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Comparison of VIDAS® GDH automated immunoassay with Cepheid GeneXpert® *C. difficile* PCR assay and an in-house PCR assay for GluD, for the detection of *C. difficile* in faecal samples

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Introduction

Laboratory diagnosis of *Clostridium difficile* infection (CDI) has traditionally involved the detection of organism specific toxins by cell-cytotoxicity assay or enzyme immunoassay. Recently the poor prognostic value of these assays has been highlighted[2] and alternative markers of CDI have been sought. Glutamate dehydrogenase (GDH) is a cell-surface associated enzyme found in many bacteria. *C. difficile*-specific GDH has been shown to be highly conserved between different PCR-ribotypes of *C. difficile*[1]. Assays targeting *C. difficile* specific GDH have been developed and a recent meta-analysis showed that GDH had a sensitivity and specificity >90% when compared to culture[8]. GDH cannot determine the toxigenic status of *C. difficile* however; it must be used as part of an algorithm for CDI detection, most commonly alongside toxin detection or polymerase chain reaction (PCR) for toxin genes. Although initial algorithms of GDH followed by either direct cell-cytotoxicity assay or cell-cytotoxicity assay on stool cultured isolates improved sensitivity, compared with standalone toxin enzyme immunoassays (EIA), the turnaround time could be up to 3 days[6, 9]. Using a toxin EIA for the second stage improves the rapidity of two-step algorithms[3, 7] but such algorithms are limited by the sub-optimal sensitivity of currently available toxin EIAs. Whilst there have been many algorithms proposed, a recent study led to guidance from the Department of Health in England advising using a two-stage algorithm, with GDH (or toxin gene PCR) as the initial screen, for detection of CDI[11].

Objectives:

To compare a new automated immunoassay, VIDAS® GDH, with the Cepheid GeneXpert® *Clostridium difficile* toxin Polymerase Chain Reaction (PCR) assay and an in-house PCR assay for GDH (*GluD)* of *C. difficile*, for the laboratory diagnosis of CDI.

Methods

One hundred and twenty seven cytotoxin positive diarrhoeal faecal samples submitted to Leeds Teaching Hospitals NHS Trust. Also, 300 samples submitted for routine *C. difficile* testing were collected between June and August 2011 (100 at each of 3 test sites in Leeds, Berlin and St Etienne). All samples were diarrhoeal (took the shape of the container), were <5 days old and stored at 4 C before commercial assay testing, before freezing at -20 C. The commercial assays, VIDAS® GDH and GeneXpert® *C. difficile*, were performed onsite at Leeds and St Etienne. Only the VIDAS® GDH was performed onsite in Berlin, the GeneXpert® *C. difficile* was performed in Leeds. All commercial assays were performed as per manufactures' instructions. The VIDAS® GDH assay was performed on a VIDAS instrument (bioMerièux, France), whilst the GeneXpert® *C. difficile* assay was performed on a smart cycler (Cepheid, France).

All samples (Berlin and St Etienne) were shipped at -20 C to Leeds for testing with an in-house GDH real-time PCR assay; samples from Leeds were initially tested before storage at -20 C. Briefly, samples were diluted in STAR buffer (Roche, Germany) including 1/10 chloroform, before being spun in a centrifuge at 1500g for 10 minutes. DNA was extracted from the supernatant using the Qiagen DX kit on the Corbett QiaXtrator (Qiagen Ltd, UK). An internal control of *Yersinia ruckeri*, an enteric pathogen of Salmonid fish species, was added to each sample before extraction to check for inhibition. DNA template was added to Brilliant QPCR multiplex mastermix (Agilent, UK), and primers and probes for *GluD* and *Yersi* (table 1). PCR was performed on a Stratagene MX3000P (Agilent, UK) with the following thermoprofile; 95 C for 10 mins, followed by 45 cycles of 95 C for 30 secs, 60 C for 30 secs and 72 C for 30 secs. Based upon limit of detection studies (data not shown), samples with a CT value for the internal control (*Yersi)* and no CT value or a CT of >40 cycles for *GluD* were recorded as negative. Samples with a CT value for *Yersi* and a CT value of <40 cycles for *GluD* were recorded as positive. Samples with no CT value for *Yersi* were recorded as inhibited and repeated where there was sufficient sample. If the negative control was positive (i.e. showing contamination) the samples were re-extracted and PCR was performed again.

Samples that were positive by the VIDAS® GDH assay but negative by *GluD* PCR were thawed, re-extracted and re-tested using the *GluD* PCR assay. In addition they were tested using a CE marked GDH assay (Alere, USA) and cultured directly onto ChromID® *C. difficile* agar (bioMerièux, France) and onto Brazier's agar (Oxoid, UK) following alcohol shock in 50:50 v/v absolute ethanol and water. If samples were positive by repeat *GluD* PCR testing, or negative on repeat *GluD* PCR testing but positive by the Alere assay and/or culture, or indeterminate on additional testing, the sample status was determined to be GDH positive. In three cases the initial GDH PCR was positive but with a CT >38.0, the repeat GDH PCR was negative and all additional tests were negative. These samples were classified as positive as it was considered that the repeat negative result could have been due to very low numbers; as demonstrated by the initial high CT value. Results were analysed both before and after repeat testing.

Sensitivity, specificity, positive and negative predictive values, and Pearson's correlation were calculated for the VIDAS GDH assay compared with the GeneXpert *C. difficile* PCR assay, and the in-house PCR assay for *GluD* (GDH).

Table 1. Primers and probes used for in-house GDH real-time PCR assay

Target	Primer or probe	Nucleotide sequence (5'-3')
Yersinia ruckeri 16s	YersiF1[4]	GGAGGAAGGGTTAAGTGTTA
Yersinia ruckeri 16s	YersiR1[4]	GAGTTAGCCGGTGCTTCTT
Yersinia ruckeri 16s	YersiP1[4]	CY5-GCGACTAACGTCAATGTTCAGTGC-BHQ2
GluD	GluDF3[5]	GTCTTGGATGGTTGATGAGTAC
GluD	GluDR2[5]	TTCCTAATTTAGCAGCAGCTTC
GluD	GluDP1[5]	FAM-AAGCCAGTTGAATTTGGTGG-BHQ1

Results

Table 2. Sensitivity, specificity, positive and negative predictive values and Pearson's correlation of the VIDAS GDH assay compared with two different PCR assays.

Comparator assay (n = 300)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Pearson's correlation
GeneXpert *C. difficile*[$]	98.5 (94.2 – 99.7)	88.1 (81.8 – 92.5)	87.6 (81.0 – 92.2)	98.6 (94.5 – 99.8)	0.86
In-house GDH PCR*	92.6 (86.5 – 96.2)	91.0 (84.8 – 94.9)	90.7 (84.2 – 94.7)	92.9 (87.0 -96.4)	0.84
In-house GDH PCR after discrepant analysis^	92.6 (86.5-96.2)	95.8 (90.6-98.3)	95.5 (89.9-98.1)	93.2 (87.4-96.5)	0.89

[$] n = 296 (invalids removed)
* n =280 (insufficient samples removed)
^ n = 278 (removed 2 samples where status could not be determined)

Table 3. VIDAS GDH false-negative sample characterisation

Sample No.	Frozen/Fresh on initial GDH PCR testing	Commercial GDH assay result	VIDAS GDH assay result	VIDAS GDH VT	Xpert toxin PCR result	In-house GDH PCR assay result	In-house GDH PCR assay CT value	Repeat In-house GDH PCR assay result	Repeat In-house GDH PCR assay CT value	Culture on Brazier's agar	Culture on Chrom ID® agar	Final decision on stool status
L9	Fresh	Positive	Negative	0.07	Positive	Positive	37.44	Positive	38.81	Positive	Positive	Positive
L27	Fresh	Positive	Negative	0.00	Negative	Positive	39.58	Negative	No CT	Negative	Negative	Positive
L44	Fresh	Negative	Negative	0.00	Negative	Positive	39.04	Negative	No CT	Negative	Negative	Positive
L48	Fresh	Negative	Negative	0.00	Negative	Positive	38.08	Negative	No CT	Negative	Negative	Positive
S67	Frozen	Negative	Negative	0.01	Negative	Positive	38.68	Negative	40.31	Negative	Negative	Positive
S70	Frozen	Negative	Negative	0.00	Negative	Positive	34.63	Positive	38.08	Insufficient to culture	Insufficient to culture	Positive
S77	Frozen	Negative	Negative	0.00	Negative	Positive	39.34	Negative	No CT	Negative	Negative	Positive
B39	Frozen	Negative	Negative	0.00	Negative	Positive	39.92	Positive	28.92	Positive	Positive	Positive
B47	Frozen	Negative	Negative	0.00	Negative	Positive	39.36	Insufficient	-------	Insufficient to culture	Insufficient to culture	Positive
B98	Frozen	Negative	Negative	0.00	Negative	Positive	39.10	Negative	No CT	Negative	Negative	Positive

Table 4. VIDAS GDH false-positive sample characterisation

Sample No.	Frozen/Fresh on initial GDH PCR testing	Commercial GDH assay result	VIDAS GDH assay result	VIDAS GDH VT	Xpert toxin PCR result	In-house GDH PCR assay result	In-house GDH PCR assay CT value	Repeat In-house GDH PCR assay result	Repeat In-house GDH PCR assay CT value	Culture on Brazier's agar	Culture on Chrom ID® agar	Final decision on stool status
L7	Fresh	Positive	Positive	2.77	Positive	Negative	42.09	Inhibited	--------	Positive	Positive	Positive
L97	Fresh	Positive	Positive	0.26	Positive	Negative	No ct	Negative	No CT	Negative	Negative	Negative
S7	Frozen	Positive	Positive	2.69	Positive	Negative	No ct	Negative	No CT	Positive	Positive	Positive
S19	Frozen	Positive	Positive	4.34	Positive	Negative	No ct	Negative	No CT	Insufficient to culture	Insufficient to culture	Negative
S22	Frozen	Positive	Positive	5.00	Positive	Negative	No ct	Negative	40.59	Insufficient to culture	Insufficient to culture	Unable to determine
S39	Frozen	Equivocal	Positive	1.61	Positive	Negative	No ct	Positive	39.80	Insufficient to culture	Insufficient to culture	Unable to determine
S43	Frozen	Positive	Positive	2.58	Positive	Negative	No ct	Negative	No CT	Insufficient to culture	Insufficient to culture	Negative
S53	Frozen	Positive	Positive	5.83	Positive	Negative	42.11	Positive	32.21	Insufficient to culture	Insufficient to culture	Positive
S57	Frozen	Positive	Positive	0.39	Negative	Negative	No ct	Positive	38.87	Negative	Negative	Positive
S90	Frozen	Negative	Positive	0.19	Negative	Negative	No ct	Negative	No CT	Negative	Negative	Negative
B9	Frozen	Positive	Positive	0.82	Negative	Negative	No ct	Insufficient	--------	Insufficient to culture	Insufficient to culture	Negative
B12	Frozen	Positive	Positive	6.31	Positive	Negative	No ct	Positive	26.21	Positive	Positive	Positive
B75	Frozen	Positive	Positive	5.40	Negative	Negative	No ct	Negative	No CT	negative	negative	Negative

Discussion

The VIDAS GDH assay has been compared here with two PCR assays (toxin gene detection & GDH gene detection). It showed a sensitivity and specificity of 92.6 and 95.8%, respectively, compared with PCR for GDH. VIDAS GDH had a sensitivity and specificity of 98.5 and 88.1%, respectively, compared with Cepheid *C. difficile* PCR. The GDH PCR assay was used as a comparator to examine the VIDAS GDH assays ability to detect GDH from a sample, not diagnose CDI. The comparison with the Cepheid *C. difficile* PCR assay demonstrates that, as expected, the specificity decreases to 88.1% when comparing with a method detecting potentially toxigenic *C. difficile*. This comparison demonstrates the equivalent sensitivity of the VIDAS GDH assay.

GDH detection in faecal samples has been shown to have a positive predictive value (PPV) (93.5%), when compared with culture of *C. difficile* from faecal samples [10], although it does not indicate the presence of a toxigenic isolate. The positive predictive value of GDH reduced in that study however, when compared with cytotoxigenic culture (67.5%) [10]. The poor PPVs seen in comparison with direct cytotoxin indicate that GDH should not be used as a standalone assay for the diagnosis of CDI[8].

VIDAS GDH has shown a high negative predictive value (NPV) in comparison to culture and direct cytotoxigenic culture, which correlates with the high NPV seen with other GDH EIA's[6-7, 9-10]. Indeed, the high sensitivity of these methods has lead to the inclusion of GDH as a first (screening) assay of two-stage algorithms for CDI diagnosis, although there is little consensus on the best algorithm [3, 6-7, 9-10]. A recent study involving ~12,500 faecal samples demonstrated that inclusion of GDH as the first line assay enables the detection of patients carrying *C. difficile* who are not toxin positive; i.e. potential *C. difficile* excretors[11]. These patients may be possible sources of organism transmission, and so may pose an infection control risk. Identification of these patients would enable infection prevention precautions to be put in place.

Conclusions

- **The VIDAS® GDH assay has comparable accuracy to the GeneXpert® *C. difficile* PCR assay and our in-house GluD PCR assay**
- **The VIDAS® GDH assay could be an option as a first line (screening) test in a two-stage *C. difficile* testing algorithm**
- **The optimal combination of tests depends on the clinical question to be answered**

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