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Evaluation of the Qiagen *artus* C. difficile QS-RGQ Kit for Detection of *Clostridium difficile* Toxins A and B in Clinical Stool Specimens

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We compared the Qiagen *artus* C. difficile QS-RGQ kit, a new nucleic acid amplification test for the detection of *Clostridium difficile* toxins in stool specimens, with the Cepheid Xpert *C. difficile* test. The sensitivity, specificity, positive predictive value, and negative predictive value for the Qiagen *artus* C. difficile QS-RGQ test were 100%, 89.5%, 60.9%, and 100%, and those for the Cepheid Xpert *C. difficile* test were 100%, 90%, 62.2%, and 100%, respectively.

Diagnosis of *Clostridium difficile* infection (CDI) is based on clinical presentation and laboratory tests. In recent years, nucleic acid amplification tests (NAATs) for the direct detection of *C. difficile* toxin genes in stool samples have become commercially available and are a highly sensitive alternative to the less sensitive enzyme immunoassays (EIAs), the time-consuming toxigenic culture (TC), and the cell culture cytotoxicity neutralization assay (CCNA). Qiagen has released a CE-IVD-marked (European certification for *in vitro* diagnostic devices) and FDA-cleared assay for the extraction and simultaneous detection of *C. difficile* toxin A and toxin B genes in stool specimens using the QIAsymphony RGQ platform. In this study, we compared the Qiagen *artus* C. difficile QS-RGQ kit (Qiagen *artus* test) with the Cepheid Gene Xpert *C. difficile* test (Cepheid Xpert test) and toxigenic culture. To our knowledge, this is the first evaluation of the Qiagen *artus* C. difficile QS-RGQ kit.

(These data were presented in part as a poster at the 54th Interscience Conference on Antimicrobial Agents and Chemotherapy, 5 to 9 September 2014, Washington, DC.)

From January 2014 to May 2014, 201 loose stool specimens submitted for *C. difficile* testing from inpatients at the University Hospital Cologne, a 1,400-bed tertiary-care facility, were included in the study. All stool samples were analyzed upon delivery or after overnight storage at −20°C. Specimens were analyzed by the Qiagen *artus* C. difficile QS-RGQ kit and the Cepheid Gene Xpert *C. difficile* test strictly according to the manufacturers' instructions. As a reference method, every stool sample was processed for enriched toxigenic culture (TC). The stool sample was plated on cycloserine-cefoxitin-fructose agar (CCFA) after alcohol shock. Suspicious colonies were confirmed by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry. Toxin detection was performed using the toxin A/B enzyme immunoassay (EIA) (Ridascreen *Clostridium difficile* toxin A/B assay; r-biopharm). Isolates negative for toxin A/B by EIA were further tested by PCR (in-house PCR adapted from that described by Houser et al. [1], the Cepheid Xpert test, and the Qiagen *artus* test). Samples negative for TC and positive for only one NAAT were defined as discrepant samples. Stored DNA from discrepant samples was retested with the Qiagen *artus* test. Retesting with the Cepheid Xpert test was not possible, as the original stool samples were not stored. In addition, the charts of patients with discrepant results were reviewed to clarify the diagnosis of CDI. We determined diarrhea (defined as three or more loose stools in 24 h) and the clinical *C. difficile* score by using a clinical prediction scale for hospital-onset CDI (2).

From the 201 stool samples, two samples were reported as invalid by the Cepheid Xpert test (1%). Both were negative by TC and the Qiagen *artus* test. We excluded these samples from performance calculation. No sample was reported as invalid by the Qiagen *artus* test. Of the remaining 199 samples, 28 (14%) were positive by TC and both of the NAATs. In addition, 21 samples were positive by at least one NAAT but remained negative by TC. In 11 of these samples, no growth was detected on CCFA. For 10 samples, suspicious colonies on CCFA were identified by MALDI-TOF as species other than *C. difficile* and toxin A/B EIA results were negative. For one sample, suspicious colonies were identified as *C. difficile* but the toxin A/B EIA and toxin PCR results for this isolate remained negative. Of the 21 TC-negative samples, 14 were positive by both PCR assays and 7 were positive by one PCR assay only (Qiagen *artus* test, $n = 4$; Cepheid Xpert test, $n = 3$). No culture-positive, PCR-negative sample was observed. The sensitivities, specificities, positive predictive values, and negative predictive values were, respectively, 100%, 89.5%, 60.9%, and 100% for the Qiagen *artus* test and 100%, 90%, 62.2%, and 100% for the Cepheid Xpert test, compared to TC (Table 1). The overall agreement between the two NAATs was excellent (kappa, 0.9). Of the 46 stool samples that were positive by the Qiagen *artus* test, 40 were reported *tcdA* positive and *tcdB* positive, five were reported *tcdA* positive and *tcdB* negative, and one sample was reported *tcdA* negative and *tcdB* positive.

Stored DNA eluates from the seven discrepant samples were retested by the Qiagen *artus* test. Cycle threshold (C_T) values of the internal controls were not different between the first and second runs (30.52 ± 1.7 versus 29.48 ± 0.33). Results of the retest and of the retrospective analysis of patient charts are shown in Table 2.

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TABLE 1 Comparison of the Qiagen *artus* C. difficile QS-RGQ kit and the Cepheid Xpert *C. difficile* test to toxigenic culture

Assay and result	No. of samples with indicated result by toxigenic culture		Performance[a] compared with toxigenic culture			
	Positive	Negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Qiagen *artus* C. difficile QS-RGQ kit			100	89.5	60.9	100
Positive	28	18				
Negative	0	153				
Total ($n = 199$)[b]	28	171				
Cepheid Xpert *C. difficile* test			100	90	62.2	100
Positive	28	17				
Negative	0	154				
Total ($n = 199$)[b]	28	171				

[a] PPV, positive predictive value; NPV, negative predictive value.
[b] Two samples were excluded from analysis due to invalid results by the Cepheid Gene Xpert *C. difficile* test.

This is the first study evaluating the performance characteristics of the Qiagen *artus* C. difficile QS-RGQ kit in a routine clinical setting. As described previously (1, 3–5), we found that PCR was more sensitive than TC. This has also been observed when CCNA was used as a reference method (6). Nevertheless, the use of a less sensitive EIA for toxin detection in TC can lead to falsely low sensitivity of TC (7, 8). In our study, we identified only one sample that was positive for *C. difficile* by culture but negative by toxin EIA. This isolate was also negative for toxin A/B by PCR. Therefore, we consider the 14 samples with positive results by the two PCR assays but negative results by TC in our study to be truly positive for toxigenic *C. difficile*. As all NAATs may detect colonization instead of infection, an accurate clinical diagnostic workup is mandatory to interpret positive results. In our study, seven samples were positive by one NAAT only. Five of the seven patients were clinically diagnosed as having CDI. Retesting of the stored DNA eluates of these samples by the Qiagen *artus* test resulted in new discrepancies compared to the results of TC and clinical outcomes (Table 2). The reason for the discrepancies remains unresolved. Since we could show that the C_T values of the internal controls were similar between the two test runs, degradation of DNA in the sample is not likely. DNA in these stool samples is seemingly close to the limit of detection, and discrepant results have to be expected as stochastic events. Individual sample-specific factors (for example, inhibitors) may also be influencing the PCR process. Thus, we propose that retesting of the stored DNA with the Qiagen *artus* test is not useful to clarify discrepant results. The Qiagen *artus* test detects two different targets, *tcdA* (toxin A) and *tcdB* (toxin B). The contribution of toxin A and toxin B to disease is not well understood. To date only strains that are either *tcdA* and *tcdB* positive or *tcdA* negative and *tcdB* positive have been shown to be pathogenic in humans (9), although *in vitro* studies suggest a pathogenic role for *tcdA*-positive, *tcdB*-negative isolates (10). In our study, we identified five stool samples with *tcdA*-positive, *tcdB*-negative results by the Qiagen *artus* test. Four of these isolates were positive for *tcdB* by the Cepheid Xpert test, indicating that a *tcdB* gene is present but was not detected by the Qiagen *artus* test. In accordance with previously published data (1), our results suggest that the analytical sensitivity of the *tcdA* target might be higher than the analytical sensitivity of the *tcdB* target, leading to an overestimation of the number of *tcdA*-positive, *tcdB*-negative strains. In line with the manufacturer's instructions, we therefore recommend that strains be interpreted and reported as positive for toxigenic *C. difficile* if either target is positive by the Qiagen *artus* test.

TABLE 2 Characterization of specimens with discrepant results by the Qiagen *artus* C. difficile QS-RGQ kit and the Cepheid Gene Xpert *C. difficile* test

Specimen	Result[a] of:				
	Initial test			Qiagen *artus* retest	CDI score[b]
	Qiagen *artus*	Cepheid Xpert	TC		
p88	*tcdB*+, *tcdA*+	Negative[c]	Negative	Negative[c]	Positive
p101	*tcdB*+, *tcdA*+	Negative[c]	Negative	Negative[c]	Positive
p104	Negative[c]	*tcdB*+, RT027+, *cdt*+	Negative	*tcdB*+, *tcdA*−	Positive
p125	*tcdB*+, *tcdA*−	Negative[c]	Negative	*tcdB*−, *tcdA*+	Negative
p132	*tcdB*−, *tcdA*+	Negative[c]	Negative	Negative[c]	Negative
p176	Negative[c]	*tcdB*+, RT027−, *cdt*−	Negative	Negative[c]	Positive
p188	Negative[c]	*tcdB*+, RT027−, *cdt*−	Negative	Negative[c]	Positive

[a] Qiagen *artus*, Qiagen *artus* C. difficile QS-RGQ assay; Cepheid Xpert, Cepheid Gene Xpert *C. difficile* test; TC, toxigenic culture. *tcdB*+ or *tcdB*−, gene target for toxin B detected or not detected, respectively; *tcdA*+ or *tcdA*−, gene target for toxin A detected or not detected; RT027+ or RT027−, *tcdC* gene deletion at nucleotide 117 indicating ribotype 027 detected or not detected; *cdt*+ or *cdt*−, binary toxin gene detected or not detected.
[b] The clinical plausibility of *C. difficile* infection (CDI) was determined using a clinical prediction scale for hospital-onset CDI (2).
[c] Negative for all genes tested in the indicated assay.

Our data show that under routine conditions, the Qiagen *artus* C. difficile QS-RGQ kit performs equally as well as the Cepheid Xpert *C. difficile* test. In our study, both of these NAATs showed identical sensitivities and specificities. Optimization of workflow depends on laboratory environment, clinical demands, and platform availability.

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