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Comparison of the Vidas *C. difficile* GDH Automated Enzyme-Linked Fluorescence Immunoassay (ELFA) with Another Commercial Enzyme Immunoassay (EIA) (Quik Chek-60), Two Selective Media, and a PCR Assay for *gluD* for Detection of *Clostridium difficile* in Fecal Samples

K. A. Davies,[a] C. E. Berry,[a] K. A. Morris,[a] R. Smith,[b] S. Young,[b] T. E. Davis,[c] D. D. Fuller,[c] R. J. Buckner,[c] M. H. Wilcox[a]

Leeds Teaching Hospitals Trust Microbiology Department, Leeds, United Kingdom[a]; Tricore Reference Laboratory, Albuquerque, New Mexico, USA[b]; Indiana University Department of Pathology and Laboratory Medicine, Indianapolis, Indiana, USA[c]

Prevention and management of *Clostridium difficile* infection (CDI) can be improved by rapid and reliable diagnostics. The Vidas *C. difficile* glutamate dehydrogenase assay had performance comparable to that of the Quik Chek-60 assay (overall agreement, 95%) and a sensitivity of >93%; thus, it is suitable as the first test in two-stage algorithms for a CDI diagnosis.

Accurate and timely diagnosis of *Clostridium difficile* infection (CDI) is a key step in optimizing patient management and reducing cross-infection risk, and diagnosis has historically relied on the detection of *C. difficile* toxins in fecal samples (1). An alternative target, *C. difficile*-specific glutamate dehydrogenase (GDH), first identified in 1991 by Lyerly et al., has been shown to be highly conserved between PCR ribotypes of *C. difficile* (2, 3). Current United Kingdom and European guidance recommends GDH as a possible first assay in a two-stage diagnostic algorithm for CDI, most commonly alongside toxin detection (1, 4, 5). The poor prognostic value of current *C. difficile* toxin enzyme immunoassays (EIA) means that they should not be used as standalone assays for the diagnosis of CDI (6–8).

We prospectively tested fecal samples routinely submitted for *C. difficile* testing between July 2012 and January 2013 to laboratories in one United Kingdom hospital (Leeds Teaching Hospital NHS Trust, Leeds, United Kingdom) and two U.S. hospitals (Wishard Health Services, Indianapolis, IN, USA, and Tricore Reference Laboratory, Albuquerque, NM, USA). Samples included were <3 days old and had been refrigerated to follow the international good practices for *C. difficile* diagnosis. All samples, once made anonymous, were tested at the receiving laboratory using a new enzyme-linked fluorescence assay (ELFA), the Vidas *C. difficile* GDH assay (bioMérieux, France), a comparator GDH EIA, Quik Chek-60 (Techlab, USA), and two culture methods. Samples were frozen at −70°C before shipping to Leeds for testing with an in-house PCR assay for the GDH gene *gluD*. Samples used in this service evaluation were residual diagnostic material and did not require ethical approval or consent in the United Kingdom. In the United States, approval of the ethics committee (institutional review board) was granted, while the requirement for informed consent was waived.

Fecal samples were directly inoculated onto *C. difficile* chromID culture media (bioMeriéux, France). In addition, fecal samples were alcohol shocked in 50% alcohol before inoculation on to cycloserine-cefoxitine fructose agar (CCFA) (Remel, USA). All plates were incubated at 37°C in an anaerobic environment for 24 h (chromID) or 48 h (CCFA) before inspection for suspect colonies (according to the manufacturers' instructions). The identity was confirmed with a Microgen latex agglutination kit (Microgen, United Kingdom) for the United Kingdom site or with Gram stain and Prodisc kits (Remel, USA) for U.S. sites. Both commercial

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Address correspondence to M. H. Wilcox, mark.wilcox@nhs.net.

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TABLE 1 Sequence of primers and probes used in GDH (*gluD*) PCR assay[a]

Oligonucleotide name	Sequence	5′ modification[b]	3′ modification[c]
Yersi F1	GGAGGAAGGGTTAAGTGTTA		
Yersi R1	GAGTTAGCCGGTGCTTCTT		
Yersi P1	GCGAGTAACGTCAATGTTCAGTGC	Cy5	BHQ2
gluD F3	GTCTTGGATGGTTGATGAGTAC		
gluD R2	TTCCTAATTTAGCAGCAGCTTC		
gluD P1	AAGCCAGTTGAATTTGGTGG	FAM	BHQ1

[a] Shown are the sequences of primer and probes, and their modifications, that were used in the GDH PCR assay at Leeds. *Yersi* primers and probes and *gluD* primers were described previously (9, 10), and the *gluD* probe was designed in house (Leeds).
[b] Cy5, cyanine 5; FAM, 6-carboxyfluorescein.
[c] BHQ, black hole quencher.

TABLE 2 Baseline characteristics of patients included in the study

Characteristic	No. (%) of patients at:			
	All sites	Leeds	Wishard	Tricore
Total	1,914 (100.0)	524 (27.4)	466 (24.3)	924 (48.3)
Age				
Child <2 yr	3 (0.2)	0 (0.0)	3 (0.6)	0 (0.0)
Child 2–12 yr	79 (4.1)	12 (2.3)	19 (4.1)	48 (5.2)
Adolescent 13–21 yr	58 (3.0)	11 (2.1)	17 (3.6)	30 (3.2)
Adult 22–59 yr	757 (39.6)	149 (28.4)	217 (46.6)	391 (42.3)
Adult ≥60 yr	1,017 (53.1)	352 (67.2)	210 (45.1)	455 (49.2)
Age class				
Pediatric (<22 yr)	140 (7.3)	23 (4.4)	39 (8.4)	78 (8.4)
Adult (≥22 yr)	1,774 (92.7)	501 (95.6)	427 (91.6)	846 (91.6)
Gender				
Female	1,123 (58.7)	296 (56.5)	267 (57.3)	560 (60.7)
Male	790 (41.3)	228 (43.5)	199 (42.7)	363 (39.3)
Nature of specimen				
Formed	47 (2.5)	0 (0.0)	0 (0.0)	47 (5.1)
Liquid	1,186 (62.0)	380 (72.5)	363 (77.9)	443 (47.9)
Semiformed	681 (35.6)	144 (27.5)	103 (22.1)	434 (47.0)

immunoassays were performed as per the manufacturers' instructions.

In-house PCR specific for *C. difficile gluD* was performed at Leeds on samples that had been frozen at −70°C, either at Leeds or before transportation from the other sites. Briefly, samples were defrosted and then diluted 1/10 in 1 ml STAR buffer (Roche, Germany) with the addition of 1/10 chloroform before being spun at 16,000 × *g* for 10 min in a centrifuge. An internal control (*Yersinia ruckeri*) was added to each sample before DNA was extracted on the QiaXtractor using the DX kit (Qiagen Ltd., United Kingdom). Template DNA was added to the Brilliant QPCR multiplex master mix (Agilent, United Kingdom) along with primers and probes for *gluD* and *Yersi* (Table 1). Amplification was performed on a Stratagene MX3000P (Agilent, United Kingdom) using the following thermocycling conditions: 95°C for 10 min followed by 45 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s. A previous evaluation determined that a *gluD* cycle time value of <35 cycles indicated a positive PCR result (data not shown).

In total, 1,914 samples were tested during the study; 1,906 had complete data for all the assays and were used for comparisons. Over half of the samples (53.1%) came from patients aged >60 years (Table 2), with slightly more women than men (ratio, 1.42 females:1 male). Liquid feces made up 62% of the total samples tested across all three sites (Table 2); only one site (Tricore) tested formed fecal samples (2.5% of the total).

Of the two reference culture media used, the chromID *C. difficile* medium was more sensitive than the Remel CCFA medium (67.0% versus 62.5%) compared with GDH PCR. The 95% confidence interval (CI) of the difference between the sensitivities of the assays did not include zero (difference, −4.5%; 95% CI, −7.5% to −1.4%), indicating a significant difference between the sensitivity of the two culture media (11).

The Vidas *C. difficile* GDH assay was 93.0% sensitive and 91.8% specific compared with chromID *C. difficile* (Table 3). The assay was slightly more sensitive compared with Remel CCFA (95.8%) but, conversely, was less specific (90.0%) (Table 3). The

TABLE 3 Sensitivities and specificities of GDH EIAs in comparison with those of two commercial selective *C. difficile* culture media

Comparator assay (total no. of samples tested)	Result of comparator assay (no. of samples)				EIA[a]							
					Vidas *C. difficile* GDH				Quik Chek-60			
	Positive	Negative	Equivocal	Invalid	Result (no. of tested samples)	Sensitivity (% [95% CI])	Specificity (% [95% CI])	Overall agreement (% [95% CI])	Result (no. of tested samples)	Sensitivity (% [95% CI])	Specificity (% [95% CI])	Overall agreement (% [95% CI])
ChromID medium (1,914)	357	1,557	0	0	Positive (332); negative (1,429)	93.0 (89.9–95.2)	91.8 (90.3–93.0)	92.0 (90.7–93.1)	Positive (332; negative (1,505)	93.0 (89.9–95.2)	96.7 (95.6–97.4)	96.0 (95.0–96.8)
Remel CCFA (1,914)	313	1,601	0	0	Positive (300); negative (1,441)	95.8 (93.0–97.6)	90.0 (88.4–91.4)	91.0 (89.6–92.2)	Positive (303); negative (1,520)	96.8 (94.2–98.3)	94.9 (93.8–95.9)	95.2 (94.2–96.1)

[a] Overall agreement of the Vidas *C. difficile* GDH assay (bioMérieux, France) or the Quik Chek-60 assay (Techlab, USA) when compared with two different reference culture media for *C. difficile* (comparator assays).

TABLE 5 Effect of analyzing equivocal GDH PCR results as positive, negative, or void on the performance of the Vidas *C. difficile* GDH assay using AUROC analysis[a]

Equivocal GDH PCR result	AUROC of Vidas *C. difficile* GDH assay compared with GDH PCR (%)	95% CI
Positive	0.83	0.80–0.86
Negative	0.86	0.83–0.88
Void[b]	0.86	0.84–0.89

[a] AUROC, area under receiver operator curve.
[b] GDH PCR equivocal results analyzed as void were removed from the analysis.

overall levels of agreement of the Vidas GDH assay with chromID *C. difficile* and Remel CCFA were 92% and 91%, respectively. There was geographical variance in the performance of the assay; sensitivity was lower at Leeds Teaching Hospitals NHS Trust but not statistically different from the two U.S. sites, while specificity was statistically lower at Tricore (data not shown). It should be noted, however, that neither medium performed as well as the Vidas *C. difficile* GDH assay compared with the GDH PCR assay (Table 4). In a three-way comparison, the 95% CI of the differences between sensitivities of the assays did not include zero, indicating a significant difference of the sensitivity of the Vidas *C. difficile* GDH assay versus both culture media (Table 4) (11). The sensitivity of the Vidas *C. difficile* GDH assay did not alter significantly if the equivocal GDH PCR results were treated as positive, negative, or void (Table 5). While the Vidas *C. difficile* GDH assay had higher sensitivity, it was significantly less specific than both culture media (Table 4), indicating that this assay may be useful only as a screening assay (for example, as part of a two-step diagnostic algorithm).

In this multicenter comparison study, we found that the Vidas *C. difficile* GDH assay was comparable in performance to the commercially available GDH EIA (Quik Chek-60, Techlab, USA), with an overall agreement of 95% (Table 6). The Vidas assay has the advantage of being automated, with good traceability and more comprehensive quality control than the Quik Chek. It is, however, slower (40 min run time) and requires a larger sample volume (200 μl versus 25 μl).

There are some limitations to our study. We did not use a gold standard reference method for a CDI diagnosis; however, GDH alone cannot reliably diagnose CDI and is diagnostic only in conjunction with a toxin detection assay (6). Culture and PCR for the *gluD* gene, therefore, are more representative comparators when assessing GDH detection assays. This does pose some difficulties when comparing our results with other publications, as most incorporated a reference method for diagnosing CDI. Notably, however, a meta-analysis showed that, for the three studies that compared a GDH assay with culture, the sensitivities of the GDH assays examined were 95.0%, 93.4%, and 93.5%, that is, comparable with the Vidas *C. difficile* GDH assay studied here (12).

GDH assays have proved to be useful as the first assay of a two-stage algorithm for CDI diagnosis (4, 5). It is important to emphasize that the second assay should detect toxins A and B of *C. difficile*, as detection of the toxin has been shown to correlate with both mortality and severity of infection (6, 13). The Vidas *C. difficile* GDH assay is a sensitive method that makes it suitable as a first assay in these recommended diagnostic algorithms.

TABLE 4 Three-way comparison between Vidas GDH assay and commercial media using GDH PCR as the reference method[a]

Assay performance	Vidas GDH		Remel CCFA			ChromID *C. difficile*		
	%	95% CI	%	95% CI	GDH minus Remel (95% CI)	%	95% CI	GDH minus ChromID (95% CI)
Sensitivity	71.2	66.7 to 75.3	62.5	57.8–67.0	8.7 (5.6 to 11.9)	67.0	62.4–71.3	4.2 (1.7 to 6.8)
Specificity	89.4	87.7 to 90.9	96.8	95.8–97.6	−7.4 (−8.9 to −6.1)	95.1	93.9–96.1	−5.7 (−7.2 to −4.3)

[a] The two *C. difficile* reference culture media are compared to the GDH PCR assay. The analysis presented used GDH PCR equivocal results as positive results.

TABLE 6 Agreement between the Vidas GDH assay and the commercial comparator assay Quik Chek-60 or the GDH *gluD* PCR assay

Comparator assay (total no. of samples tested)[a]	Result of comparator assay (no. of samples)				Vidas *C. difficile* GDH			
	Positive	Negative	Equivocal	Invalid	Result (no. of tested samples)	Positive agreement (% [95% CI])	Negative agreement (% [95% CI])	Overall agreement (% [95% CI])
Quik Chek 60 (1,914)	384	1,530	0	0	Positive (374); negative (1,444)	97.4 (95.3–98.6)	94.4 (93.1–95.4)	95.2 (94.2–96.1)
GDH PCR (1,906)[b]								
Equivocal as positive	424	1,482	45	0	Positive (302); negative (1,325)	71.2 (66.7–75.3)	89.4 (87.7–90.9)	85.4 (83.7–86.9)
Equivocal as negative	379	1,527	45	0	Positive (289); negative (1,454)	76.3 (71.7–80.3)	88.9 (87.2–90.3)	86.4 (84.7–87.8)

[a] Shown are the levels of agreement between the Vidas *C. difficile* GDH assay (bioMérieux, France) and the commercial comparator assay Quik Chek 60 (Techlab, USA) or the GDH PCR assay.
[b] Results for the comparison of the Vidas *C. difficile* GDH assay (bioMérieux, France) and the GDH PCR assay have been analyzed with the equivocal GDH PCR results included as either a positive or a negative result.

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REFERENCES

1. **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. http://dx.doi.org/10.1111/j.1469-0691.2009.03098.x.
2. **Lyerly DM, Barroso LA, Wilkins TD.** 1991. Identification of the latex test-reactive protein of *Clostridium difficile* as glutamate dehydrogenase. J Clin Microbiol **29:**2639–2642.
3. **Carman RJ, Wickham KN, Chen AM, Lawrence AM, Boone JH, Wilkins TD, Kerkering TM, Lyerly DM.** 2012. Glutamate dehydrogenase (GDH) is highly conserved among *Clostridium difficile* ribotypes. J Clin Microbiol **50:**1425–1426. http://dx.doi.org/10.1128/JCM.05600-11.
4. **United Kingdom Department of Health.** 2014. *Clostridium difficile*: updated guidance on diagnosis and reporting. http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_132927.
5. **Wren MWD, Sivapalan M, Kinson R, Shetty NP.** 2009. Laboratory diagnosis of *Clostridium difficile* infection. An evaluation of tests for fecal toxin, glutamate dehydrogenase, lactoferrin and toxigenic culture in the diagnostic laboratory. Br J Biomed Sci **66:**1–5.
6. **Planche T, Davies K, Coen P, Finney J, Monahan I, Morris K, O'Connor L, Oakley S, Pope C, Wren M, Shetty N, Crook D, Wilcox M.** 2013. Differences in outcome according to *Clostridium difficile* testing method: a prospective multicentre diagnostic validation study of *C. difficile* infection. Lancet Infect Dis **13:**936–945. http://dx.doi.org/10.1016/S1473-3099(13)70200-7.
7. **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 and cytotoxigenic culture methods. J Clin Microbiol **47:**3211–3217. http://dx.doi.org/10.1128/JCM.01082-09.
8. **Planche T, Aghaizu A, Holliman R, Riley P, Poloniecki J, Breathnach A, Krishna S.** 2008. Diagnosis of *Clostridium difficile* infection by toxin detection kits: a systematic review. Lancet Infect Dis **8:**777–784. http://dx.doi.org/10.1016/S1473-3099(08)70233-0.
9. **Lund M, Nordentoft S, Pedersen K, Madsen M.** 2004. Detection of *Campylobacter* spp. in chicken fecal samples by real-time PCR. J Clin Microbiol **42:**5125–5132. http://dx.doi.org/10.1128/JCM.42.11.5125-5132.2004.
10. **Paltansing S, van den Berg RJ, Guseinova RA, Visser CE, van der Vorm ER, Kuijper EJ.** 2007. Characteristics and incidence of *Clostridium difficile*-associated disease in The Netherlands, 2005. Clin Microbiol Infect **13:**1058–1064. http://dx.doi.org/10.1111/j.1469-0691.2007.01793.x.
11. **Clinical and Laboratory Standard Institute.** 2008. User protocols for evaluation of qualitative test performance; approved guideline—2nd ed. CLSI document EP12-A2. Clinical and Laboratory Standard Institute, Wayne PA.
12. **Shetty N, Wren MWD, Coen PG.** 2011. The role of glutamate dehydrogenase for the detection of *Clostridium difficile* in fecal samples: a meta-analysis. J Hosp Infect **77:**1–6. http://dx.doi.org/10.1016/j.jhin.2010.07.024.
13. **Longtin Y, Trottier S, Brochu G, Paquet-Bolduc B, Garenc C, Loungnarath V, Beaulieu C, Goulet D, Longtin J.** 2013. Impact of the type of diagnostic assay on *Clostridium difficile* infection and complication rates in a mandatory reporting program. Clin Infect Dis **56:**67–73. http://dx.doi.org/10.1093/cid/cis840.