the QCC-EIA sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Results: Of the 210 stool samples tested, 43 (20.5%) were positive by QCC-EIA, while 31 (14.8%) were positive by GeneXpert assay. The sensitivity and specificity of the QCC-EIA were found to be 100 and 93%, respectively; the PPV and NPV were 72 and 100%, respectively. The binary toxin was detected in 12 (38.7%) and *tcdC* gene deletion in 3 (9.6%).

Conclusions: The low specificity of QCC-EIA makes it less reliable as a confirmatory test for CDI diagnosis. This test may be used as a screening test in a two-step algorithm when combined with a molecular assay or another confirmatory test.

Keywords: *Clostridium difficile*, enzyme immunoassay, GeneXpert PCR assay

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INTRODUCTION

Clostridium difficile is a major etiological agent of healthcare associated infections worldwide. *C. difficile* infection is associated with significant patient mortality, morbidity, prolonged hospitalization, and increased healthcare cost. Rapid and accurate identification of toxigenic *C. difficile* is important for early commencement of appropriate management and implementation of infection control measures with resultant improvement in patient outcomes.^[1] Laboratory tests available for *C. difficile* diagnosis include stool culture, enzyme immunoassays (EIAs) for toxin detection, glutamate dehydrogenase (GDH) assay for antigen detection, cell culture cytotoxicity neutralization assay (CCNA), and molecular assays. However, the choice of test is influenced by cost, sensitivity and specificity, turn-around-time, and ease of performance in the diagnostic laboratory. For example, although both stool culture and CCNA have high sensitivity, they are more expensive and have longer turn-around-time. In contrast, EIA for detection of toxins A and B is a simple inexpensive test but low sensitivities have been reported depending on the type of kit, study population, and gold standard test used.^[2,3] The Society for Healthcare Epidemiology of America and Infectious Disease Society of America guidelines indicate that EIAs should no longer be considered as adequate stand-alone tests for the diagnosis of *C. difficile* infection.^[4] Tests like cell CCNAs and toxigenic culture are unattractive confirmatory tests in the diagnostic laboratory because they are complex and tedious. Molecular assays represent better alternative as confirmatory tests as they are easier to perform and have higher specificity and sensitivity.^[5,6]

Recently, a two-step testing algorithm for detecting toxigenic *C. difficile* directly from the stool of symptomatic patients was proposed.^[7] This algorithm is based on a GDH EIA for initial screening, followed by toxin A+B detection using EIA or molecular assay for confirmation. The TechLab *C. difficile* Quik Chek Complete® (QCC-EIA) (Techlab, Blacksburg, VA, USA) is a rapid EIA for the simultaneous detection of *C. difficile* GDH antigen and toxins A/B, while the GeneXpert *C. difficile* polymerase chain reaction (PCR) assay (Cepheid, CA, USA) is a real-time PCR test that detects the toxin B gene (*tdcB*), the binary toxin gene (*cdt*), and the *tdcC* gene deletion at nt 117 in *C. difficile*.^[6] In this study, we have carried out an evaluation of the utilization of the QCC-EIA versus the GeneXpert *C. difficile* PCR assay in this two-step algorithm.

MATERIALS AND METHODS

Stool samples

A total of 210 stool samples obtained from patients presenting with diarrhea and clinically suspected of *C. difficile* infection were tested. These were samples submitted to the microbiology laboratory at King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia from June 2014 to June 2015 as part of routine clinical investigations. Presence of diarrhea was an entry criterion. All stool samples were liquid stool and no formed stool was accepted for the study. The detection of *C. difficile* toxin was carried out using QCC-EIA and GeneXpert *C. difficile* PCR assay. Samples were stored and refrigerated at 2–8°C.

Quik chek complete-enzyme immunoassay

The detection of GDH antigen and/or toxins by QCC-EIA was carried out in accordance with the manufacturers' instructions, including the use of appropriate controls, as specified in the package insert. Briefly, ~25 ml of stool sample was added to a tube containing the diluent and conjugate and the mixture was transferred to the device sample well. After incubation for 15 min at room temperature, the wash buffer followed by the substrate were added to the reaction window. The results were read after 10 min. The GDH antigen and/or toxins were reported as positive if a clear visible band was seen on the antigen and/or toxin side of the device display window, respectively, as per manufacturer guidelines.

GeneXpert C. difficile PCR assay: A sterile cotton tipped swab was dipped into the watery stool sample and placed in sample reagent and vortexed at high speed for 10 seconds at room temperature. All liquid from the sample reagent was transferred into the "S" chamber of GeneXpert *C. difficile* cartridge as stipulated in the package insert. Reagent one was added to chamber one and reagent two to chamber two of the test cartridge. The cartridge barcode was scanned and the cartridge was placed in the GeneXpert instrument for the PCR run. The resulting data were interpreted as positive, negative, or invalid as per manufacturer recommendations.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Inc., Chicago, Illinois, USA). GeneXpert *C. difficile* PCR assay was used as the reference standard to calculate the assay sensitivity, specificity, PPV and NPV.

RESULTS

Majority of the stool samples ($n = 110$; 52.4%) were from males. The mean age (\pm SD) of the patient population

was 42 (± 24.3) years. Most of the specimens were from inpatients ($n = 171$; 81.4%) mainly from the medical ward ($n = 61$). Of the 210 stool samples tested, 43 (20.5%) were positive by QCC-EIA, while 31 (14.8%) were positive by GeneXpert *C. difficile* PCR assay. Among those positive by GeneXpert *C. difficile* PCR assay, 100% ($n = 31$) were positive for toxin B, 38.7% ($n = 12$) for binary toxin, and 9.6% ($n = 3$) for tcdC gene deletion. All 31 samples positive by GeneXpert *C. difficile* PCR assay were also positive by QCC-EIA and were considered as true positives [Table 1]. There were 12 false positive findings but no false negatives [Table 1]. Based on these findings, the sensitivity of QCC-EIA was 100%, whereas the specificity was 93% [Table 2]. In addition, the PPV was 72%, while the NPV was 100%. Table 2 shows the calculation of the parameters.

DISCUSSION

We have evaluated the performance of QCC-EIA test versus GeneXpert *C. difficile* PCR assay for the detection of *C. difficile* toxin in the stool of patients suspected of CDI. Our findings indicate that although the QCC-EIA has high sensitivity for the *C. difficile* toxin detection in stool samples, it lacks specificity. In contrast to our findings, previous reports suggest that *C. difficile* toxin EIA lacks sensitivity but shows better specificity compared to molecular test and the cell culture cytotoxin neutralization assay for detection of *C. difficile* toxin.^[8-10] However, emerging data now indicate that sensitivity and specificity of the EIA assay might be related to the *C. difficile* ribotype present in the stool sample being tested.^[11] We speculate that this might explain the differences in the sensitivity and specificity of the QCC-EIA assay observed in our study

Table 1: Comparison of QCC-EIA with GeneXpert *C. difficile* PCR assay

	GeneXpert <i>C. difficile</i> PCR assay		Total
	Positive	Negative	
QCC-EIA			
Positive	31 (TP)	12 (FP)	43
Negative	0 (FN)	167 (TN)	167
Total	31	179	210

TP: True positive; FP: False positive; FN: False negative; TN: True negative

Table 2: Sensitivity, specificity, and positive and negative predictive value of QCC-EIA

Parameter	Equation	
Sensitivity	$TP/TP+FN$ (31/31+0)	100%
Specificity	$TN/TN+FP$ (167/167+12)	93%
Positive predictive value (PPV)	$TP/TP+FP$ (31/31+12)	72%
Negative predictive value (NPV)	$TN/TN+FN$ (167/167+0)	100%

TP: True positive; FP: False positive; FN: False negative; TN: True negative

compared to other reports. The virulent *C. difficile* strain BI/NAP-1/027 carries a mutation in the *tcdC* genetic locus resulting in a truncated protein *tcdC*, which leads to hyper-production of toxins A and B.^[12,13] In our study, the *tcdC* gene deletion was identified in three stool samples and to our knowledge, this is the first positive report of *tcdC* deletion and binary toxin in stool samples collected from Saudi patients. This finding is suggestive of the presence of *C. difficile* strain BI/NAP-1/027 in our setting. This is of concern as *C. difficile* BI/NAP1/027 is associated with high-level fluoroquinolone resistance and outbreaks. These findings indicate the need for urgent work to determine the prevalent ribotypes associated with *C. difficile* infection in Saudi Arabia. Available report on *C. difficile* ribotypes in Saudi Arabia revealed that 027, BT1, V, and A2 have been identified in retail food.^[14]

Another finding in our study is the impact of test performance on the predictive value of the QCC-EIA. Although the QCC-EIA has a high NPV that correlates with absence of disease in patients suspected of CDI, the PPV is only 72%. This data implies that a *C. difficile* QCC-EIA positive result would require another confirmatory test to obtain a definitive diagnosis of CDI. Some laboratories have utilized the *C. difficile* toxin EIA in a two-step procedure for diagnosis of *C. difficile* infection.^[5,10] This algorithm involves *C. difficile* EIA as a screening test and a PCR assay as confirmatory test. While the two-step algorithm may be cost saving, it is more laborious and prolongs the turn-around-time for reporting final results when compared to a stand-alone PCR for *C. difficile* toxin.^[10] In view of superior performance characteristic and a shorter turn-around-time, many clinical laboratories have now implemented the molecular assay. The American Society for Microbiology guidance document for laboratory detection of *C. difficile* toxin recommends the use of molecular assay as a stand-alone test, while the *C. difficile* toxin EIA results should be confirmed with either a toxigenic culture or a molecular assay.^[15]

CONCLUSION

In conclusion, the QCC-EIA is useful as a screening test for the detection of *C. difficile* toxin A + B in stool samples. Due to its low specificity, a second confirmatory test is required, which may include a molecular assay. The GeneXpert *C. difficile* PCR assay demonstrates a superior accuracy of toxin B detection, has a shorter turn-around-time with simplicity of sample preparation, and it can detect gene targets associated with hyper-virulent strains of *C. difficile*. Utilization of the GeneXpert *C. difficile* PCR in the laboratory diagnosis of CDI will be invaluable in

promoting early initiation of management interventions, enhancing patient outcomes and reducing the incidence of healthcare associated infections.

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Conflicts of interest

There are no conflicts of interest.

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