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Comparison of the Vidas *C. difficile* and Quik Chek-60 glutamate dehydrogenase assays for the detection of *Clostridium difficile* in faecal samples

Sir,

Clostridium difficile infection (CDI) may vary from a mild, self-limiting diarrhoea to life-threatening pseudomembranous colitis and toxic megacolon.[1] With the emergence of more severe disease it has become crucial that diagnostic testing be rapid, sensitive and specific so that appropriate treatment may be initiated as quickly as possible. Glutamate dehydrogenase (GDH), also known as 'common antigen', is a sensitive marker for the detection of *C. difficile* in faecal samples.[2,3] Strategies to test for the presence of CDI in American and European guidelines [Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) and European Society of Clinical Microbiology and Infectious Diseases (ESCMID)] suggest a two-step algorithm in which the first step is a faecal GDH assay.[2,3] A positive GDH test is usually followed by either an enzyme immune assay (EIA) to detect the presence of *C. difficile* toxin(s), or a nucleic acid amplification test (NAAT) to detect the presence of the *tcdA* or *tcdB* genes which encode toxins A and B, respectively.[4]

A meta-analysis showed that detection of *C. difficile* GDH in faeces had a high diagnostic accuracy for presence of the organism and, when compared to culture, sensitivities and specificities of >90%.[5] *Clostridium difficile* GDH is encoded by the *gluD* gene which is highly conserved in both toxigenic and non-toxigenic members of the species.[6] Recently, 77 different ribotypes of *C. difficile* were tested and all carried *gluD* and all produced *in vitro* levels of GDH that were readily detected by various commercial kits tested.[6] This study confirmed earlier results by Goldenberg *et al.*[7] that there was no effect of ribotype on the detection of GDH produced by *C. difficile* and refuted earlier claims that suggested GDH assay performance was dependent upon strain type.[8] bioMérieux have released a version of the *C. difficile* GDH assay, an enzyme-linked fluorescence immunoassay (ELFA), to run on their automated Vidas platform. In this study, we compared the Vidas *C. difficile* GDH assay (bioMérieux, France) to the C. Diff Quik Chek-60 GDH assay (Techlab, USA) using direct culture of *C. difficile* on ChromID *C. difficile* agar (bioMérieux) as the gold standard. We have previously shown that direct culture of *C. difficile* from faeces on ChromID *C. difficile* agar gave results similar to those obtained after alcohol shock.[9]

A total of 403 faecal samples received by the PathWest Enteric Laboratory were tested on the day of collection with the Techlab Quik Chek-60 kit and then stored at 4°C for further testing within 24 h. The samples were brought to room temperature, re-tested with the bioMérieux Vidas *C. difficile* GDH assay and cultured on bioMérieux ChromID *C. difficile* agar.[9] Both GDH assays were performed according to the manufacturer's instructions. The Quik Chek-60 is a manual ELISA while the only manual steps with the Vidas platform are sample dilution and centrifugation before addition into the reagent strip. All agar plates were incubated for 24 h in an anaerobic chamber (Don Whitley Scientific, UK) (80% N_2, 10% CO_2 and 10% H_2) and checked for growth at 24 and 48 h. All discordant samples were cultured in Robertson's Cooked Meat medium containing gentamicin (5 mg/L), cycloserine (200 mg/L) and cefoxitin (10 mg/L) for 7 days at 35°C, then alcohol-shocked and plated onto pre-reduced cycloserine cefoxitin fructose agar supplemented with 0.1% sodium taurocholate (TCCFA) and incubated anaerobically for 48 h.[9] All presumptive *C. difficile* colonies on TCCFA were sub-cultured onto blood agar (BA) plates and checked for purity.

Presumptive *C. difficile* colonies were identified by their characteristic morphology on BA; natural chartreuse colony fluorescence under UV light on BA; and their ability to produce L-proline-aminopeptidase. Further confirmation of species identity was via a species specific PCR for toxin A (*tcdA*), toxin B (*tcdB*) and binary toxin genes (*tcdB*).[10] PCR ribotyping was performed[11] and banding patterns used to identify specific ribotypes (RTs) by comparison with a reference library of 50 RTs that included 15 reference strains from the European Centre for Disease Prevention and Control (ECDC) and a collection of the most prevalent PCR RTs currently circulating in Australia (B. Elliott, unpublished data). Interpretation of PCR ribotyping band patterns was performed by dendrogram and cluster analysis using the Dice coefficient within BioNumerics software package v.6.5 (Applied Maths, Belgium).

The Quik Chek-60 gave 51 positives and the Vidas GDH 76, with 42 and 46, respectively, being true positives. *Clostridium difficile* was grown from 48 samples, of which 34 were toxigenic. The Quik Chek-60 gave six false negatives while the Vidas gave two (Table 1). Two samples that were negative with both assays grew toxigenic *C. difficile* on ChromID agar and, after enrichment culture, only one further sample grew a non-toxigenic strain. After resolution of

Table 1 A comparison of assay results for the Vidas *C. difficile* (GDH) automated enzyme-linked fluorescence immunoassay (ELFA), C. Diff Quik Chek-60 and culture

Vidas *C. difficile* ELFA (GDH)	C. Diff Quik Chek-60 (GDH)	Culture	No. of specimen
Neg	Neg	Neg	319
Neg	Neg	Pos	2
Neg	Pos	Neg	6
Pos	Neg	Neg	27
Pos	Neg	Pos	4
Pos	Pos	Neg	3
Pos	Pos	Pos	42
Total			403

Neg, negative; Pos, positive.

Table 2 Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of C. Diff Quik Chek-60 and Vidas *C. difficile* GDH when culture was used as the gold standard before and after enrichment culture

	Before enrichment culture		After enrichment culture	
	Vidas *C. difficile* GDH	C. Diff Quik Chek-60	Vidas *C. difficile* GDH	C. Diff Quik Chek-60
Sensitivity	95.8% (85.7−99.4)	87.5% (74.0−94.8)	95.9% (84.8−99.3)	87.7% (74.5−94.9)
Specificity	91.6% (88.3−94.4)	97.5% (95.1−98.7)	92.1% (88.6−94.6)	97.7% (95.4−98.9)
PPV	60.5% (49.3−72.1)	82.3% (68.6−91.1)	62.7% (50.7−73.3)	84.3% (70.9−92.5)
NPV	99.4% (97.6−99.9)	98.3% (96.1−99.3)	99.4% (97.6−99.9)	98.3% (96.1−99.3)

Confidence limits (95%) are in parentheses.

discordant results, the final sensitivity, specificity, positive predictive values (PPVs) and negative predictive values (NPVs) for the Vidas GDH were 95.9%, 92.1%, 61.3% and 99.4% and for the Quik Chek-60 87.7%, 97.7%, 81.3% and 98.3%. The inclusion of the final true positive for each assay after enrichment culture did not alter sensitivity, specificity, PPV and NPV significantly (Table 2). When the *C. difficile* isolates were ribotyped, 13 isolates belonged to either RT 014 or the 014/020 group, the most common RT found in Australia.[12] Only one isolate was positive for binary toxin (RT 244) and 14 could not be assigned to a RT by comparison to our reference collection.

The bioMérieux Vidas GDH assay uses established automated technology with standardised interpretation of results. The Quik Chek-60 is a manual ELISA method which can be read on a microplate reader or is read visually as either positive (any yellow colour) or negative. Both assays gave NPVs of >98%; therefore, both are good screening tests to detect the presence of *C. difficile* in faecal samples. However, the Vidas GDH assay had a higher sensitivity (95.9%) than the Quik Chek-60 (87.7%), and a lower specificity (92.1% versus 97.7%), so there is a greater probability that the Vidas GDH assay will not miss true positives though there will be a larger number of false positives. This was seen here with the Vidas GDH finding 46 (97.9%) true positives and the Quik Chek-60 42 (89.3%).

In calculating PPV and NPV a prevalence of 50% is normally used as the 'standardised predictive value'[13] to reduce prevalence bias and to enable rapid comparisons of one diagnostic test with another. In this instance both assays had similarly high NPVs, but the Quik Chek-60 had a higher PPV by 21%. This again is an indicator of the higher number of false positives found with the Vidas GDH assay. However, GDH is not exclusively found in *C. difficile* and a number of other enteric organisms such as other clostridia, *Bacteroides* and *Peptostreptococcus* can react in the test,[14] although more recent versions of the test using monoclonal antibodies may be more specific.[5] In another recent evaluation of the Vidas GDH assay, where culture on TCCFA was the gold standard, the sensitivity and specificity were above 95% and the same NPV of 99.4% was recorded.[15] Recently Davies *et al.*[16] also compared the Vidas GDH assay with Quik Chek-60 and concluded that the assays had very similar performance characteristics with an overall agreement of 95% and a sensitivity of >93%.

Both assays had a high sensitivity or specificity as well as good NPVs and so would be excellent screening assays in a 2-step or 3-step algorithm for the detection of *C. difficile*. The number of initial positive results would be greater with the Vidas GDH assay than with the Quik Chek-60 due to the lower specificity and lower PPV, but because of the higher sensitivity it would be less likely that a true positive was missed. The Vidas GDH assay had the added advantage of being automated, and therefore a quicker and easier assay to use, with standardised interpretation of the results. However, in terms of everyday use both assays would be easy to integrate into a routine laboratory, are quick (turnaround time 1−2 h) and are relatively cheap as a screening tool.

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A case of *Nocardia mexicana* cerebral abscess highlights deficiencies in susceptibility testing and the utility of direct molecular identification

Sir,

We report a novel case of *Nocardia mexicana* brain abscess highlighting the utility of polymerase chain reaction (PCR) direct from clinical samples to achieve a species level identification and the divergence between validated susceptibility test methods for *Nocardia* and the Australian Therapeutic Guidelines with regards to meropenem.

An 81-year-old male presented to a regional hospital with new onset of dysarthria. He had a history of diabetes mellitus controlled with oral medication, polymyalgia rheumatica, subtotal gastrectomy three years earlier for stage IA gastric adenocarcinoma and untreated, indolent extranodal marginal zone B-cell lymphoma of bronchial associated lymphoid tissue. Non-contrast cerebral computed tomography (CT) scan showed changes consistent with small vessel ischaemic disease only. Subsequent magnetic resonance imaging (MRI) with contrast revealed a 14 × 12 mm ring enhancing lesion with surrounding oedema in the left temporal region. CT scan of the lungs showed unchanged right upper lobe ground glass changes and reduction in size of a left upper lobe soft tissue lesion that had been first noted one month earlier. He had low serum albumin, normocytic anaemia and thrombocytopenia but normal C-reactive protein. He had mild hyponatraemia with normal creatinine. He was commenced on trimethoprim-sulphamethoxazole (SXT) at 160/800 mg twice daily orally for a presumed brain abscess although no cultures were taken at that stage. Over the next six weeks he had persistent dysphasia with progressive cognitive decline and was no longer able to care for himself at home. Repeat MRI demonstrated increase in the size of the ring enhancing lesion to 25 × 22 mm which was now bulging into the lateral ventricle (Fig. 1). There was no significant change in his blood parameters.

He was referred to our centre and the lesion was aspirated. Purulent fluid was obtained with Gram's stain revealing many leukocytes as well as fine filamentous, branching, beaded Gram positive rods. He was commenced on meropenem 2 g 8 hourly and the SXT dose was increased to 320/1600 mg twice daily intravenously in line with the Australian Therapeutic Guidelines for treatment of cerebral nocardiosis.[1] The perioperative period was complicated by abscess rupture into the ventricle accompanied by minor intraventricular haemorrhage. No evidence of lymphoma was identified by cytological examination of aspirated fluid.

Given his pre-operative antibiotic treatment, there was concern that growth from the aspirate may be inhibited. Therefore, fluid was submitted for direct PCR and sequencing of the entire 16S rRNA gene using universal primers fD1 and rP2.[2] The 1399 bp consensus sequence (KU530187.1) was compared to NCBI reference strains in GenBank using the nucleotide-nucleotide Basic Local Alignment Search Tool (nBLAST) with a 99.79% sequence similarity to *Nocardia mexicana* (NR_117332.1 1393/1396 bp) and >0.5% distance from the next closest species match *Nocardia thraciencis* strain A2019 (NR_109057.1 1386/1399 bp). The closest type strain was *Nocardia mexicana* DSM 44952 (NR_104778.1 1393/1399 bp). Several of the GenBank *N. mexicana* sequences, including the type strain, have an unresolved base coded 'K' (G/T) which aligned with a 'T' in our sequence accounting for one of the base mismatches.

Following 6 days in room air at 37°C, a single colony was noted on chocolate agar. Subculture onto Middlebrook 7H10 produced dry, rough colonies pitting the agar with aerial hyphae, a brownish purple pigment and earthy odour. The same organism was isolated on subculture from Mycobacteria Growth Indicator Tubes (BACTEC MGIT 960; BD, USA) which flagged positive after 2 weeks incubation at both 32°C and 36°C. No reliable identification was achieved by matrix assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS; MALDI Biotyper system, Bruker) using the standard bacterial extraction method. The isolate was submitted for sequence identification but susceptibility testing was delayed because of the need to perform multiple subcultures to achieve an adequate inoculum. Susceptibility testing (Table 1) was performed by broth microdilution (BMD) using the Sensititre Rapid Growing Mycobacteria plate (TREK Diagnostics, Thermo Scientific, USA). The test was read at 72 h due to the slow growing, clumping nature of the organism. As meropenem is not included in the Sensititre tray, additional testing for meropenem and imipenem was performed by Etest (bioMerieux) giving minimum inhibitory concentrations (MICs) of 4 mg/L and >32 mg/L, respectively.[3] Trimethoprim-sulphamethoxazole BMD MIC of 4/76 mg/L was elevated