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C.difficile (including epidemiology)

Three-centre evaluation of laboratory *Clostridium difficile* detection algorithms and the EntericBio® realtime *C. difficile* assay

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ABSTRACT

The comparatively high cost of laboratory detection methods for *Clostridium difficile* infection (CDI) coupled to a low prevalence rate has resulted in testing algorithms that use cheaper and relatively sensitive screening methods, followed by more specific confirmatory methods. The aim of this prospectively-conducted study from two centres in the UK, and one in the Republic of Ireland was to determine the efficacy of the EntericBio® realtime *C. difficile* Assay (EBCD) for the detection of toxigenic *C. difficile* in stool samples.

The EBCD was compared to the in-use testing methods for *Clostridium difficile* (CD) detection in each centre. In the two UK centres, the EBCD was compared to the *C.diff* Quik Chek Complete® kit (Techlab), and discrepancies were tested further using The Xpert® *C. difficile* PCR assay (Cepheid) and PCR ribotyping after cultivation using the spore culture method, respectively. In the Irish centre, EBCD comparison was to an algorithm of C. DIFF CHEK™-60 test (Techlab) for screening followed by *C. difficile* Premier ™ Toxins A&B assay (Meridian Bioscience®) in the case of positive results; discrepancies were tested using the Xpert® *C. difficile* PCR assay.

In a retrospective analysis of data, a total of 947 stool samples were tested, of which eight (0.8%) proved inhibitory to the EBCD assay. Of the 939 valid tests conducted, reported sensitivities of the EBCD were 94.7%, 100% and 97.9%, respectively; specificities were 99.6%, 100% and 100%, respectively; positive predictive values were 94.7%, 100% and 100%, respectively, and negative predictive values were 99.6%, 100% and 99.8%, respectively. The CD positivity rates in the current study ranged between 6.6% and 8.2%.

1. Introduction

More than 2.5 million cases of healthcare-associated infections (HAI) occur in the European Union and European Economic Area (EU/EEA) each year [1]. HAI have been estimated to cost the United Kingdom National Health Service (NHS) £1 billion annually in recent years [2]. *Clostridium difficile* infection (CDI) is one of the most important HAI [3]. The spectrum of CDI varies from mild diarrhoea to pseudomembranous colitis and toxic megacolon [4]. Furthermore, one EU-based study reported when analysing data between 2000 and 2010 that CDI mortality at 30 days ranged from 2% to 42% [5]. Diagnosis of CDI is made on the combination of clinically suggested disease supported by laboratory test results. Laboratory confirmation of CDI relies on toxigenic *Clostridium difficile* (CD) detection, and a variety of strategies are used currently for this purpose. Traditional CD tests, such as toxigenic culture and cytotoxin neutralization (CTN) assays, have traditionally been identified as the gold standard methods for clinical diagnosis of CD [6]. However, CTN assays are time-consuming, thereby suggesting a need for alternative, sensitive, rapid-detection methods of diagnosing CDI. Furthermore, unfortunately, as a method, toxigenic culture is not standardized and thus can incorporate bias into data analysis [7] Enzyme immunoassays (EIA) to detect the toxin A and toxin B of CD have been used widely. However, a screening test

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which detects glutamate dehydrogenase, a cell wall antigen of *C. difficile*, was found to be significantly more sensitive than EIA making it an effective screening test for CDI [8]. The glutamate dehydrogenase (GDH) test, reported as having a high negative predictive value (NPV) of >99% [9], is a useful first test as it is relatively inexpensive and quick to perform. Because it lacks a correspondingly high specificity, further testing is required on GDH positive samples. The use of molecular-based assays to detect CD toxin genes has now become common, but these assays are expensive when compared to alternative methods. Understandably, algorithms that combine a set of CD detection and confirmation tests have been developed, such as that recommended by Schmidt & Gilligan (2009) [8].

In light of these detection methods being expensive or slow to generate a result as a single test, or being logistically complicated as testing algorithms, the purpose of the current study was to determine whether the EntericBio® realtime *C. difficile* Assay (EBCD) (Serosep Ltd., Limerick, Ireland) might be a suitable single-test alternative to the selected CD testing algorithms used in three separate laboratories. The findings following individual prospective studies in three centres, were compared retrospectively and are presented here as a joint paper.

2. Materials and methods

Test Centres conducted this prospective study of the performance efficacy of the EBCD when compared to their established testing methods for *C. difficile* detection.

For this paper the Microbiology Department at Sandwell and Birmingham Hospitals NHS Trust was designated Centre 1, and this study was conducted during September and October, 2014. The Microbiology Department at the Antrim Area Hospital, Northern Trust was designated Centre 2, in which the study was conducted during October and November, 2015. The Microbiology Department at Cork University Hospital was designated Centre 3, whose study was conducted between January and May, 2013.

3. Patient stool sample acceptance criteria for this study in the three testing centres

In Centres 1 and 2, stool samples from patients >2 years of age bearing a request for CD testing, and which showed a fluid or semi-formed appearance were included in a prospective study. In Centre 3, patients aged between 2 and 16 years were not routinely tested for CD but acceptance criteria regarding stool consistency were the same as elsewhere.

The testing methods and algorithms used in each of the three centres were used for evaluation of the EBCD; all are shown together in Fig. 1.

4. Ethics

Ethical approval was granted in the case of each of the three centres to conduct an anonymised study.

5. Methods

The CD faecal detection methods used in one or more of the three centres included in this study are outlined briefly below, and all methods were conducted according to the manufacturers' instructions. EBCD, which is the only one of the assays that has not previously been described in studies elsewhere, is outlined in some detail.

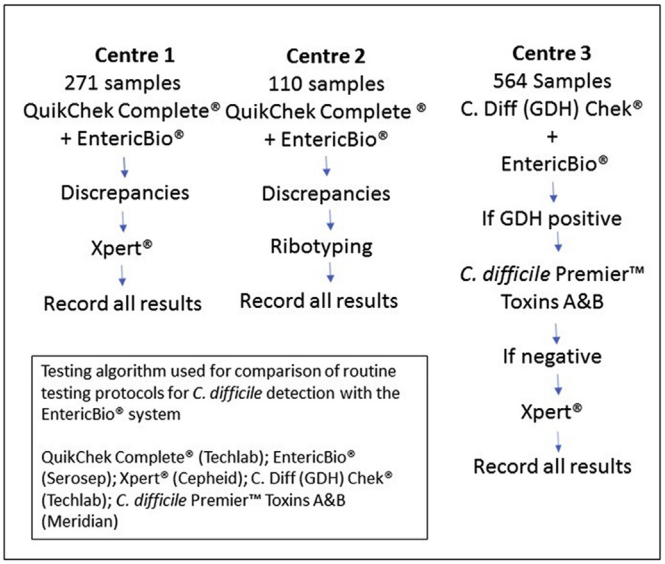

Fig. 1. Method for evaluation of the performance of the EntericBio® realtime *C. difficile* Assay when compared to established testing algorithms for *Clostridium difficile* detection in three clinical microbiology laboratories.

6. The C. DIFF CHEK™-60 test (Techlab, Blacksburg, VA)

This is an enzyme immunoassay (EIA) used as a screening test to detect glutamate dehydrogenase (GDH), an antigen produced by CD. The test does not distinguish toxigenic from nontoxigenic strains of CD, but was used in this study as a screening test for samples suspected of containing CD for which further assays were then used to detect the presence of CD toxins. The test took approximately 65–70 min to complete.

7. The *C. difficile* Premier ™ Toxins A&B assay (Meridian Bioscience®, Paris, France)

This EIA, which detects both toxins A and B, took approximately 35–40 min to complete following the selection of the higher incubation temperature of 37 °C and using a Stat-Fax-2200 heated plate shaker (Awareness Technology, NY, USA).

8. *C.diff* Quik Chek Complete® kit (Techlab)

This cassette EIA simultaneously detects both GDH antigen and toxins A & B of CD in faecal specimens in a test which took approximately 30 min to complete.

9. The Xpert® *C. difficile* PCR assay (Cepheid, Sunnyvale, CA)

This is a PCR assay, which uses a test cartridge, for the detection of the toxin B gene (*tcdB*), which is associated with toxigenic CD. Additionally, presumptive identification of 027/NAP1/BI strains of CD is by detection of binary toxin (CDT) gene sequences and the single base pair deletion at nucleotide 117 in the *tcdC* gene. The *tcdC* gene encodes for a negative regulator in CD toxin production. This test took approximately 50 min to generate a result.

10. Ribotyping

This method was used by Centre 2 for the four samples for which the routine CD testing method and the EBCD disagreed. Application of the spore culture method of cultivation, DNA extraction and