

An evaluation of the Gastro-Tect™ *C.difficile* toxin A+B antigen detection kit

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Introduction:

Clostridium difficile is one of the most frequently identified causes of nosocomial gastrointestinal disease. It has been implicated as a causative agent in antibiotic-associated diarrhea, antibiotic-associated colitis, and pseudomembranous colitis. *C. difficile*-associated diarrhoea (CDAD) is most often associated with prior antibiotic therapy, but an immunocompromised state, bowel surgery, and bowel stasis are also predisposing factors. CDAD may also occur when no known risk factors are present. Many strains of *C. difficile* produce two protein exotoxins, A and B, which are thought to be the primary cause of colonic mucosal injury and inflammation. Toxin A exerts primarily enterotoxic effects, while toxin B is primarily cytopathic. Most recently strains of *C. difficile* that produce a third toxin, binary toxin, have been described, and such strains are currently causing a large outbreak in North America (1).

Several laboratory techniques exist to aid in the diagnosis of CDAD. The organism may be detected by culture, enzyme immunoassay (EIA) for the *C. difficile* glutamate dehydrogenase common antigen, or metabolic end product analysis for isocaproic acid by gas-liquid chromatography. However, these methods are nonspecific in that both toxigenic and nontoxigenic strains of *C. difficile* are identified. PCR protocols have also been described but are not in widespread use. The current standard in the USA is the cytotoxin B tissue culture assay. It is most commonly performed directly on faeces, where it typically displays $\leq 85\%$ sensitivity. This sensitivity may be increased to $>99\%$ by combining tissue culture with culture of *C. difficile* either in broth or on CCFA, but this increase comes at a cost of increased turnaround time and expense. Tissue culture assay is not standardized, requires tissue culture facilities, and has a turnaround time of at least 24 h to 2 days (2).

In recent years, various EIAs for the detection of *C. difficile* toxins have become available. These newer tests have been reported to approach the sensitivity of tissue culture assay, are specific, and offer significant advantages in terms of turn around time, cost, and ease of performance (2). The Gastro-Tect™ *C.difficile* toxin A+B antigen detection kit is one of the newest of these kits available in Australia. As the name suggests, it detects both toxin A and toxin B. The results of a small evaluation of this kit are reported here.

Methods:

A total of 64 faecal samples was tested. These were samples submitted to the Enteric Laboratory at PathCentre specifically for testing for *C. difficile*, however, they were not consecutive samples. Positive samples by the reference method (tissue culture assay) were stored at -20°C until all samples were tested at roughly the same time. This increased the prevalence of disease artificially to around 30% which has implications for the sensitivity of the test.

The kit was used as per the manufacturer's instructions and results read manually.

All samples that were positive by the reference method but negative by the kit, and *vice versa*, were retested with the kit, together with a small selection of other samples.

Results:

The positive and negative controls provided gave appropriate results.

The results are summarized in the following Table.

	Cytotoxin positive	Cytotoxin negative	Totals
EIA positive	12	4	16
EIA negative	7	41	48
Totals	19	45	64

The sensitivity, specificity, PPV and NPV were 63%, 91%, 75%, and 85%, respectively.

A total of 23 samples was tested twice. One cytotoxin positive, EIA negative result was EIA positive on repeat, and one cytotoxin negative, EIA negative result was EIA positive on repeat testing.

The raw data are attached.

Comments:

The kit was easy to use and faster than many EIA kits in that only a 30 minute incubation step was required.

The yellow colour was easy to read, but an ELISA reader may make the task even easier, and less subjective.

The sensitivity and specificity figures are similar to other EIAs although it should be remembered that the sensitivity will decrease in a population in which the prevalence of disease is lower.

There are large gaps in our knowledge of the world-wide prevalence of toxin A negative toxin B positive strains of *C.difficile*. Although these are the best studied of the variant *C.difficile* strains, information about them is sparse. This is probably because many diagnostic laboratories test only for toxin A using a kit, in which case the strain will not be detected, or toxin B using tissue culture, in which case the strain will not be distinguished from a toxin A positive toxin B positive strain. However, these strains do cause significant disease and should be detected by the Gastro-Tect™ kit.

This was a small number of specimens and thus the results, while satisfactory, should be interpreted with this in mind.

References:

1) Riley TV. Nosocomial diarrhoea due to *Clostridium difficile*. *Curr Opinions Infect Dis* 2004; 17: 323-327.

2) Turgeon DK, Novicki TJ, Quick J, *et al*. Six rapid tests for direct detection of *Clostridium difficile* and its toxins in fecal samples compared with the fibroblast cytotoxicity assay. *J Clin Microbiol* 2003; 41: 667-670.

Raw data for *Clostridium difficile* Toxin A+B Antigen Detection Kit (GASTRO-TECT)

No	Sample ID	Cytotoxin	G-T EIA	Repeat
1	5000144	+	+3	
2	5000655	+	-	-
3:	5000681	+	+2	
4	5000639	+	+1	
5	5000167	+	+2	
6	4014372Z	+	+2	
7	4015079Q	+	+3	
8	4013117Y	+	-	-
9	4013581N	+	-	+2
10	4013591Z	+	-	-
11	798	+	-	-*
12	5000696	+	-	-*
13	5000707	+	+2	+2*
14	5001259	+	-	- *
15	4015980P	+	+1	+1 *
16	4015080R	+	+1	+1 *
17	4015951T	+	+3	+3 *
18	392	+	-	-*
20	4015078P	+	+2	+2 *
21	5001271	-	-	+1 *
22	5001270	-	-	- *
23	5001264	-	-	- *
24	5001282	-	+1	+1 *
25	5001286	-	-	- *
26	5001222	-	+3	+1 *
27	5001277	-	-	-*
28	5001258	-	-	-*
29	5001225	-	+2	+2 *
30	5001243	-	-	-*
31	5001195†	-	-	
32	5001276	-	-	
33	5001275	-	-	
34	5001278	-	-	
35	5001287	-	-	
36	5001289	-	-	
37	5001290	-	-	
38	5001254	-	-	
39	5001293	-	-	
40	5001260	-	-	
41	5001266	-	-	
42	5001176	-	-	

No	Sample ID	Cytotoxin	G-T EIA	Repeat
43	5001194	-	-	
44	5001244	-	-	
45	5001220	-	-	
46	5001221	-	-	
47	5001196	-	-	
48	5001288	-	-	
49	5001262	-	-	
50	5001165	-	-	
51	5001191	-	-	
52	5001245	-	-	
53	5001257	-	-	
54	5001265	-	-	
55	5001192	-	-	
56	5001193	-	-	
57	5001208	-	-	
58	5001256	-	-	
59	5001162	-	-	
60	5001199	-	-	
61	5001195†	-	-	
62	5001223	-	-	
63	255	-	-	
64	227	-	-	
65	Negative control		-	.*
66	Positive control (Toxin A)		+4	+4*
67	Positive control (Toxin B)		+4	+4*
† Two samples with the same number. * PBS was used as washing buffer.				