

Evaluation of three commercial enzyme immunoassay kits for detecting faecal *Clostridium difficile* toxins

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Abstract

The detection of faecal cytotoxicity using tissue culture was compared with three commercial *Clostridium difficile* enzyme immunoassay (EIA) kits; Premier *C difficile* toxin A (Meridian Diagnostic, Inc.); CD-TOX *C difficile* toxin A (Porton Cambridge); and Cytoclone A + B EIA (Cambridge Biotech Corporation). Of 160 faecal samples examined by all four methods, 52 (32.5%) were cytotoxic, 44 (27.5%) were positive by Premier, 48 (30%) by CD-TOX EIA, and 50 (31.3%) with Cytoclone. When compared with detection of cytotoxicity by tissue culture assay, the following performance indices were obtained: Premier, sensitivity 84.1%, specificity 99.1%, positive predictive value (PPV) 97.8%, negative predictive value (NPV) 93%; CD-TOX, sensitivity 92.3%, specificity 88.0%, PPV 78.7%, NPV 95.9%; Cytoclone, sensitivity 96.2%, specificity 93.5%, PPV 87.7%, NPV 98.1%. EIA results were available within three hours, whereas the results of the cytotoxin assay were available after 24-48 hours. All three kits provided satisfactory results and, although relatively expensive, all could be used in the laboratory effectively to screen for diarrhoeal disease associated with *C difficile*.

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Clostridium difficile is the major cause of pseudomembranous colitis and antibiotic associated diarrhoea.¹ Culture of the organism or demonstration of cytotoxicity remain the most commonly used tests for the laboratory diagnosis of *C difficile* associated diarrhoea. Culture of the organism is sensitive, although time consuming, but lacks specificity as non-toxicogenic strains will also be detected.² Detection of cytotoxicity using a tissue culture assay has been used as the "gold standard" for the diagnosis of *C difficile* associated diarrhoea because of its high sensitivity and specificity. However, many smaller laboratories are not in a position to undertake tissue culture techniques due to the lack of experience or equipment to perform the test. Recently developed commercial enzyme immunoassay (EIA) kits are now available to detect the presence of toxins A or B.³⁻⁶ These kits base the detection of toxin A or B on the use of specific antibody bound to a solid phase (microtitre well). We report here an evaluation of three EIA kits for

the detection of *C difficile* toxins in faecal samples.

Methods

Faecal specimens were collected between January and July 1993, from patients in Sir Charles Gairdner Hospital (SCGH), a 680 bed general teaching hospital. Specimens referred to SCGH from other hospitals and laboratories were also included. After routine culture for *C difficile* and testing for cytotoxin in tissue culture, the specimens were frozen at -20°C and later tested in batches with the three commercial kits. Tissue culture assays were also repeated to assess any degradation of the toxin(s) following the freeze/thaw cycle.

Vero cells were used to detect *C difficile* cytotoxicity. Cells were grown in Eagle's minimal essential medium supplemented with 3% fetal calf serum, 10 mg/l of gentamicin, and 10 mg/l of vancomycin. Cell lines were grown in an atmosphere of 10% CO₂ at 37°C. Faecal filtrates were prepared for detection of cytotoxicity by emulsifying about 1 g of faeces in 3 ml of saline and centrifuging at 2500 rpm for five minutes. After filtering through a 0.45 µm Millipore filter, 100 µl of filtrate was added to two wells of a multiwell (16 mm) Costar tissue culture plate seeded with Vero cells. To one well 100 µl of *Clostridium sordellii* antitoxin (Wellcome) was added. After 48 hours of incubation, the cells were examined for typical changes (rounding). Wells exhibiting over 80% rounding were considered positive.

Direct and enrichment culture for *C difficile* was performed by the methods described before.⁷

Kits used in the study were: (i) Premier *C difficile* Toxin A (Meridian Diagnostic, Inc.); (ii) CD-TOX *C difficile* Toxin A (Porton Cambridge); (iii) Cytoclone Toxin A + B EIA (Cambridge Biotech Corporation). All kits were used according to the manufacturer's instructions. All kits recommended the preparation of a 20% faecal suspension in the relevant diluent. All results were read by eye on a white background and colour intensity was compared with that of the negative control.

Results

One hundred and sixty faecal specimens were cultured for *C difficile* and tested by tissue culture assay and the three commercial kits. Fifty two (32.5%) were positive by tissue culture assay when tested after freeze/thawing. Seven

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Performance indices of EIA kits compared with tissue culture assay

	Premier	CD-TOX	Cytoclone A + B
Sensitivity	84.6%	92.3%	96.2%
Specificity	99.1%	88.0%	93.5%
PPV	97.8%	78.7%	87.7%
NPV	93.0%	95.9%	98.1%

samples were initially positive, but negative after freeze/thawing. Seven specimens were positive by culture but negative with all the kits and on cytotoxicity testing. Two of these specimens yielded cytotoxigenic strains of *C difficile* on culture and in vitro testing. The remaining five isolates were non-cytotoxigenic.

Of the 52 specimens positive on repeat cytotoxicity testing, 44 were positive with the Premier kit. Of the remaining 108 specimens negative by tissue culture assay, 107 were negative with the Premier kit. The one Premier positive, cytotoxicity negative specimen was culture negative but positive with the Cytoclone. All specimens negative by Premier kit were negative for *C difficile* by culture.

Of the 52 specimens positive by tissue culture, 48 were positive with the CD-TOX kit. Of the 108 specimens negative by tissue culture, 95 were negative with CD-TOX. Of the 13 CD-TOX positive specimens but negative for cytotoxicity, five yielded a cytotoxigenic strain of *C difficile* by culture and subsequent in vitro testing.

Fifty of the 52 specimens positive by tissue culture were positive with the Cytoclone kit. Of the 108 specimens negative by tissue culture, 101 were negative with the Cytoclone kit. Two of these specimens yielded cytotoxigenic strains of *C difficile*.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the three commercial kits compared with the tissue culture assay are shown in the table.

Discussion

Traditionally, the detection of *C difficile* toxin in the laboratory has been performed by tissue culture assay because of its extreme sensitivity and specificity. However, many small laboratories do not have either the experience or the facilities to perform tissue culture techniques. The incubation period for detection of cytotoxin in tissue culture may also be up to 48 hours. A toxin detection method which does not use tissue culture and which needs less incubation time would be ideal.

Rapid EIA techniques for the detection of *C difficile* toxin(s) are now commercially available. This study evaluated three: Premier *C difficile* toxin A (Meridian Diagnostic, Inc.); CD-TOX *C difficile* toxin A (Porton Cambridge); and Cytoclone *C difficile* A + B EIA (Cambridge Biotech Corporation). They were compared with our reference method for cytotoxin detection, a tissue culture assay using Vero cells. Overall, the kits performed exceptionally well with very little difference

between kits. The Premier kit was the most specific (99.1%), with a sensitivity of 84.6% and the Cytoclone was the most sensitive (96.2%), with a specificity of 93.5%.

With respect to the CD-TOX results, of 13 specimens negative by tissue culture but positive by CD-TOX, five yielded cytotoxigenic strains of *C difficile*. With this taken into consideration, the specificity of the CD-TOX kit increased from 88.0% to 92.6%. Similar results were obtained with Cytoclone. In this case, of seven specimens negative by tissue culture but positive by Cytoclone, two were culture positive for cytotoxigenic strains of *C difficile*. This improved the specificity of the Cytoclone from 93.5% to 95.4%. After taking these changes into consideration, the kit with the best performance indices was the Cytoclone with a sensitivity of 96.2% and a specificity of 93.5%.

It is interesting to speculate as to the reasons why some specimens were negative by tissue culture but positive by EIA methods. Seven culture positive specimens which were initially positive by tissue culture but negative after freeze/thawing were EIA (toxin A and B) positive. This suggests that the freeze/thawing cycle decreased the biological activity, but not the immunological activity of the toxin(s).

Several studies have evaluated various EIA kits for the detection of *C difficile* toxin A and toxin B since they became available.³⁻⁶ Several of these have assessed the Premier kit. Di Persio *et al*⁸ compared the Premier kit with a stool cytotoxicity assay and achieved figures for sensitivity and specificity of 84.1 and 98.9%, respectively. In a multicentre study of 504 samples, the sensitivity and specificity of the Premier kit were 86.6 and 99.0%, respectively, while the PPV and NPV were 94.7 and 97.4%, respectively.⁶ Borriello *et al*³ evaluated the Premier kit with 101 specimens of which 69 were positive by isolation of organism or cytotoxicity assay. Forty nine of these 69 were positive using the Premier kit. *C difficile* was cultured from 25% of specimens negative by cytotoxin or Premier assay, possibly suggesting a lack of sensitivity of the kit. Finally, Delmee *et al*⁴ determined the sensitivity and specificity of the Premier EIA kit to be 88 and 95%, respectively, when compared with tissue culture using Hela cells in a study of 228 specimens. Our results are similar to those quoted above, with good specificity (99.1%) and reasonable sensitivity (84.6%).

Fewer investigators have evaluated the Cytoclone A + B EIA kit. This test has the potential advantage of being able to detect both toxin A and toxin B. Most strains of *C difficile* produce both toxins, but the relative amounts of toxin produced may vary between strains. Doern *et al*⁵ compared this test with a cytotoxicity assay and recorded both a high sensitivity (94.9%) and specificity (98.9%). While we found a slightly higher sensitivity (96.2%), our specificity was slightly lower (93.5%). The sensitivity recorded was the best of the three kits evaluated and may be a reflection of the Cytoclone A + B kit's ability to detect both toxins. In addition, a high NPV

(98.1%) was calculated, suggesting that the Cytoclone A + B kit would make a useful screening test.

To our knowledge the CD-TOX kit has not been evaluated before. Although the figures for specificity and PPV were not as high as those for the other two kits, the high NPV (96.0%) would also make the CD-TOX a good screening test.

Procedures for using the Premier and the CD-TOX kits were similar. With the Premier kit, results were available in about two hours 15 minutes, while with the CD-TOX, results take only about one hour 15 minutes. The Cytoclone kit was slightly more time consuming involving a centrifugation step and also more wash steps than the other kits, and results were not available for about three hours.

Both the Premier and the Cytoclone kits cost about \$9.40 (roughly £4.60) per specimen while the CD-TOX kit cost is about \$6.25 (roughly £3.00) per specimen. These costs do not allow for controls and it should be noted that each batch of specimens must have at least one positive and one negative control. The CD-TOX kit actually recommends the use of four controls with each batch, thus increasing the relative cost per specimen. It is difficult to estimate costs of tissue culture techniques as there are many hidden factors requiring consideration. The laboratory must also have appropriate facilities to maintain the cell lines and have staff familiar with these techniques.

In conclusion, small laboratories without facilities for tissue culture should find the EIA kits an excellent alternative, with all three kits

performing well. An automatic plate washer and reader would further enhance the efficiency of these techniques. The CD-TOX kit is the least expensive, but if the recommendations of the manufacturer are followed, and four controls per batch are used, the cost per specimen is substantially increased. The Cytoclone kit gave the most impressive performance indices, but the test takes slightly longer to complete. All kits can be recommended and it must be left to individual laboratories to decide whether tissue culture methods or EIA kits meet the logistic needs or financial requirements of their laboratory.

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Imerslund-Grasbeck syndrome in a Chinese family with distinct skin lesions refractory to vitamin B₁₂

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Abstract

Two brothers in a Chinese family with selective malabsorption of vitamin B₁₂ associated with proteinuria (Imerslund-Grasbeck syndrome) presented with widespread mottled skin pigmentation, termed poikiloderma. In contrast to anaemia, this pigmentary disturbance remained unresponsive to vitamin B₁₂ replacement. This is different from the reported hyperpigmentation sometimes seen in vitamin B₁₂ deficiency which is reversible following treatment. As far as is known, an irreversible and persistent skin disorder has not been reported in this syndrome before.

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Familial selective malabsorption of vitamin B₁₂ associated with proteinuria was first described by Imerslund (1960)¹ and Grasbeck *et al* (1960)² (Imerslund-Grasbeck syndrome). It is inherited as an autosomal recessive trait and is prevalent among small inbred communities such as the Lapps in Scandinavia and North African Jews.³ Only a few cases had been reported in Orientals.⁴ We have seen two young men in a Chinese family with this syndrome associated with a skin disorder, known as poikiloderma. The anaemia in both patients responded to vitamin B₁₂ treatment but the skin lesions did