JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2009, p. 3729–3731 Vol. 47, No. 11
0095-1137/09/$12.00 doi:10.1128/JCM.01280-09
Copyright © 2009, American Society for Microbiology. All Rights Reserved.

Comparison of a Commercial Multiplex Real-Time PCR to the Cell Cytotoxicity Neutralization Assay for Diagnosis of *Clostridium difficile* Infections[▽]

Haihui Huang,[1,2] Andrej Weintraub,[2] Hong Fang,[2] and Carl Erik Nord[2]*

Institute of Antibiotics, Huashan Hospital, Fudan University, 12 Wulumuqi Zhong Road, Shanghai 200040, China,[1] and Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital, Huddinge, SE-141 86 Stockholm, Sweden[2]

Received 30 June 2009/Returned for modification 17 August 2009/Accepted 31 August 2009

A commercial multiplex real-time PCR assay (Cepheid Xpert *C. difficile* assay) for the diagnosis of *Clostridium difficile* infection was evaluated. The sensitivity and specificity of the Cepheid assay were 97.1% and 93.0% for fresh stools, using the cell cytotoxicity neutralization assay as the reference. Using PCR ribotyping as the reference for ribotype 027 strains, the corresponding figures were 100% and 98.1%, respectively.

Clostridium difficile infection (CDI) has increased in frequency and severity in North America and Europe over the last 5 years, largely due to the emergence of the epidemic PCR ribotype 027 strain (10, 11). The diagnosis of CDI is usually based on a clinical history of recent antimicrobial usage and diarrhea in combination with laboratory tests (9). Therefore, rapid and accurate microbiological diagnosis is urgently needed. The Cepheid Xpert *C. difficile* assay (Sunnyvale, CA) is a real-time multiplex PCR assay performed on the Cepheid GeneXpert Dx system. Proprietary primers specific for the toxin B gene (*tcdB*), binary toxin genes (*cdtA* and *cdtB*), and *tcdC* gene single-base deletion at nucleotide 117 were designed to detect toxigenic *C. difficile* and the presumptive PCR ribotype 027 strain. The purpose of this study was to evaluate the Cepheid Xpert *C. difficile* multiplex real-time PCR assay for the detection of toxigenic *C. difficile* strains and the presumptive ribotype 027.

There were four serial investigations in the present study. In investigation 1, 205 frozen *C. difficile* strains collected during 2007 and 2008 were analyzed. In investigation 2, 195 frozen stool specimens belonging to different categories were selected based on direct cell cytotoxicity neutralization assay (CCNA) and toxigenic anaerobic culture results. Because PCR ribotype 027 is uncommon in Sweden, 40 frozen stool specimens collected in the United States were also analyzed. In investigation 3, 30 pairs of fresh-frozen stool specimens were analyzed. The fresh stool was analyzed within 24 h of collection, and then the leftover was stored at −20°C for 3 days and retested. In investigation 4, 220 consecutive fresh, unformed stool specimens (Bristol Stool Chart grade 5 to 7) from patients older than 2 years were analyzed within 24 h of collection. Eligible participants were those symptomatic patients who had a stool sample submitted to the Karolinska University Hospital for routine *C. difficile* testing.

Unrepeated strains and stools were determined for *C. difficile* test by CCNA with a commercial *C. difficile* toxin/antitoxin kit (TechLab, Blacksburg, VA). For the stool specimens, anaerobic cultures on selective taurocholate cycloserine-cefoxitin-fructose agar plates were also performed (13). All isolates were typed by PCR ribotyping (19).

Concurrently, the Cepheid Xpert *C. difficile* assay was performed according to protocols provided by the manufacturer. Each kit contained single-use disposable cartridges with integrated reaction chambers and reagents. A sterile Copan swab was dipped into the stool specimen or used to pick one fresh *C. difficile* colony from the blood agar plate, and the stool sample or colony was resuspended in sample buffer and then transferred to the cartridge. The cartridge was placed in the GeneXpert Dx module and run. Every PCR run included a sample-processing control and a probe check control. Each day, a positive control (*C. difficile* ATCC 9689 or the ribotype 027 strain) and a negative control (diluted *C. difficile*-negative stool sample) provided by Cepheid were tested. Results were automatically interpreted by the software as follows: "*C. difficile* positive," "*C. difficile* 027 NAP1 presumptive positive," "*C. difficile* negative," "invalid," "error," or "no result." If any of the test results was "invalid," "error," or "no result," the sample was retested.

CCNA and strain typing of the isolates were used as the reference standards for all of the investigations. If discrepant results for stool specimens were obtained with the Cepheid assay and CCNA, the results of CCNA for recovered isolates were taken into consideration. Sensitivity, specificity, positive and negative predictive values, and 95% confidence interval (CI) were calculated using SAS.

The agreement between the Cepheid assay and CCNA for 205 isolates, 235 frozen stool specimens, and 220 fresh stools was 99.5%, 89.4%, and 93.6%, respectively (Table 1). Mixed populations of isolates with different toxin profiles (toxin B negative and toxin B positive) and different PCR ribotypes were found in four fecal samples. The real-time PCR cycle threshold values for frozen-thawed samples matched those for fresh samples (data not shown). In investigation 4, the initial evaluation of the stool specimens yielded 95.7% sensitivity and

* Corresponding author. Mailing address: Division of Clinical Microbiology, Karolinska University Hospital Huddinge, SE-141 86 Stockholm, Sweden. Phone: 46 8 585 878 38. Fax: 46 8 585 879 33. E-mail: carl.erik.nord@ki.se.

▽ Published ahead of print on 9 September 2009.

TABLE 1. Comparison between the Cepheid Xpert *C. difficile* assay and CCNA

Investigation (no. of samples)	CCNA result	No. of samples with indicated Cepheid Xpert *C. difficile* assay result		Performance [% (95% CI)] of Cepheid Xpert *C. difficile* assay with CCNA[a]			
		Toxin B positive	Toxin B negative	Sensitivity	Specificity	PPV	NPV
1 (205)				100	94.4 (83.9–105.3)	99.5 (98.4–100.5)	100
	Positive	187	0				
	Negative	1	17				
2 (235)				94.4 (90.9–98.0)	78.4 (69.0–87.8)	90.5 (86.0–94.9)	86.6 (78.4–94.8)
	Positive	152	9				
	Negative	16	58				
4 (220)				97.1 (91.6–102.7)	93.0 (89.3–96.7)	72.3 (59.6–85.1)	99.4 (98.3–100.6)
	Positive	34	1				
	Negative	13	172				

[a] PPV, positive predictive value; NPV, negative predictive value.

87.3% specificity, respectively. The discrepancies between the assays are listed in Table 2. Upon reevaluation by toxigenic anaerobic culture, the sensitivity and specificity increased to 97.1% and 93.0%, respectively.

Only one PCR ribotype 027 presumptive positive strain was detected by the Cepheid assay in investigation 1; this result was verified by PCR ribotyping. In investigation 2, the presumptive ribotype 027 strain was detected in 28 of the 235 samples by the Cepheid assay; 24 were verified by PCR ribotyping. The sensitivity, specificity, and positive and negative predictive values of the Cepheid assay were 100%, 98.1% (95% CI, 96.3 to 99.9%), 85.7% (95% CI, 72.8 to 98.7%), and 100%, respectively. No ribotype 027 strain was detected in investigations 3 and 4.

The difficulty of choosing an optimal test for the diagnosis of CDI has long been known. Toxigenic *C. difficile* detection by CCNA is considered to be the "gold standard" but is time consuming (24 to 48 h) and requires cell culture facilities. Anaerobic culture has sensitivity approaching 100%, but the false-positive rate exceeds 10% because of the high rate of asymptomatic carriage (8, 15). Toxin enzyme immunoassays are more rapid but are associated with widely varying sensitivities (69% to 99%) and specificities (92% to 100%), making their reliability questionable if used as stand-alone assays (1, 2, 14). A two-stage testing strategy with an initial rapid screening test to identify positive samples that were then confirmed by a reference method was suggested, but it still delays detection and has been reported to have varying results in some institutions (5, 6, 16).

TABLE 2. Characterization of specimens with initial discrepant results in the Cepheid Xpert *C. difficile* assay versus CCNA in investigation 4[a]

No. of samples	Result of: Initial testing			Isolate testing		Final *C. difficile* assignment[c]
	Cepheid assay[b]	CCNA	Culture	Cepheid assay	CCNA	
1	Neg	Pos	Neg			Pos
12	Pos	Neg	Neg			Neg
12	Pos	Neg	Pos	Pos	Pos	Pos
1	Pos	Neg	Pos	ND	Neg	Neg

[a] Neg, negative; Pos, positive; ND, not done (the isolate could not be recovered).
[b] Cepheid Xpert *C. difficile* assay for toxin B.
[c] The final *C. difficile* assignment was based on the combination of CCNA results for stool specimens and isolates.

Several reports of the use of real-time PCR for the detection of toxigenic *C. difficile* have appeared over the past few years. In the present study, the overall agreement between the results of the Cepheid assay and CCNA was similar to the results of previous in-house or commercial real-time PCR evaluations (3, 17–18, 21–22). The discrepancies between Cepheid assay and CCNA results may represent false positives or false negatives. This could be due to the fact that the Cepheid assay detects the gene encoding toxin B rather than the functional toxin. Hence, false-negative results may be due to aberrant *tcdB* genes (12) and the number of nontoxigenic strains being higher than the number of toxigenic strains, since multiple strains (determined by PCR ribotyping) may coexist simultaneously in the stool (20). False-positive results may be due to antibiotic treatment prior to receiving a sample for analysis; in this case, the DNA might still be present but toxin and/or culture might be negative. Finally, it has been reported that toxin is not uniformly distributed in stool samples (4).

One of the significant advantages of the Cepheid assay is predicting the presence of the ribotype 027 strain. It can greatly facilitate the tracing of outbreaks, and with the recognition of ribotype 027 in an institution, quinolone restriction may be an important component of the infection control strategy (7). In investigation 2, the Cepheid assay was 100% sensitive and 98.1% specific for the diagnosis of ribotype 027, using PCR ribotyping as the reference. However, in investigations 3 and 4 in which fresh fecal samples from Swedish patients were analyzed, no ribotype 027 strain was found. More ribotype 027-positive fresh samples are needed for further evaluation.

Another important advantage of the Cepheid assay in the clinical microbiology field is the rapidity that it offers. The procedure takes 1 h from specimen processing in the laboratory to reporting the results and is easier to perform than CCNA or conventional PCR.

In conclusion, the Cepheid Xpert *C. difficile* assay offers sensitivity and specificity for toxin B detection that are comparable to those of the CCNA reference method. With the

results available within 1 h, it provides prompt and precise laboratory diagnosis.

We thank the Swedish Institute for Infectious Disease Control for support in PCR ribotyping.

This work was supported by grants from the Scandinavian Society for Antimicrobial Chemotherapy and from the Stockholm County Council.

We declare no conflict of interest.

REFERENCES

1. **Alcala, L., L. Sanchez-Cambronero, M. P. Catalan, M. Sanchez-Somolinos, M. T. Pelaez, M. Marin, and E. Bouza.** 2008. Comparison of three commercial methods for rapid detection of *Clostridium difficile* toxins A and B from fecal specimens. J. Clin. Microbiol. **46:**3833–3835.
2. **Bartlett, J. G., and D. N. Gerding.** 2008. Clinical recognition and diagnosis of *Clostridium difficile* infection. Clin. Infect. Dis. **46**(Suppl. 1)**:**S12–S18.
3. **Belanger, S. D., M. Boissinot, N. Clairoux, F. J. Picard, and M. G. Bergeron.** 2003. Rapid detection of *Clostridium difficile* in feces by real-time PCR. J. Clin. Microbiol. **41:**730–734.
4. **Eltringham, I. J.** 2009. Diagnosis of *Clostridium difficile* infection by toxin detection kits. Lancet Infect. Dis. **9:**141–142.
5. **Fenner, L., A. F. Widmer, G. Goy, S. Rudin, and R. Frei.** 2008. Rapid and reliable diagnostic algorithm for detection of *Clostridium difficile*. J. Clin. Microbiol. **46:**328–330.
6. **Gilligan, P. H.** 2008. Is a two-step glutamate dehydrogenase antigen-cytotoxicity neutralization assay algorithm superior to the premier toxin A and B enzyme immunoassay for laboratory detection of *Clostridium difficile*? J. Clin. Microbiol. **46:**1523–1525.
7. **Kallen, A. J., A. Thompson, P. Ristaino, L. Chapman, A. Nicholson, B. T. Sim, F. Lessa, U. Sharapov, E. Fadden, R. Boehler, C. Gould, B. Limbago, D. Blythe, and L. C. McDonald.** 2009. Complete restriction of fluoroquinolone use to control an outbreak of *Clostridium difficile* infection at a community hospital. Infect. Control Hosp. Epidemiol. **30:**264–272.
8. **Kelly, C. P., and J. T. LaMont.** 1998. *Clostridium difficile* infection. Annu. Rev. Med. **49:**375–390.
9. **Kelly, C. P., and J. T. LaMont.** 2008. *Clostridium difficile*—more difficult than ever. N. Engl. J. Med. **359:**1932–1940.
10. **Martin, H., B. Willey, D. E. Low, H. R. Staempfli, A. McGeer, P. Boerlin, M. Mulvey, and J. S. Weese.** 2008. Characterization of *Clostridium difficile* strains isolated from patients in Ontario, Canada, from 2004 to 2006. J. Clin. Microbiol. **46:**2999–3004.
11. **McFarland, L. V.** 2009. Renewed interest in a difficult disease: *Clostridium difficile* infections—epidemiology and current treatment strategies. Curr. Opin. Gastroenterol. **25:**24–35.
12. **Mehlig, M., M. Moos, V. Braun, B. Kalt, D. E. Mahony, and C. von Eichel-Streiber.** 2001. Variant toxin B and a functional toxin A produced by *Clostridium difficile* C34. FEMS Microbiol. Lett. **198:**171–176.
13. **Mundy, L. S., C. J. Shanholtzer, K. E. Willard, D. N. Gerding, and L. R. Peterson.** 1995. Laboratory detection of *Clostridium difficile*. A comparison of media and incubation systems. Am. J. Clin. Pathol. **103:**52–56.
14. **Novak-Weekley, S. M., and M. H. Hollingsworth.** 2008. Comparison of the premier toxin A and B assay and the TOX A/B II assay for diagnosis of *Clostridium difficile* infection. Clin. Vaccine Immunol. **15:**575–578.
15. **Peterson, L. R., and P. J. Kelly.** 1993. The role of the clinical microbiology laboratory in the management of *Clostridium difficile*-associated diarrhea. Infect. Dis. Clin. N. Am. **7:**277–293.
16. **Planche, T., A. Aghaizu, R. Holliman, P. Riley, J. Poloniecki, A. Breathnach, and S. Krishna.** 2008. Diagnosis of *Clostridium difficile* infection by toxin detection kits: a systematic review. Lancet Infect. Dis. **8:**777–784.
17. **Sloan, L. M., B. J. Duresko, D. R. Gustafson, and J. E. Rosenblatt.** 2008. Comparison of real-time PCR for detection of the *tcdC* gene with four toxin immunoassays and culture in diagnosis of *Clostridium difficile* infection. J. Clin. Microbiol. **46:**1996–2001.
18. **Stamper, P. D., R. Alcabasa, D. Aird, W. Babiker, J. Wehrlin, I. Ikpeama, and K. C. Carroll.** 2009. Comparison of a commercial real-time PCR assay for *tcdB* detection to a cell culture cytotoxicity assay and toxigenic culture for direct detection of toxin-producing *Clostridium difficile* in clinical samples. J. Clin. Microbiol. **47:**373–378.
19. **Stubbs, S. L. J., J. S. Brazier, G. L. O'Neill, and B. I. Duerden.** 1999. PCR targeted to the 16S-23S rRNA gene intergenic spacer region of *Clostridium difficile* and construction of a library consisting of 116 different PCR ribotypes. J. Clin. Microbiol. **37:**461–463.
20. **van den Berg, R. J., H. A. Ameen, T. Furusawa, E. C. Claas, E. R. van der Vorm, and E. J. Kuijper.** 2005. Coexistence of multiple PCR-ribotype strains of *Clostridium difficile* in faecal samples limits epidemiological studies. J. Med. Microbiol. **54:**173–179.
21. **van den Berg, R. J., E. J. Kuijper, L. E. van Coppenraet, and E. C. Claas.** 2006. Rapid diagnosis of toxinogenic *Clostridium difficile* in faecal samples with internally controlled real-time PCR. Clin. Microbiol. Infect. **12:**184–186.
22. **van den Berg, R. J., N. Vaessen, H. P. Endtz, T. Schulin, E. R. van der Vorm, and E. J. Kuijper.** 2007. Evaluation of real-time PCR and conventional diagnostic methods for the detection of *Clostridium difficile*-associated diarrhoea in a prospective multicentre study. J. Med. Microbiol. **56:**36–42.