Exploring the accuracy and practicality of a new two-step algorithm for C. difficile testing

A two-step approach for diagnosing C. difficile has been found to be both cost-effective and significantly more sensitive than other methods in use.

INTRODUCTION

Rapid, accurate reporting of C. difficile is essential for improving patient outcomes and minimising healthcare-associated infections. However, several recent studies in the UK, Europe and the US have shown that current laboratory testing, based on enzyme immunoassay (EIA) detection of C. difficile toxins, may miss as many as 50% of positive patients (Fenner et al, 2008; Reller et al, 2007).

A systematic review of C. difficile toxin detection kits (Planche et al, 2008) concluded that ‘no single assay reliably fulfilled the criteria we preset for an acceptable test to detect C. difficile toxin’ and suggested a two-stage strategy to improve diagnosis.

A team at University College Hospital, London, has evaluated a two-step algorithm that incorporates an initial rapid screening test to detect the C. difficile antigen, glutamate dehydrogenase (GDH) (Wren et al, 2009). Faecal samples that are GDH-positive were tested for C. difficile toxins A/B and cultured for toxigenic strains. The study showed that GDH testing has a negative predictive value (NPV) of 99.5%.

Professor Mike Wren, consultant biomedical scientist at UCH, said: ‘Stand-alone faecal toxin testing is missing up to one-third of patients with proven serious disease, and is missing an additional 30% of patients with diarrhoea who are carrying toxigenic C. difficile. This has important clinical and infection control implications and there is clearly a need to reassess the laboratory approach to diagnosing diseased patients’.

AN ONGOING PROBLEM

Infection rates have risen substantially since reporting began, from 20,556 cases in 2000 to 49,785 in 2007 (Health Protection Agency, 2008a). Tackling this issue is a top priority (Department of Health, 2008; HPA, 2008b). The HPA funded a new initiative in 2007 to investigate and optimise management of C. difficile at local level (Donaldson, 2007). This project – the Clostridium difficile Ribotyping Network for England (CDRNE) – consists of six regional microbiology laboratories, including UCH, that provide culture and ribotyping services to infection control teams.

The challenge facing every hospital is the rapid, accurate diagnosis of C. difficile infection on a day-to-day basis.

STANDARD METHODS

Only strains of C. difficile that produce the toxins A (TcdA) and/or B (TcdB) cause disease. It is therefore essential that laboratory tests are able to quickly identify the presence of these toxigenic organisms in faecal samples.

The gold-standard method for detecting C. difficile toxins is the cell cytotoxicity neutralisation assay (CCNA). However, with a turnaround time of two days and a need for cell culture facilities and expertise, it is not a realistic option for many laboratories.

The CCNA is also less sensitive for detecting cases than the use of toxigenic culture (isolation of the organism and testing the isolate for toxin), which is the ‘alternative gold standard’ proposed by Professor Mark Wilcox from the CDRNE Reference Laboratory in Leeds. However, this takes at least 48 hours and, with increasing demand for same-day results, over 80% of UK microbiology laboratories have now abandoned culture and rely solely on rapid EIAs to detect C. difficile toxins A/B in faecal samples.

A number of commercial EIA tests are available. However, while rapid and easy to perform, EIAs have a low sensitivity compared with CCNA or culture and reports from prestigious centres have recently cited false negative rates as high as 50%. Ongoing studies at UCH show that toxin testing alone in its laboratory would miss over 30% of positive patients. There are also reports that EIA gives false positive results (Van den Berg et al, 2007).

THE TWO-STEP APPROACH

The UCH laboratory set out to investigate a new two-step algorithm that would reduce false negatives and positives to provide useful clinical results within the working day.

The study compared the new algorithm with the ‘alternative gold standard’ of culture and toxin testing, and has examined 1,007 samples (Wren et al, 2009). Results show the GDH screen has an NPV of 99.5% with very few false positives.

This algorithm includes an initial negative screening based on detecting the GDH antigen. Known as ‘the common antigen’, GDH is a constitutive metabolic enzyme produced in large quantities by all toxigenic and non-toxigenic strains, making it an excellent marker for the organism.
The team is using an easy-to-perform EIA that uses a GDH-specific mouse monoclonal antibody. As a first screening assay, it has an excellent NPV, allowing negative results, without further testing, to be reported with confidence (Wren et al, 2009; Fenner et al, 2008). As the GDH assay does not distinguish between toxigenic and non-toxigenic strains, GDH-positive specimens are then tested by a rapid toxin A/B test. Toxin positives are reported and cultured to allow further testing such as antibiotic susceptibility, ribotyping and molecular determination. Toxic testing of isolates also adds weight to decision-making.

RAPID DIAGNOSIS

Patients with C. difficile can develop serious disease including colitis, toxic megacolon and colonic perforation. They also present a significant risk to patients around them. While all those with diarrhoea should be nursed in isolation until the cause is diagnosed, high bed occupancy rates often make this difficult and infected patients are treated on open wards.

The anaerobic, spore-forming bacterium is present in the healthy guts of 3% of adults and 66% of neonates, only becoming a problem when the normal balance of gut flora is altered, allowing the organism to multiply rapidly (European Centre for Disease Protection and Control, 2008).

The diarrhoea associated with C. difficile causes widespread dissemination of the spores, which are highly resistant to surface cleaning agents and can be extremely persistent in the environment. As a result, other patients, staff and visitors are exposed to contamination, dramatically increasing the risk of cross-infection. The time to diagnosis is critical.

IMPROVED PATIENT CARE

A positive toxin result must always be considered in the context of the patient’s clinical condition. Not everyone carrying the organism develops disease and toxin-positive patients may be carriers and their symptoms unrelated to the presence of the organism. They do not require treatment but, as excreters of the organism, they present a potential risk to others and should be managed accordingly.

Patients with C. difficile-associated disease may have mild symptoms that will resolve without treatment after stopping their antibiotic or may develop serious infection that requires immediate intervention.

The bowel is extremely sensitive to C. difficile toxins and the disease can quickly progress to colitis and more severe manifestations (Barbut et al, 2005). Early antibiotic treatment (vancomycin or metronidazole) is very effective in halting its progress and minimising the need for surgery (colectomy). However, the relapse rate is high, reported as up to 30% (Monaghan et al, 2008), and treatment may itself lead to problems and contribute to the rate and severity of relapses. Managing a positive result therefore requires close cooperation between the laboratory and doctors. Rapid, reliable results allow practitioners to make informed clinical decisions and react quickly.

DISEASE OR CARRIAGE?

Many leading authorities believe that over-treatment of C. difficile is contributing to the problem and may be causing more disease.

Therefore, faced with a positive toxin result, healthcare staff must quickly decide whether the patient is suffering from true C. difficile disease or is just a carrier of the organism. To help with this, Professor Wren is looking to determine whether a rapid test for lactoferrin can prove a useful adjunct to GDH and toxin testing.

Previous studies have shown that patients with severe C. difficile infection contain high levels of lactoferrin in their faeces and that this association is statistically significant (Vaishnavi et al, 2000). The results of the lactoferrin test are available in 10 minutes, so there is no delay in reporting positives. The ongoing study at UCH shows that approximately 21% of patients with a positive toxigenic isolate but a negative direct faecal toxin test have evidence of intestinal inflammation (Wren et al, 2009).

COST BENEFITS

From the laboratory point of view, Professor Wren calculates that the cost per test for the two-step GDH screen followed by toxin A/B is very little more than performing only the toxin test, allowing a lactoferrin assay to be incorporated within the current budget. With accurate reporting and a better clinical picture, there is less repeat testing of negatives.

The financial benefits to the overall hospital budget are difficult to quantify but patient-related cost reductions include reduced antibiotic usage, fewer bowel investigations, such as colostomies and CT scans, and fewer colectomies.

CONCLUSION

Professor Wren says: ‘Antigen (GDH) testing screens out negatives with over 99% NPV and, by testing all GDH positives for toxins A/B and lactoferrin, followed up with toxigenic culture, we hopefully provide a more complete diagnostic picture. This algorithm utilises rapid, easy-to-use tests, costs very little more than current methods and introduces major benefits in terms of improved patient care.’

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