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Evaluation of a New Automated Homogeneous PCR Assay, GenomEra *C. difficile*, for Rapid Detection of Toxigenic *Clostridium difficile* in Fecal Specimens

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We evaluated a new automated homogeneous PCR assay to detect toxigenic *Clostridium difficile*, the GenomEra *C. difficile* assay (Abacus Diagnostica, Finland), with 310 diarrheal stool specimens and with a collection of 33 known clostridial and nonclostridial isolates. Results were compared with toxigenic culture results, with discrepancies being resolved by the GeneXpert *C. difficile* PCR assay (Cepheid). Among the 80 toxigenic culture-positive or GeneXpert *C. difficile* assay-positive fecal specimens, 79 were also positive with the GenomEra *C. difficile* assay. Additionally, one specimen was positive with the GenomEra assay but negative with the confirmatory methods. Thus, the sensitivity and specificity were 98.8% and 99.6%, respectively. With the culture collection, no false-positive or -negative results were observed. The analytical sensitivity of the GenomEra *C. difficile* assay was approximately 5 CFU per PCR test. The short hands-on (<5 min for 1 to 4 samples) and total turnaround (<1 h) times, together with the high positive and negative predictive values (98.8% and 99.6%, respectively), make the GenomEra *C. difficile* assay an excellent option for toxigenic *C. difficile* detection in fecal specimens.

Toxin-producing *Clostridium difficile* is the most common cause of hospital-acquired and antibiotic-associated diarrhea (1, 2). The main virulence factors of *C. difficile* are *C. difficile* toxin A (TcdA) and *C. difficile* toxin B (TcdB), which are encoded by the genes *tcdA* and *tcdB*, respectively (3, 4). In addition, a separate binary toxin is produced by a small group of isolates with or without TcdA and/or TcdB (5, 6), and it has been suggested that the binary toxin may play a part in the recurrence of *C. difficile* infection (CDI) (7). As the role of the binary toxin in CDI currently is not well understood, it has not become a common target for diagnostic assays.

CDI is prevalent in many hospitals and health care facilities throughout the world. It is associated with increases in hospitalization times and mortality rates, leading to augmented health care costs (2, 6, 8). In order to limit the transmission of *C. difficile* and to decrease the burden of CDI, rapid reliable detection of toxigenic *C. difficile* isolates and good infection prevention practices are required.

Cytotoxigenic cultures and cytotoxin assays are considered standard diagnostic methods. These are sensitive but have several drawbacks, including long turnaround times and labor-intensive sample preparation and analysis, which limit their clinical utility (9–13). An alternative approach is the detection of *C. difficile* toxins or glutamate dehydrogenase (GDH) in stool samples by immunochromatographic antigen (IA) tests or enzyme immunoassays (EIAs) (14–16). These assays are rapid but are less sensitive and less specific than toxigenic cultures. Moreover, relying only on GDH detection reveals nothing about the toxigenic nature of the possible *C. difficile* isolates. To improve the detection of CDI using IA tests or EIAs, a two-step diagnostic algorithm has been advocated recently (17). This approach includes a preliminary screening test and an additional test (e.g., culture or nucleic acid amplification assay) for confirmation. The strength of this combined practice is a high negative predictive value in areas with low CDI prevalence (17). With the increasing frequency of cases of CDI in many countries, however, this approach may lack sufficient sensitivity and increase the workload of laboratory personnel.

The direct molecular detection of genes encoding *C. difficile* toxin A and/or toxin B has become a diagnostic target of interest (18–20). Molecular assays are more sensitive than IA tests or EIAs and in some studies are even more sensitive than cytotoxigenic cultures or cytotoxin assays (21, 22). Some of these methods have been commercialized and made suitable for use in clinical microbiological laboratories (14, 23–27). The main advantage of molecular assays, in addition to their high sensitivity and specificity, is the short turnaround time, compared with cytotoxigenic cultures or cytotoxin assays. Moreover, sensitive, rapid, and well-performing molecular tests are cost-effective, decreasing the need for retesting and reducing unnecessary treatment and isolation of patients (28, 29).

In this study, we investigated the performance of a new PCR assay, the GenomEra *C. difficile* assay (Abacus Diagnostica, Turku, Finland), for direct detection of toxigenic *C. difficile* in fecal specimens. The GenomEra *C. difficile* assay is a CE-marked diagnostic nucleic acid test for the qualitative detection of toxigenic *C. difficile* in stool samples. It detects the toxin B gene (*tcdB*) using rapid thermal cycling and homogeneous time-resolved fluorescence detection technology, which has proved to be resistant to aqueous quenching and other background effects (30). The GenomEra

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TABLE 1 Characteristics of deep-frozen *Clostridium difficile* strain collection specimens ($n = 15$) analyzed using the GenomEra *C. difficile* assay

C. difficile ribotypes	Virulence genes	GenomEra *C. difficile* assay result (*tcdB*)
001, 002, 005, 011, 012, 014, 017, 018, 020, 056	*tcdA*, *tcdB*	+
023, 027, 045, 078	*tcdA*, *tcdB*, *cdtB*, deletion in *tcdC*	+
033	*cdtB*, deletion in *tcdC*	−

CDX instrument has shown excellent performance in previous studies with other pathogenic organisms (31–33).

(These results were presented in part at the 23rd European Congress of Clinical Microbiology and Infectious Diseases, 27 to 30 April 2013, Berlin, Germany [34].)

MATERIALS AND METHODS

Clinical and culture collection samples. This study was conducted at the clinical microbiology laboratory of Vaasa Central Hospital, where approximately 2,200 fecal specimens are screened for CDI each year. Of these specimens, approximately 20% are positive with the routinely used toxigenic culture method. A total of 310 loose stool specimens (one per patient) were collected prospectively from inpatients at Vaasa Central Hospital during August and September 2012, according to routine hospital practice for antibiotic-associated diarrhea. The patients' mean age was 72 years, with ages ranging from 7 to 95 years. Specimens were tested by toxigenic culture immediately or were stored at 4°C and tested within 24 h after being receiving in the laboratory. An aliquot was stored at −70°C for later testing with the GenomEra *C. difficile* assay.

In addition, specimens from a deep-frozen collection ($n = 33$) of known toxigenic and nontoxigenic *C. difficile* strains ($n = 15$) (Table 1) as well as other clostridial and nonclostridial strains ($n = 18$) were analyzed with the GenomEra *C. difficile* assay. Species identification of the culture collection specimens had been performed by standard laboratory methods, e.g., API 20A and API 20E (bioMérieux, Marcy l'Etoile, France) and RapID ANA (Thermo Fisher Scientific, Lenexa, KS) systems, when the strains were first isolated. The PCR ribotyping of *C. difficile* isolates and the definition of virulence genes had been performed in the Bacteriology Unit of the National Institute for Health and Welfare (THL), as previously described (35, 36). All strains were cultured on fastidious anaerobe agar plates (Lab M Ltd., Heywood, Lancashire, United Kingdom) or chocolate agar plates (Lab M Ltd.) at 35°C, under ambient or anaerobic conditions, for 16 h (nonanaerobic isolates) or 48 h (anaerobic isolates) prior to analysis with the GenomEra *C. difficile* assay.

Toxigenic culture. Toxigenic culture was performed by plating the specimens on cycloserine-cefoxitin egg-yolk (CCEY) agar (Oxoid Limited, Basingstoke, Hampshire, United Kingdom), followed by a 48-h anaerobic incubation at 35°C. Presumed growth of *C. difficile* was confirmed by Gram staining, UV light analysis, and IA testing (Wampole C.Diff Quik Chek Complete; Techlab, Blacksburg, VA) targeting *C. difficile*-specific glutamate dehydrogenase (GDH). The toxigenic nature of the suspected isolate was confirmed by the aforementioned IA test (Wampole C.Diff Quik Chek Complete), although detection of GDH and toxins A and B from colonies is an "off-label" approach and is not approved by the manufacturer.

Detection of toxigenic *C. difficile* by the GenomEra *C. difficile* PCR assay. The GenomEra *C. difficile* assay was performed according to the manufacturer's instructions. Briefly, 1 μl of thawed stool was collected with a sterile loop and diluted in a tube containing 1 ml of GenomEra sample buffer (Abacus Diagnostica). Four hundred microliters of this solution was then aliquoted into a second tube containing glass beads and was vortex-mixed for 5 min. Finally, 35 μl of the sample solution was transferred into the single-use disposable test chip, containing all PCR reagents in dried form. The thermal cycling and homogeneous detection were performed automatically in the GenomEra CDX instrument, and results were reported within 50 min.

Analytical sensitivity was estimated by preparing 10-fold dilutions of freshly cultured, known toxigenic *C. difficile* isolates (PCR ribotypes 001, 002, 027, and 078) in sample tubes containing 0.9% NaCl. Each preparation was then diluted 50-fold into the GenomEra sample buffer and mixed with glass beads prior to analysis. PCR runs were performed in duplicate, and colony counts from each analyzed dilution were determined by duplicate plating onto fastidious anaerobe agar plates (Lab M Ltd., United Kingdom).

Analysis of discrepant results. In cases with discrepant toxigenic culture and GenomEra *C. difficile* PCR assay results, specimens were sent to the bacteriology unit of the National Institute for Health and Welfare for confirmation with the GeneXpert *C. difficile* PCR assay (Cepheid). Two similar results from the total of three methods (toxigenic culture and two different PCR tests) were used to determine the positivity/negativity of the sample, partly based on the assumption that the detection sensitivity of the molecular methods may exceed that of toxigenic culture (22).

Analysis by the GeneXpert system was performed according to the manufacturer's instructions. Briefly, the tip of a sterile swab was immersed in unformed stool and inserted into a sample reagent tube. The stem of the swab was snapped. The tube was closed and vortex mixed for 10 s, and the liquid was transferred to a sample cartridge containing all PCR reagents. The cartridge was inserted into the GeneXpert module, and the analysis was performed automatically using GeneXpert software.

RESULTS

PCR performance characteristics. The hands-on times for the GenomEra *C. difficile* assay were approximately 3 min for one sample and 5 min for four samples (the capacity of the instrument). The assay run-time was 50 min. Thus, the total turnaround time for one to four samples was less than 1 hour. Test results for *tcdB* are reported by the GenomEra software in numerical form, from −15 (negative) to +100 (strongly positive), with a written conclusion of "*C. difficile tcdB* negative," "borderline," or "positive." The zone for inconclusive borderline results is −5 to +5. The estimated analytical sensitivity for intact *C. difficile* cells was 5 CFU per PCR, as the minimal detectable amount of viable cells in the mimicked stool samples varied from 1.40×10^2 to 1.50×10^2 cells/μl for each ribotype analyzed. However, this was only presumptively assessed, as a limited number of replicates were used in this study.

Analysis of clinical specimens and culture collection isolates. A total of 310 stool specimens were included. Of these, 80 (25.8%) were *tcdB*-positive with the GenomEra *C. difficile* assay (Table 2). However, toxin-producing *C. difficile* was isolated from 77 specimens (25.2%) in culture. Two of the three GenomEra-positive but culture-negative stool specimens were confirmed to be *tcdB*-positive with the reference PCR assay, the GeneXpert *C. difficile* assay, while one was positive only with the GenomEra assay. Additionally, one specimen was toxin-positive by culture and initially yielded an inconclusive borderline *C. difficile tcdB* result (*tcdB* score, −4) with the GenomEra assay but after retesting gave a negative test result (*tcdB* score, −15). Unfortunately, this specimen was accidentally discarded before being tested with the GeneXpert system.

The results indicated sensitivity and specificity values of 98.8% and 99.6%, respectively, when the GenomEra *C. difficile* assay was

TABLE 2 Toxigenic *Clostridium difficile* screening results for 310 fecal specimens using the GenomEra *C. difficile* PCR assay and toxigenic culture

GenomEra *C. difficile* assay result	No. with toxigenic culture result of:	
	Negative	Positive
Negative	229	1[b]
Positive	3[a]	77

[a] Two of the three specimens with positive GenomEra *C. difficile* assay results but negative toxigenic culture results were confirmed as positive with the GeneXpert *C. difficile* PCR assay (Cepheid).
[b] One specimen was culture positive but gave an inconclusive borderline result with the GenomEra assay and a negative result after retesting. This specimen was not tested with the GeneXpert assay.

performed directly from fecal specimens. The 95% confidence interval for sensitivity was 96.4 to 100.0%, and that for specificity was 98.8 to 100.0%. Positive and negative predictive values were 98.8% and 99.6%, respectively. No false-positive or false-negative test results were observed from the culture collection using the GenomEra *C. difficile* assay, as all *tcdB*-containing *C. difficile* isolates were detected as toxin-positive (Table 1). Furthermore, the *C. difficile* isolate not carrying the *tcdB* and *tcdA* genes, other clostridial species (*Clostridium bifermentans*, *Clostridium butyricum*, *Clostridium cadaveris*, *Clostridium clostridioforme*, *Clostridium histolyticum*, *Clostridium innocuum*, *Clostridium novyi*, *Clostridium perfringens*, *Clostridium ramosum*, *Clostridium septicum*, *Clostridium sporogenes*, and *Clostridium tertium*), and nonclostridial isolates (*Bacteroides fragilis*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumonia*, *Klebsiella oxytoca*, and *Staphylococcus epidermidis*) all gave negative *tcdB* results with this new PCR assay.

DISCUSSION

Due to the increased incidence and severity of toxin-producing *C. difficile* infections (37), CDI surveillance and prevention in hospitals and other health care facilities should be effective and readily available. The early accurate identification of toxigenic *C. difficile* helps to direct resources and measures to appropriate targets. The development of nucleic acid amplification assays has recently enabled significant improvements in the detection of toxigenic *C. difficile* isolates in fecal specimens (14, 19, 23, 24, 27, 36). Some of these methods, however, involve multiple manual or automated sample preparation steps, rendering the tests laborious and/or time-consuming.

Here we evaluated a new homogeneous PCR assay, the GenomEra *C. difficile* assay, for rapid reliable detection of toxigenic *C. difficile* in stool samples with minimal hands-on time. This new PCR assay detects the *tcdB* gene alone, which has proved to be a slightly better target than *tcdA* for screening for CDI (5, 38). The clinical sensitivity of the GenomEra *C. difficile* assay proved to be high (98.8%); only one inconclusive borderline result, with a negative result upon repeat testing, was observed among the culture-positive stool samples. The one false-negative result obtained with the GenomEra *C. difficile* assay may be the consequence of freezing and thawing of that particular fecal specimen prior to PCR analysis, as only scarce growth of toxin-positive *C. difficile* was observed in the primary culture and the specimen was *C. difficile*-negative in a reculture performed at the time of the GenomEra analysis. Furthermore, the GenomEra *C. difficile* assay detected two positive specimens that were confirmed as positive with another PCR assay and hence showed supposedly false-negative results in toxigenic cultures. The specificity of the GenomEra *C. difficile* assay was high (99.6%), with only one false-positive result (toxigenic culture- and GeneXpert-negative). No false-positives were observed for the culture collection isolates.

Laboratories of any size can easily adopt the GenomEra CDX instrument. The capacity of the assay is adequate for laboratories performing up to approximately 8,000 (Monday through Friday) to 11,000 (every day) *C. difficile* analyses per year (32 sample analyses could be performed within one 8-h workday). In laboratories with smaller numbers of *C. difficile* samples per year, the capacity of the instrument may be used to test for additional microbiological targets, in order to take full advantage of this molecular equipment. Using a method that permits the detection of other significant pathogens as well saves investment costs and training expenses for laboratory personnel. Other tests currently available for the GenomEra platform include tests for the detection of *Staphylococcus aureus* and a marker of methicillin resistance in blood culture, plate culture, and swab samples (31, 32).

Nucleic acid amplification assays are an interesting alternative for the detection of toxigenic *C. difficile*, being more sensitive than IA tests and EIAs and significantly faster than the standard methods (24, 36, 39). However, the suitability of these assays alone for the detection of CDI is under debate. Knowing that the asymptomatic carriage of toxigenic *C. difficile* among children (40, 41) and elderly inpatients, as well as patients in extended-care facilities (i.e., nursing homes) (42, 43), can be common and taking into account the fact that molecular assays detect only toxin genes and not the actual presence of toxins in the intestine, it has been assumed that molecular assays are not able to discriminate between CDI and asymptomatic colonization (44). However, Humphries et al. recently demonstrated that a toxin EIA performed poorly in comparison with a molecular assay (39). The presence of fecal toxin measured by the EIA did not correlate with the severity of CDI, and direct screening for CDI with the molecular assay proved to be superior to the EIA. We argue that attention should be paid to the appropriate indications for specimen collection and laboratory testing. For example, use of the Bristol stool chart (chart specimen types 5 to 7) and specific diagnostic criteria (45) would decrease the risk of unnecessary sampling and "false-positive" detection of asymptomatic *C. difficile* carriage by molecular methods. In cases of unexplained diarrhea, other microbial pathogens also should be considered, and sufficient precise clinical information should be provided to the laboratory (21). If a positive result for toxigenic *C. difficile* is obtained for a stool sample, then experts in clinical microbiology should inform the clinicians and discuss if additional tests, such as toxin detection by EIA or culture, are needed.

Cost estimations were not performed in this study, as various diagnostic methods should have been included for reliable comparison. In recently published studies in which such calculations were performed, the full benefits of molecular assays or IA tests/EIAs used alone or in two-step algorithms were not comprehensively assessed (15, 16, 24). In the studies by Walkty et al. and Culbreath et al., a GDH/EIA two-step algorithm was found to be the most economical approach to screening for CDI, but only the costs of reagents and consumables were taken into account (15, 16). In contrast, in the study by Chapin et al., greater savings were actually attained with direct molecular screening, compared with

both the IA test and the GDH/EIA two-step algorithm, because of enhanced performance in CDI detection overall (24). Also in our study, significant overall improvements in the detection of toxigenic *C. difficile* were achieved with a molecular assay. In addition to the high sensitivity and specificity, the GenomEra *C. difficile* assay provided results within 1 hour, whereas toxigenic culture required at least 2 days for complete results. The workload of laboratory personnel also was decreased with the GenomEra assay, as only 5 min of sample handling was required for four samples, compared with 20 to 40 min of hands-on time for toxigenic culture.

In conclusion, the GenomEra *C. difficile* assay proved to be an excellent option for screening for toxigenic *C. difficile* in fecal specimens, offering excellent clinical sensitivity and specificity. The short hands-on time and minimal total turnaround time are advantages over some other molecular assays on the market (14, 23, 25–27). Furthermore, the user-friendly application and small space requirements make this small-scale system ideal, especially for small and medium-sized laboratories that lack room for larger PCR systems. A rapid PCR method enables the improvement of patient outcomes for CDI, provided that the overall process is optimized with appropriate sample selection, collection, and transportation and prompt reporting of the results to physicians.

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REFERENCES

1. **Bartlett JG, Gerding DN.** 2008. Clinical recognition and diagnosis of *Clostridium difficile* infection. Clin. Infect. Dis. **46**(Suppl 1):S12–S18.
2. **Karas JA, Enoch DA, Aliyu SH.** 2010. A review of mortality due to *Clostridium difficile* infection. J. Infect. **61:**1–8.
3. **Kuehne SA, Cartman ST, Heap JT, Kelly ML, Cockayne A, Minton NP.** 2010. The role of toxin A and toxin B in *Clostridium difficile* infection. Nature **467:**711–713.
4. **Lyras D, O'Connor JR, Howarth PM, Sambol SP, Carter GP, Phumoonna T, Poon R, Adams V, Vedantam G, Johnson S, Gerding DN, Rood JI.** 2009. Toxin B is essential for virulence of *Clostridium difficile*. Nature **458:**1176–1179.
5. **Elliott B, Squire MM, Thean S, Chang BJ, Brazier JS, Rupnik M, Riley TV.** 2011. New types of toxin A-negative, toxin B-positive strains among clinical isolates of *Clostridium difficile* in Australia. J. Med. Microbiol. **60:**1108–1111.
6. **Warny M, Pepin J, Fang A, Killgore G, Thompson A, Brazier J, Frost E, McDonald LC.** 2005. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. Lancet **366:**1079–1084.
7. **Stewart DB, Berg A, Hegarty J.** 2013. Predicting recurrence of *C. difficile* colitis using bacterial virulence factors: binary toxin is the key. J. Gastrointest. Surg. **17:**118–124.
8. **Dodek PM, Norena M, Ayas NT, Romney M, Wong H.** 21 January 2013. Length of stay and mortality due to *Clostridium difficile* infection acquired in the intensive care unit. J. Crit. Care [Epub ahead of print.] doi:10.1016/j.jcrc.2012.11.008.
9. **Arroyo LG, Rousseau J, Willey BM, Low DE, Staempfli H, McGeer A, Weese JS.** 2005. Use of a selective enrichment broth to recover *Clostridium difficile* from stool swabs stored under different conditions. J. Clin. Microbiol. **43:**5341–5343.
10. **Bliss DZ, Johnson S, Clabots CR, Savik K, Gerding DN.** 1997. Comparison of cycloserine-cefoxitin-fructose agar (CCFA) and taurocholate-CCFA for recovery of *Clostridium difficile* during surveillance of hospitalized patients. Diagn. Microbiol. Infect. Dis. **29:**1–4.
11. **Clabots CR, Gerding SJ, Olson MM, Peterson LR, Gerding DN.** 1989. Detection of asymptomatic *Clostridium difficile* carriage by an alcohol shock procedure. J. Clin. Microbiol. **27:**2386–2387.
12. **Marler LM, Siders JA, Wolters LC, Pettigrew Y, Skitt BL, Allen SD.** 1992. Comparison of five cultural procedures for isolation of *Clostridium difficile* from stools. J. Clin. Microbiol. **30:**514–516.
13. **Brazier JS.** 1998. The diagnosis of *Clostridium difficile*-associated disease. J. Antimicrob. Chemother. **41**(Suppl 3):29–40.
14. **Bruins MJ, Verbeek E, Wallinga JA, Bruijnesteijn van Coppenraet LE, Kuijper EJ, Bloembergen P.** 2012. Evaluation of three enzyme immunoassays and a loop-mediated isothermal amplification test for the laboratory diagnosis of *Clostridium difficile* infection. Eur. J. Clin. Microbiol. Infect. Dis. **31:**3035–3039.
15. **Culbreath K, Ager E, Nemeyer RJ, Kerr A, Gilligan PH.** 2012. Evolution of testing algorithms at a university hospital for detection of *Clostridium difficile* infections. J. Clin. Microbiol. **50:**3073–3076.
16. **Walkty A, Lagacé-Wiens PR, Manickam K, Adam H, Pieroni P, Hoban D, Karlowsky JA, Alfa M.** 2013. Evaluation of an algorithmic approach in comparison with the Illumigene assay for laboratory diagnosis of *Clostridium difficile* infection. J. Clin. Microbiol. **51:**1152–1157.
17. **Crobach MJ, Dekkers OM, Wilcox MH, Kuijper EJ.** 2009. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): data review and recommendations for diagnosing *Clostridium difficile*-infection (CDI). Clin. Microbiol. Infect. **15:**1053–1066.
18. **Alonso R, Muñoz C, Peláez T, Cercenado E, Rodríguez-Creixems M, Bouza E.** 1997. Rapid detection of toxigenic *Clostridium difficile* strains by a nested PCR of the toxin B gene. Clin. Microbiol. Infect. **3:**145–147.
19. **Bélanger SD, Boissinot M, Clairoux N, Picard FJ, Bergeron MG.** 2003. Rapid detection of *Clostridium difficile* in feces by real-time PCR. J. Clin. Microbiol. **41:**730–734.
20. **Kato H, Kato N, Watanabe K, Iwai N, Nakamura H, Yamamoto T, Suzuki K, Kim SM, Chong Y, Wasito EB.** 1998. Identification of toxin A-negative, toxin B-positive *Clostridium difficile* by PCR. J. Clin. Microbiol. **36:**2178–2182.
21. **Dubberke ER, Han Z, Bobo L, Hink T, Lawrence B, Copper S, Hoppe-Bauer J, Burnham CA, Dunne WM, Jr.** 2011. Impact of clinical symptoms on interpretation of diagnostic assays for *Clostridium difficile* infections. J. Clin. Microbiol. **49:**2887–2893.
22. **Eastwood K, Else P, Charlett A, Wilcox M.** 2009. Comparison of nine commercially available *Clostridium difficile* toxin detection assays, a real-time PCR assay for *C. difficile tcdB*, and a glutamate dehydrogenase detection assay to cytotoxin testing and cytotoxigenic culture methods. J. Clin. Microbiol. **47:**3211–3217.
23. **Buchan BW, Mackey TL, Daly JA, Alger G, Denys GA, Peterson LR, Kehl SC, Ledeboer NA.** 2012. Multicenter clinical evaluation of the Portrait toxigenic *C. difficile* assay for detection of toxigenic *Clostridium difficile* strains in clinical stool specimens. J. Clin. Microbiol. **50:**3932–3936.
24. **Chapin KC, Dickenson RA, Wu F, Andrea SB.** 2011. Comparison of five assays for detection of *Clostridium difficile* toxin. J. Mol. Diagn. **13:**395–400.
25. **Le Guern R, Herwegh S, Grandbastien B, Courcol R, Wallet F.** 2012. Evaluation of a new molecular test, the BD Max Cdiff, for detection of toxigenic *Clostridium difficile* in fecal samples. J. Clin. Microbiol. **50:**3089–3090.
26. **Shin BM, Mun SJ, Yoo SJ, Kuak EY.** 2012. Comparison of BD GeneOhm Cdiff and Seegene Seeplex ACE PCR assays using toxigenic *Clostridium difficile* culture for direct detection of *tcdB* from stool specimens. J. Clin. Microbiol. **50:**3765–3767.
27. **Terhes G, Urbán E, Sóki J, Nacsa E, Nagy E.** 2009. Comparison of a rapid molecular method, the BD GeneOhm Cdiff assay, to the most frequently used laboratory tests for detection of toxin-producing *Clostridium difficile* in diarrheal feces. J. Clin. Microbiol. **47:**3478–3481.
28. **Babady NE, Stiles J, Ruggiero P, Khosa P, Huang D, Shuptar S, Kamboj M, Kiehn TE.** 2010. Evaluation of the Cepheid Xpert *Clostridium difficile* Epi assay for diagnosis of *Clostridium difficile* infection and typing of the NAPI strain at a cancer hospital. J. Clin. Microbiol. **48:**4519–4524.
29. **Sharp SE, Ruden LO, Pohl JC, Hatcher PA, Jayne LM, Ivie WM.** 2010. Evaluation of the C.Diff Quik Chek Complete Assay, a new glutamate dehydrogenase and A/B toxin combination lateral flow assay for use in rapid, simple diagnosis of *Clostridium difficile* disease. J. Clin. Microbiol. **48:**2082–2086.
30. **von Lode P, Rosenberg J, Pettersson K, Takalo H.** 2003. A europium

chelate for quantitative point-of-care immunoassays using direct surface measurement. Anal. Chem. **75:**3193–3201.

31. **Hirvonen JJ, Nevalainen M, Tissari P, Salmenlinna S, Rantakokko-Jalava K, Kaukoranta SS.** 2012. Rapid confirmation of suspected methicillin-resistant *Staphylococcus aureus* colonies on chromogenic agars by a new commercial PCR assay, the GenomEra MRSA/SA Diagnose. Eur. J. Clin. Microbiol. Infect. Dis. **31:**1961–1968.
32. **Hirvonen JJ, von Lode P, Nevalainen M, Rantakokko-Jalava K, Kaukoranta SS.** 2012. One-step sample preparation of positive blood cultures for the direct detection of methicillin-sensitive and -resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci within one hour using the automated GenomEra CDX™ PCR system. Eur. J. Clin. Microbiol. Infect. Dis. **31:**2835–2842.
33. **Lehmusvuori A, Juntunen E, Tapio AH, Rantakokko-Jalava K, Soukka T, Lövgren T.** 2010. Rapid homogeneous PCR assay for the detection of *Chlamydia trachomatis* in urine samples. J. Microbiol. Methods **83:**302–306.
34. **Hirvonen JJ, Kaukoranta SS.** 2013. Evaluation of a new polymerase chain reaction (PCR) assay, the GenomEra *C. difficile*, for rapid detection of toxigenic *Clostridium difficile* in faecal specimens, poster 1858. Program 23rd Eur. Congr. Clin. Microbiol. Infect. Dis. European Society of Clinical Microbiology and Infectious Diseases, Berlin, Germany.
35. **Stubbs SL, Brazier JS, O'Neill GL, Duerden BI.** 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.
36. **Ylisiurua P, Koskela M, Vainio O, Tuokko H.** 2013. Comparison of antigen and two molecular methods for the detection of *Clostridium difficile* toxins. Scand. J. Infect. Dis. **45:**19–25.
37. **Lessa FC, Gould CV, McDonald LC.** 2012. Current status of *Clostridium difficile* infection epidemiology. Clin. Infect. Dis. **55**(Suppl 2):S65–S70.
38. **Rupnik M, Kato N, Grabnar M, Kato H.** 2003. New types of toxin A-negative, toxin B-positive strains among *Clostridium difficile* isolates from Asia. J. Clin. Microbiol. **41:**1118–1125.
39. **Humphries RM, Uslan DZ, Rubin Z.** 2013. Performance of *Clostridium difficile* toxin enzyme immunoassay and nucleic acid amplification tests stratified by patient disease severity. J. Clin. Microbiol. **51:**869–873.
40. **Rousseau C, Poilane I, De Pontual L, Maherault AC, Le Monnier A, Collignon A.** 2012. *Clostridium difficile* carriage in healthy infants in the community: a potential reservoir for pathogenic strains. Clin. Infect. Dis. **55:**1209–1215.
41. **Matsuki S, Ozaki E, Shozu M, Inoue M, Shimizu S, Yamaguchi N, Karasawa T, Yamagishi T, Nakamura S.** 2005. Colonization by *Clostridium difficile* of neonates in a hospital, and infants and children in three day-care facilities of Kanazawa, Japan. Int. Microbiol. **8:**43–48.
42. **Kyne L, Warny M, Qamar A, Kelly CP.** 2000. Asymptomatic carriage of *Clostridium difficile* and serum levels of IgG antibody against toxin A. N. Engl. J. Med. **342:**390–397.
43. **Riggs MM, Sethi AK, Zabarsky TF, Eckstein EC, Jump RL, Donskey CJ.** 2007. Asymptomatic carriers are a potential source for transmission of epidemic and nonepidemic *Clostridium difficile* strains among long-term care facility residents. Clin. Infect. Dis. **45:**992–998.
44. **Wilcox MH.** 2012. Overcoming barriers to effective recognition and diagnosis of *Clostridium difficile* infection. Clin. Microbiol. Infect. **18:**13–20.
45. **Pai S, Aliyu SH, Enoch DA, Karas JA.** 2012. Five years experience of *Clostridium difficile* infection in children at a UK tertiary hospital: proposed criteria for diagnosis and management. PLoS One **7:**e51728. doi:10.1371/journal.pone.0051728.