




# Evaluation of the Liat Cdiff Assay for Direct Detection of *Clostridioides difficile* Toxin Genes within 20 Minutes

David J. Hetem,<sup>a</sup> Ingrid Bos-Sanders,<sup>b</sup> Roel H. T. Nijhuis,<sup>a,b\*</sup> Sven Tamminga,<sup>a</sup> Livia Berlinger,<sup>c</sup> Ed J. Kuijper,<sup>b</sup> Joanna J. Sickler,<sup>d</sup>  Eric C. J. Claas<sup>b</sup>

<sup>a</sup>Haaglanden Medical Center, Department of Microbiology, The Hague, The Netherlands

<sup>b</sup>Leiden University Medical Center, Department of Medical Microbiology, Leiden, The Netherlands

<sup>c</sup>BioAnalytica AG, Department of Microbiology, Luzern, Switzerland

<sup>d</sup>Roche Molecular Systems, Pleasanton, California, USA

**ABSTRACT** *Clostridioides difficile* is the main causative agent of antibiotic-associated diarrhea. Prompt diagnosis is required for initiation of timely infection control measures and appropriate adjustment of antibiotic treatment. The cobas Cdiff assay for use on the cobas Liat system enables a diagnostic result in 20 minutes. A total of 252 prospective ( $n = 150$ ) and retrospective ( $n = 102$ ) stool specimens from The Netherlands, France, and Switzerland were tested on the cobas Cdiff assay using the Xpert *C. difficile* assay as a reference method. The overall positive and negative percent agreement (PPA and NPA, respectively) of the cobas Cdiff assay compared with the Xpert *C. difficile* assay was 98.0% (100/102; 95% confidence interval [CI], 93.1% to 99.5%) and 94.0% (141/150; 95% CI, 89.0% to 96.8%), respectively. When comparing the PPAs of cobas Cdiff and Xpert *C. difficile* with culture, the results were 91.7% (55/60; 95% CI, 81.9% to 96.4%) and 85.0% (51/60; 95% CI, 73.9% to 91.9%), respectively. The difference was not statistically significant. The cobas Cdiff assay offers a very rapid alternative for diagnosing *C. difficile* infection. The 20-minute turnaround time provides the potential for point-of-care testing so that adequate infection control measures can be initiated promptly.

**KEYWORDS** *Clostridioides difficile*, Liat, PCR, GeneXpert

*Clostridioides difficile* is an anaerobic Gram-positive spore-forming bacillus and a leading cause in nosocomial infectious diarrhea (1, 2). Symptoms of *C. difficile* infections (CDIs) can range from mild self-limiting diarrhea to life-threatening pseudomembranous colitis, toxic megacolon, and death. CDI is associated with an increased length of hospital stay and increased health care expenses (3). Colonization with *C. difficile*, without clinical symptoms, is suggested as a potential source of transmission, although screening for asymptomatic carriage and contact precautions are not yet recommended (4). *C. difficile* carriage in healthy adults without clinical symptoms ranges from 4% to 15%, being higher in neonates (4% to 71%) and hospitalized patients (3% to 21%) (5).

Rapid diagnosis of CDI is important to initiate the proper infection control measures and timely start of an appropriate antibiotic treatment. Current guidelines recommend multistep algorithms for the diagnosis of CDI (4, 6), with primary screening for CDI using a test with a high negative predictive value, such as nucleic acid amplification tests (NAAT) or glutamate dehydrogenase (GDH) enzyme immune assays (EIAs). If positive, the actual presence of free fecal toxins should be determined using a test with a high positive predictive value (e.g., toxin A/B EIA). The presence or absence of free fecal toxins is considered an important tool to differentiate colonization from infection. NAAT-detecting toxin-producing genes are becoming more popular as a first step screening, given their ease of use and high sensitivity (7, 8).

**Citation** Hetem DJ, Bos-Sanders I, Nijhuis RHT, Tamminga S, Berlinger L, Kuijper EJ, Sickler JJ, Claas ECJ. 2019. Evaluation of the Liat Cdiff assay for direct detection of *Clostridioides difficile* toxin genes within 20 minutes. *J Clin Microbiol* 57:e00416-19. <https://doi.org/10.1128/JCM.00416-19>.

**Editor** Andrew B. Onderdonk, Brigham and Women's Hospital

**Copyright** © 2019 Hetem et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Eric C. J. Claas, [e.claas@lumc.nl](mailto:e.claas@lumc.nl).

\* Present address: Roel H. T. Nijhuis, Meander Medical Centre, Department of Medical Microbiology, Amersfoort, The Netherlands.

**Received** 20 March 2019

**Accepted** 22 March 2019

**Accepted manuscript posted online** 3 April 2019

**Published** 24 May 2019

In some countries, testing for *C. difficile* is performed in centralized laboratories using batch processing of specimens, lengthening the time to result. Decentralized testing and point-of-care solutions can potentially reduce the time to result for *C. difficile* testing. Previous studies have shown the benefit of faster diagnostic results for patient management (9, 10). The cobas Cdiff test for use on the cobas Liat system manufactured by Roche Diagnostics (Pleasanton, CA, USA) enables point-of-care testing and a diagnostic result in 20 minutes. The Liat is a compact PCR system designed for on-demand testing in point-of-care settings, such as physician clinics, pharmacies, hospital laboratories, and satellite laboratories (11).

In this study, we evaluate the performance characteristics of the Liat system by comparing it with the Cepheid Xpert *C. difficile* (Xpert) toxin genes diagnostic assay manufactured by Cepheid (Sunnyvale, CA, USA).

## MATERIALS AND METHODS

A prospective study was conducted using predominantly unformed stool specimens that were submitted to the clinical microbiology laboratory of the Leiden University Medical Center (LUMC) for diagnosis of *C. difficile* infection. Following the routine diagnostic workflow, a laboratory-developed, internally controlled, real-time PCR assay (LDT) (7) was used to screen for the *C. difficile* toxin B gene. From January to September 2017, 75 *C. difficile*-positive specimens were included in the study. For each positive sample, a negative fecal specimen was included. The study received a waiver from the institutional review board because all samples were deidentified without using patient characteristics based on an established procedure at LUMC.

PCR-positive samples were also cultured, and all PCR-positive specimens were subjected to the Vidas *C. difficile* toxin A&B test (bioMérieux, Marcy l'Etoile, France). The presence of free feces toxin by Vidas antigen detection was considered indicative for CDI. Of each included fecal sample, three aliquots were stored at  $-20^{\circ}\text{C}$  until further testing using the Liat and Xpert assays. Culture-positive specimens were also subjected to PCR ribotyping, as described previously (12).

In addition, a selection of 25 positive and 25 negative neat fecal specimens were obtained from Laboratoire Cerba in Saint-Ouen-l'Aumône, France (obtained by Roche) and from BioAnalytica in Luzern, Switzerland. The latter collection consisted of fecal specimens in Cary-Blair medium, enabling analysis of the performance of the rapid testing devices on this matrix as well. No ribotyping or culture was performed on these samples collected retrospectively.

**Enriched culture.** A 0.5-g portion of feces was suspended in 0.5 ml 96% ethanol, incubated for an hour, and, subsequently, 1 drop was cultured anaerobically on plates with selective medium containing cefoxitin, amphotericin B, and cycloserine (CLO) and Columbia agar with colistin and nalidixic acid (CNA). For enriched culture, a preincubation cycloserine-cefoxitin-mannitol broth with taurocholate, lysozyme, and cysteine (CCMB-TAL) was performed.

**tcdB PCR (LDT).** Half a pea or 200  $\mu\text{l}$  of fecal material was resuspended in 1 ml of stool transport and recovery (STAR) buffer (Roche Diagnostics, Pleasanton, CA, USA) with Precellys beads (Bertin, Montigny-le-Bretonneux, France). After mixing for 5 min at 2,200 rpm, the sample was left for 5 minutes and subsequently centrifuged for 1 minute at 14,000 rpm. Then, 200  $\mu\text{l}$  of the supernatant was subjected to nucleic acid extraction using the Viral NA small volume kit on a MagNAPure 96 system (Roche Diagnostics) in the presence of a phocine herpesvirus (PhHV) internal control (IC), yielding a final eluate of 100  $\mu\text{l}$ . Finally, the *tcdB* gene was amplified from 10  $\mu\text{l}$  of eluate, as described previously (7).

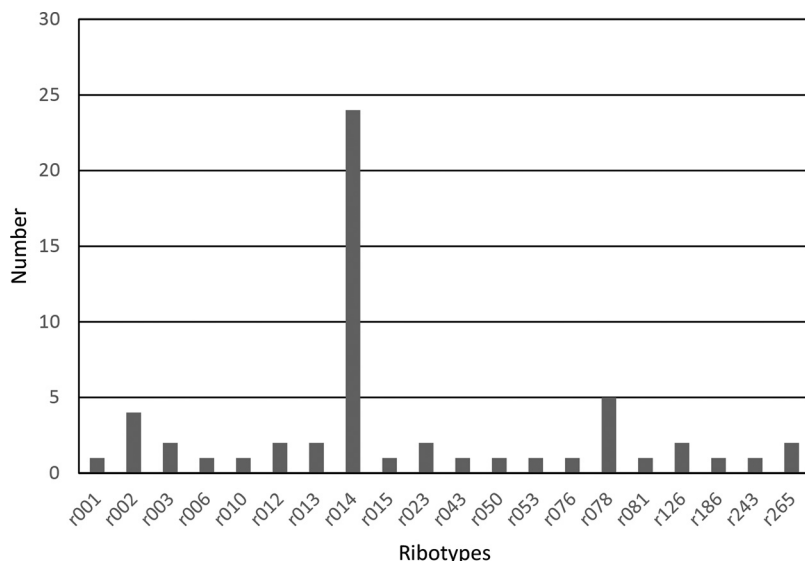
**Cobas Cdiff test.** The procedure was performed according to the manufacturer's instructions. A tip of a polyester swab was immersed in the stool specimen and then transferred to the cobas PCR medium tube. After breaking the swab, the medium was vortexed for 5 seconds, after which 200  $\mu\text{l}$  of the sample was pipetted into the Liat assay tube. Subsequently, the test was run as instructed.

**Cepheid Xpert *C. difficile*.** The procedure was carried out according to the manufacturer's instructions. The swab was immersed in feces and inserted into the sample reagent tube provided. After breaking the swab and closing the lid, the sample was vortexed for 10 seconds. Next, the complete sample was pipetted into the sample chamber of the Xpert cartridge. After transferring the cartridge to the GeneXpert instrument, the run was started. All positive samples with quantitation cycle ( $C_q$ ) of  $>37$  were called negative by the GeneXpert software.

## RESULTS

Altogether, the total sample size was 252 samples, namely, 150 for the prospective part (75 positive, 75 negative) and 102 (50 positive, 52 negative) for the retrospective part of the study.

**Prospective study.** Based on the *tcdB* screening by LDT, 75 positive samples were selected to be included in the study, with  $C_q$  values ranging from 19.2 to 39.5. Of these 75 positive samples, 19 were positive for the presence of free toxins and diagnosed as CDI and 14 were indeterminate. Culture was performed on all positive specimens irrespective of  $C_q$  value, resulting in 60 culture-positive *C. difficile* isolates. PCR ribotype



**FIG 1** PCR ribotypes obtained from the prospective study.

014 was the most prevalent ( $n = 24$ ), followed by PCR ribotype 078 ( $n = 5$ ) and PCR ribotype 002 ( $n = 4$ ). Altogether, 20 different PCR ribotypes, but no PCR ribotype 027, were detected in this study group (Fig. 1).

Subjecting all 150 samples to both the Liat and the Xpert procedures showed good overall concordance between the assays at 95.3% (143/150; 95% CI, 90.7% to 97.7%) (Tables 1 and 2).

Culture was performed on 137 of the prospective samples to support discrepancy analysis between the two assays. Compared to culture, the PPAs of Liat and Xpert were 91.7% (55/60; 95% CI, 81.9% to 96.4%) and 85.0% (51/60; 95% CI, 73.9% to 91.9%), respectively, with negative predictive values of 93.6% (73/78; 95% CI, 85.9% to 97.2%) and 89.2% (74/83; 95% CI, 80.7% to 94.2%). The difference was not statistically significant, with a  $P$  value of 0.39 from 2-sample chi-square test for testing equal PPAs with continuity correction. The NPAs were 94.8% (73/77; 95% CI, 87.4% to 98.0%) and 96.1% (74/77; 95% CI, 89.2% to 98.7%) for Liat and Xpert, respectively, when compared with those of culture.

For the seven prospective discordant samples, five culture results matched the Liat test (Table 3). Four of the six Liat positive/Xpert negative samples recorded Xpert

**TABLE 1** Positive and negative test results for clinical samples by cobas Liat Cdiff versus Xpert PCR and culture

Test result by specimen type	Liat	
	+	-
<b>Xpert</b>		
Prospective		
+	53	1
-	6	90
Retrospective		
+	47	1
-	3	51
All samples		
+	100	2
-	9	141
<b>Culture</b>		
Prospective		
+	55	5
-	4	73

**TABLE 2** Comparison of the cobas Liat Cdiff and Xpert PCR tests<sup>a</sup>

Comparison measure	Values by specimen type					
	Prospective		Retrospective		All	
	Estimate (% [n])	95% CI (%)	Estimate (% [n])	95% CI (%)	Estimate (% [n])	95% CI (%)
PPA	98.1 (53/54)	90.2–99.7	97.9 (47/48)	89.1–99.6	98.0 (100/102)	93.1–99.5
NPA	93.8 (90/96)	87.0–97.1	94.4 (51/54)	84.9–98.1	94.0 (141/150)	89.0–96.8
PPV	89.8 (53/59)	79.5–95.3	94.0 (47/50)	83.8–97.9	91.7 (100/109)	85.1–95.6
NPV	98.9 (90/91)	94.0–99.8	98.1 (51/52)	89.9–99.7	98.6 (141/143)	95.0–99.6
OPA	95.3 (143/150)	90.7–97.7	96.1 (98/102)	90.4–98.5	95.6 (241/252)	92.4–97.6

<sup>a</sup>CI, confidence interval; NPA, negative percent agreement; NPV, negative predictive value; OPA, overall percent agreement; PPA, positive percent agreement; PPV, positive predictive value.

threshold cycle (Ct) values above the 37 cutoff, ranging from 37.2 to 38.8. Vidas results were available for six of the seven discordant samples. Six were negative and one was indeterminate.

**Retrospective study.** The 102 retrospective samples also demonstrated good overall concordance between the Liat and the Xpert assays at 96.1% (98/102; 95% CI, 90.4% to 98.5%) (Tables 1 and 2).

The overall PPA and NPA between the two assays, including both prospective and retrospective samples, were 98.0% (100/102; 95% CI, 93.1% to 99.5%) and 94.0% (141/150; 95% CI, 89.0% to 96.8%), with a negative predictive value of 98.6% (141/143; 95% CI, 95.0% to 99.6%) (Table 1).

## DISCUSSION

This is the third study comparing the performance of the Liat test to routine laboratory NAAT for the detection of *C. difficile*. It is the first study in Europe and the first to compare Liat with the Xpert *C. difficile* assay. The inclusion of samples from the French and Swiss populations increases the geographic diversity of the sample set to make the findings relevant beyond The Netherlands. The test had strong agreement with Xpert for prospective and retrospective samples, with an overall PPA of 98.0% and NPA of 94.0% for all samples. These findings are in line with the results from other published studies. A prospective comparison between the Liat and Xpert *C. difficile*/Epi test on fresh samples in the US population found 94.5% and 98.0% positive and negative percent agreement (13). A second prospective study in the U.S. population using the cobas 4800 Cdiff as the reference yielded 95.4% and 99.4% PPA and NPA, respectively (11).

Culture results for the discrepant analysis found that most agreed with the Liat result (five of seven) for prospective samples. However, the clinical relevance of samples near the limit of detection and/or cutoff for PCR assays remains unclear. None of these samples were toxin A/B positive in the Vidas test, possibly indicating colonization with *C. difficile* rather than infection.

PCR ribotyping enables differentiation of *C. difficile* strains by amplification of the 16S-23S intergenic spacer regions. This is not only useful for epidemiology but also for identification of hypervirulent 027 and 078 ribotypes. Obviously, all ribotypes need to

**TABLE 3** Cobas Liat Cdiff and Xpert discordant prospective samples

Category	Population	Sample number	Liat		Xpert		Direct culture result
			Result	C <sub>T</sub> <sup>a</sup>	Result	C <sub>T</sub>	
False positive	Prospective	418-135	+	30.42	–		–
True positives		418-156	+	33.07		37.9	+
		418-173	+	33.21	–	37.2	+
		418-185	+	20.38	–		+
		418-201	+	30.55	–	38.8	+
		418-237	+	30.37	–	38	+
False negative	Prospective	418-167	–		+	37.6	+

<sup>a</sup>C<sub>T</sub>, cycle threshold.

be detected by diagnostic methods and all ribotypes encountered in this study, including some new ribotypes, could be detected by Liat.

The LDT used routinely at LUMC was the screen to select positive and negative samples for the study. This test had a significantly higher positivity rate than either Liat (59/75) or Xpert (54/75). The LDT used for screening had no cutoff and as a result continued to amplify the bacterial DNA to make the detection of very low levels of *C. difficile* possible, likely explaining the high number of positives compared with Liat and Xpert as well as culture. Both Liat and Xpert have a cutoff built into the algorithm. Setting this cutoff is done to balance sensitivity, specificity, and turnaround time (TAT) of the assay. The additional LDT-positive samples had  $C_q$  values ranging from 32 to 39, and all were negative in the toxin assay.

The study has several limitations. First, the samples were aliquoted and different aliquots were used for each test. Variability due to uneven distribution of the pathogen within the samples may have impacted results. In addition, all samples went through a freeze/thaw process before PCR testing and so were not fresh, as indicated by the instructions for use for both assays. The samples in the prospective portion were not sequential and were included in the study based on a screening that created a 50% prevalence rate not reflective of the general population. Lastly, samples were deidentified and so clinical information on the patients was not available for the analysis. This made it impossible to look at the diagnostic implications for PCR-positive and toxin A/B-negative samples.

A strength of the study was utilizing samples from multiple geographic areas in Europe to demonstrate a high level of concordance. Additionally, different fecal matrices were used in the study, indicating the wide application of the assay. In addition, the availability of a toxin A/B and culture result for the prospective studies enables assessment of how the assay could impact clinical decision making. It also highlights the ongoing diagnostic challenges of distinguishing between acute CDI and *C. difficile* colonization.

The key finding is that the fast TAT of 20 minutes demonstrated by the Liat assay does not decrease the sensitivity of the test. The typical paradigm is to assume that increased speed and ease of use can require a performance compromise for the benefit of these features. The results of this study indicate the Liat assay still delivers the performance of routine laboratory NAAT with the potential to increase the speed and access to test results. This feature makes it a valuable assay for health systems to consider utilizing for *C. difficile* testing.

## ACKNOWLEDGMENTS

We thank Shaowu Tang, Sheena Chaudhuri, and Mark Van Vessel from Roche Molecular Diagnostics for their support with statistical analysis, study logistics, and training; and Katherine Gatland, Keely Jennings, and Papia Das for formatting, proof reading and editing.

This study was funded by Roche Molecular Diagnostics, Pleasanton, CA, but they were in no way involved in generating the results.

Joanna J. Sickler is an employee of Roche Molecular Diagnostics.

## REFERENCES

1. Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, Farley MM, Holzbauer SM, Meek JI, Phipps EC, Wilson LE, Winston LG, Cohen JA, Limbago BM, Fridkin SK, Gerding DN, McDonald LC. 2015. Burden of *Clostridium difficile* infection in the United States. *N Engl J Med* 372: 825–834. <https://doi.org/10.1056/NEJMoa1408913>.
2. Smits WK, Lyras D, Lacy DB, Wilcox MH, Kuijper EJ. 2016. *Clostridium difficile* infection. *Nat Rev Dis Primers* 2:16020. <https://doi.org/10.1038/nrdp.2016.20>.
3. Zimlichman E, Henderson D, Tamir O, Franz C, Song P, Yamin CK, Keohane C, Denham CR, Bates DW. 2013. Health care-associated infections: a meta-analysis of costs and financial impact on the US health care system. *JAMA Intern Med* 173:2039–2046. <https://doi.org/10.1001/jamainternmed.2013.9763>.
4. Crobach MJT, Baktash A, Duszenko N, Kuijper EJ. 2018. Diagnostic guidance for *C. difficile* infections. *Adv Exp Med Biol* 1050:27–44. [https://doi.org/10.1007/978-3-319-72799-8\\_3](https://doi.org/10.1007/978-3-319-72799-8_3).
5. Crobach MJT, Vernon JJ, Loo VG, Kong LY, Pechine S, Wilcox MH, Kuijper EJ. 2018. Understanding *Clostridium difficile* colonization. *Clin Microbiol Rev* 31:e00021-17. <https://doi.org/10.1128/CMR.00021-17>.
6. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, Dubberke ER, Garey KW, Gould CV, Kelly C, Loo V, Shaklee Sammons J, Sandora TJ, Wilcox MH. 2018. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clin Infect Dis* 66:e1–e48. <https://doi.org/10.1093/cid/cix1085>.
7. van den Berg RJ, Kuijper EJ, van Coppenraet LE, Claas EC. 2006. Rapid diagnosis of toxinogenic *Clostridium difficile* in faecal samples with

- internally controlled real-time PCR. *Clin Microbiol Infect* 12:184–186. <https://doi.org/10.1111/j.1469-0691.2005.01301.x>.
8. Crobach MJT, Duszenko N, Terveer EM, Verduin CM, Kuijper EJ. 2018. Nucleic acid amplification test quantitation as predictor of toxin presence in *Clostridium difficile* infection. *J Clin Microbiol* 56:e01316-17. <https://doi.org/10.1128/JCM.01316-17>.
  9. Sewell B, Rees E, Thomas I, Ch'ng CL, Isaac M, Berry N. 2014. Cost and impact on patient length of stay of rapid molecular testing for *Clostridium difficile*. *Infect Dis Ther* 3:281–293. <https://doi.org/10.1007/s40121-014-0034-x>.
  10. Egilmez E, Walker GJ, Bakthavathsalam P, Peterson JR, Gooding JJ, Rawlinson W, Stelzer-Braid S. 2018. Systematic review of the impact of point-of-care testing for influenza on the outcomes of patients with acute respiratory tract infection. *Rev Med Virol* 28:e1995. <https://doi.org/10.1002/rmv.1995>.
  11. Garg SK, Lu K, Duncan J, Peterson LR, Liesenfeld O. 2017. Equivalent performance of the Cobas® Cdiff test for use on the Cobas® Liat® system and the Cobas® 4800 system. *Eur J Microbiol Immunol (Bp)* 7:310–318. <https://doi.org/10.1556/1886.2017.00034>.
  12. van den Berg RJ, Claas EC, Oyib DH, Klaassen CH, Dijkshoorn L, Brazier JS, Kuijper EJ. 2004. Characterization of toxin A-negative, toxin B-positive *Clostridium difficile* isolates from outbreaks in different countries by amplified fragment length polymorphism and PCR ribotyping. *J Clin Microbiol* 42:1035–1041. <https://doi.org/10.1128/JCM.42.3.1035-1041.2004>.
  13. Granato PA, Hansen G, Herding E, Chaudhuri S, Tang S, Garg SK, Rowell CR, Sickler JJ. 2018. Performance comparison of the cobas Liat and Cepheid GeneXpert systems for *Clostridium difficile* detection. *PLoS One* 13:e0200498. <https://doi.org/10.1371/journal.pone.0200498>.