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Short communication

ASSESSMENT OF THE UTILITY OF REPEAT STOOL TESTING FOR *CLOSTRIDIUM DIFFICILE* STOOL TOXIN USING ENZYME IMMUNOASSAY

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Abstract. The poor performance of toxin enzyme immunoassay (EIA) for laboratory testing for *Clostridium difficile* (*C. difficile*) infection (CDI) is well acknowledged. Guidelines published in recent years state that testing solely with EIA for detecting toxins A and B is sub-optimal. As a consequence, clinicians may lose confidence in the test and submit multiple samples to offset the poor sensitivity of the toxin EIA. This leads to waste of laboratory resources and is discouraged by recent guidelines. 2,489 requests for toxin EIA submitted during one year at a state general hospital in Malta were reviewed to assess the utility of repeat stool testing for *C. difficile* toxin detection using toxin EIA and also to gather data on the extent of repeat samples within 28 days of a positive test. There were a total of 1,970 diarrhoeal episodes, from which a total of 302 cases (15.3%) submitted more than one sample for repeated testing. Only 2% of these repeats tested positive after having an initial negative result for the *C. difficile* toxin EIA test. Most recent published practice guidelines recommend a two-step or three-step testing algorithm in the diagnosis of *C. difficile*-associated diarrhoea, which offers a marked increase in sensitivity when compared to that of toxin A and B EIA alone. A three-step protocol is proposed which should enable the discernment of the role of *C. difficile* in a diarrhoeal patient.

Keywords: *Clostridium difficile*, repeat stool testing, toxin enzyme immunoassay, algorithm

1 Introduction

Clostridium difficile (*C. difficile*) is a Gram-positive, spore-forming bacterium, spread by the faecal-oral route, which is an important cause of nosocomial diarrhoea in industrialised countries. The bacterium is non-invasive and only toxigenic strains cause disease, due to the production of toxins A and/or B. Carriage occurs in 5–15% of healthy adults, but may be as high as 88.4% in newborns and healthy infants, and up to 57.0% in residents of long-term care facilities (Surawicz et al., 2013).

C. difficile very rarely causes spontaneous disease in healthy young individuals. However, antibiotics may disrupt the normal flora of the gut, leading to *C. difficile* overgrowth and, subsequently, *C. difficile*-associated diarrhoea. The latter can be complicated by pseudo-

membranous colitis, megacolon, perforation of the colon and possibly death. Clinical disease as a result of *C. difficile* is described as *C. difficile* infection (CDI). The diagnosis of CDI is usually based on the clinical history of the patient in combination with laboratory tests.

For the past 30 years, the two primary reference tests were the *C. difficile* cytotoxin neutralisation assay (CCNA) and the toxigenic culture (TC). These two methods are time-consuming and require specific laboratory facilities as well as technical expertise. As a result, many clinical laboratories have replaced the use of these two methods with the enzyme immunoassay (EIA) technique, which is able to detect both toxins A and B while being less labour-intensive and more cost-effective. In addition, it allows for faster turn-around time from the receipt of the sample to the issuing of results. This test also has high specificity. However, specificity is a test characteristic derived from the proportion of true negative results out of the total number of negative results produced by the test and is not affected by the prevalence of disease in the tested population. Therefore, the high specificity of EIA toxin A/B is offset in settings where CDI is uncommon, resulting in a low positive predictive value (PPV), whereby persons who test positive are less likely to truly have the disease. The sensitivity of the toxin EIA method is 79–80%, due to low reproducibility compared to the cytotoxin assay (She, Durrant & Petti, 2009). These factors undermine the confidence clinicians have in tests for CDI detection, thus prompting them to order multiple samples per patient.

The practice guidelines published by the Society for Healthcare Epidemiology of America (SHEA) in 1995 (cited in Gerding et al., 1995) state that when *C. difficile* is clinically suspected, a single stool specimen should initially be sent for testing. If the result of this first test is negative, one to two additional stool samples should be sent for re-testing. Thus, up to three serial toxin EIA tests increase the diagnostic yield by as much as 8–10% if the initial test is negative (Deshpande et al., 2010). In view of this limited increase in diagnostic yield provided by repeat testing, the 2010 SHEA position paper suggested that repeat testing during the same episode of diarrhoea is of limited value and should be discouraged (Cohen et al., 2010). Tests of cure following a positive *C. difficile* result are not recommended. *C. difficile* toxin positive patients do not need to be retested for *C. difficile* toxin if still symptomatic within a period of 28 days unless symptoms resolve and then recur, pointing towards a need to confirm recurrent CDI (Department of Health and Health Protection Agency, 2008).

In a study carried out in 2008 by Aichinger et al. (2008), of 683 patients who had three or more repeat EIA tests performed within seven days, 605 (88.6%) had only negative results. Twenty patients (2.9%) had a negative result on the first test with subsequent positive results on the following tests. In 12 patients, a positive result was obtained by the second test, in three patients by the third test, in four by the fourth test and in one by the sixth test. The remaining 58 (8.5%)

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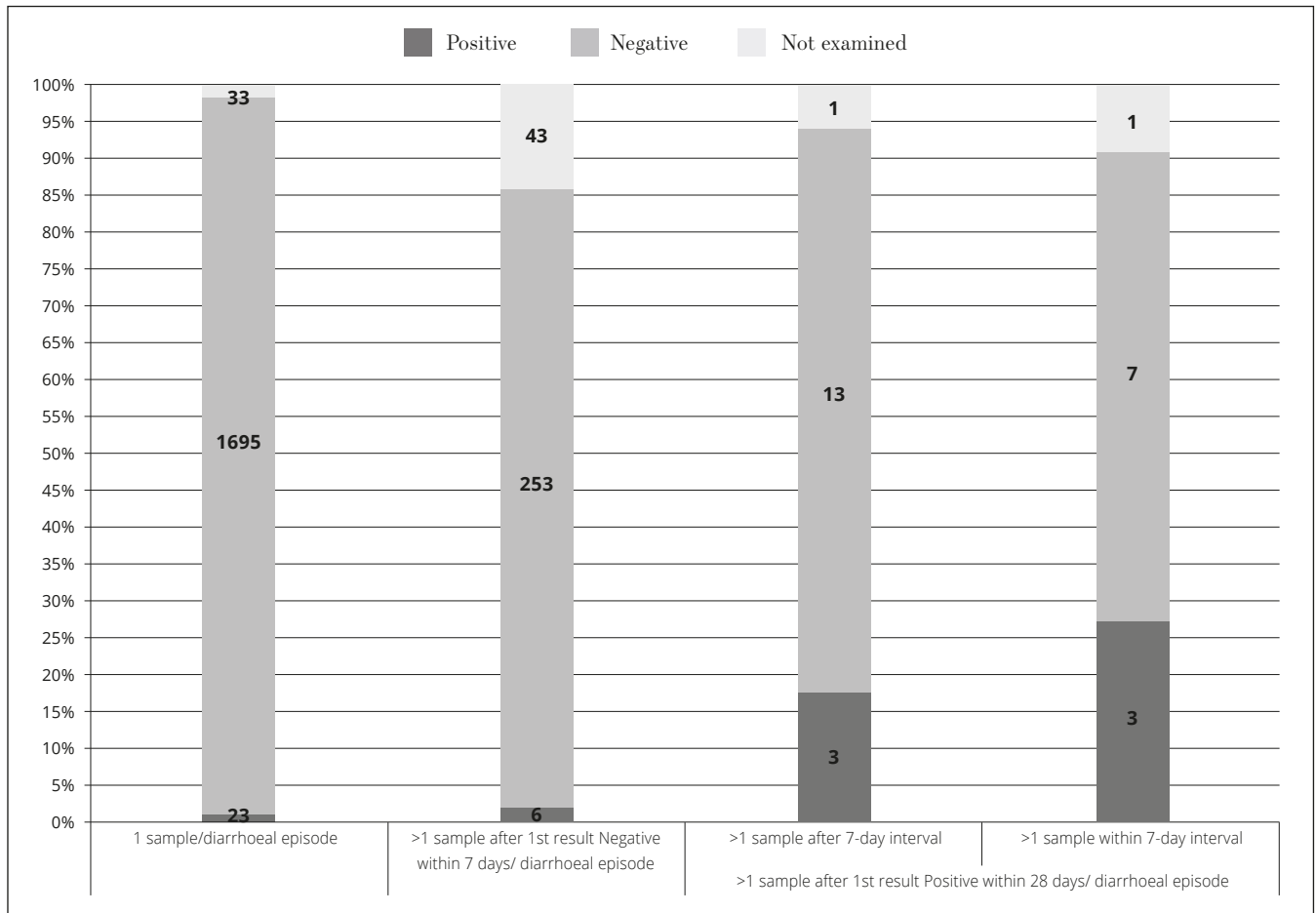


Figure 1. *C. difficile* stool testing outcome grouped according to number of samples sent per diarrhoeal episode

patients had a positive test followed by subsequent positive or negative tests (Aichinger et al., 2008). In a study by Nemat et al. (2009), out of 1165 positive cases, 1046 (89.8%) tested positive in the first test, 95 (8.2%) in the second, and only 24 (2.0%) tested positive in the third test. In the same study, a second test was ordered after an initial negative result in 1,934 cases, of which 95 (4.9%) tested positive, while in 793 episodes, a third test was ordered after two negative samples, of which only 24 (3.0%) resulted in toxin detection. These results highlight the ineffectiveness of repeat testing of stools for *C. difficile* toxin using EIA.

This study was carried out to assess the clinical value of the *C. difficile* toxin EIA technique, by determining the number of cases that had repeat stool tests performed during the same diarrhoeal episode, with a first negative stool toxin EIA result followed by a positive result. The study aimed to ascertain the diagnostic value of these repeat stool tests.

2 Methods

A retrospective study was carried out at the state general hospital in Malta, which is also a teaching hospital that covers the specialties of general medicine and surgery, geriatrics, paediatrics, nephrology, transplant, oncology and critical care. Permission to carry out the study was granted by the Chairman of the hospital's Pathology Department. The readily available and anonymous data was collected via a computer search using the laboratory information system. All requests

for *C. difficile* toxin testing between 1st July 2013 and 1st July 2014 were included in the study.

All faecal samples submitted for routine testing for *C. difficile* were processed using RIDASCREEN® *Clostridium difficile* Toxin A/B (Product C0801; R-Biopharm AG, Darmstadt, Germany). Sequential samples collected during the same diarrhoeal episode and received at the laboratory within seven days of an initial negative test result were considered repeats. In addition, samples submitted for re-testing after an initial positive result within a 28-day time frame were also assessed and included in the study.

3 Results

During the study period (1st July 2013 – 1st July 2014), the laboratory received 2,489 requests for testing stool samples for *C. difficile* toxins. These had been collected from a total of 1,689 patients. Some patients suffered more than one episode of diarrhoea. Upon grouping the requests submitted by patient and by date of submission, a total of 2,053 episodes of diarrhoea were identified. In 1,751 of these diarrhoeal episodes, only one sample was collected, from which 23 (1.3%) samples yielded a positive result (see Figure 1). There were 302 diarrhoeal episodes that were repeatedly tested, which resulted in the submission of 738 samples; the mode of the number of samples submitted per diarrhoeal episode was 2. From these 302 diarrhoeal episodes, only 6 (2.0%) had a negative result on the first test, with subsequent positive

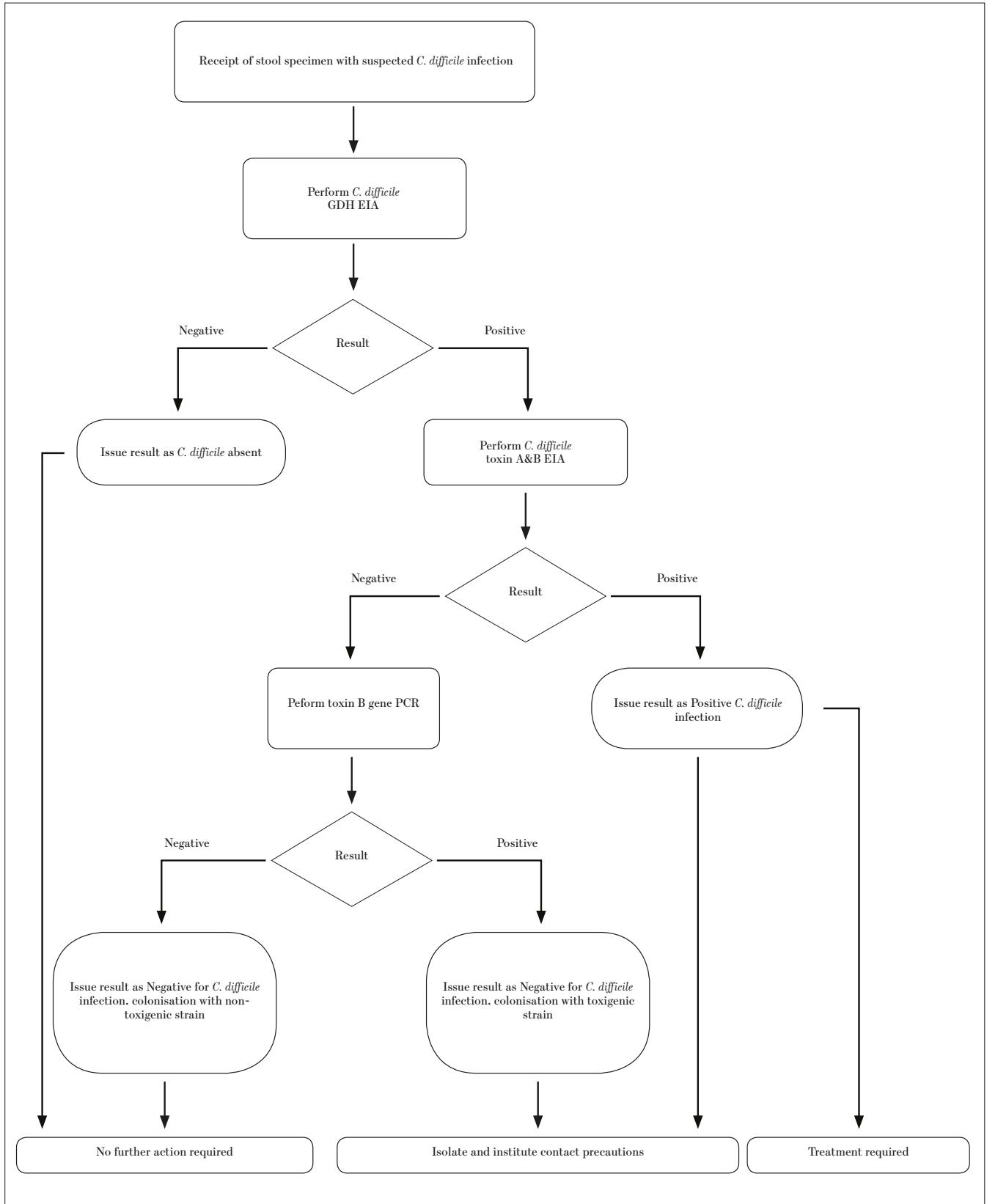


Figure 2. Proposed algorithm to diagnose *C. difficile* infection

results on a following test during the same episode. There were a total of 28 episodes in which the initial test resulted positive and repeat tests for toxin EIA were requested within a period of 28 days after the first positive result. In 11 of these episodes, the repeat test was requested within seven days of the first test.

4 Discussion

Across Europe, a daily average of 109 patients with CDI are undiagnosed due to lack of clinical suspicion or sub-optimal testing. This amounts to more than 39,000 cases which are potentially missed each year (Davies, Davis & Ashwin, 2014). Missed or imprecise diagnosis has implications for infection control practice and patient management.

The diagnosis of CDI is usually based on the clinical history in combination with laboratory tests. Various laboratory tests are currently available for the detection of *C. difficile* or its toxins. The diagnostic tests for *C. difficile* can be divided into (i) tests for *C. difficile* products (glutamate dehydrogenase (GDH), aromatic fatty acids, toxins A and/or B), (ii) culture methods for the detection of toxin-producing *C. difficile* (toxigenic culture), and (iii) tests for *C. difficile* genes polymerase chain reactions (PCR) for 16S ribonucleic acid (RNA), toxin genes, genes for GDH (Crobach et al., 2009).

Practice guidelines for the best testing strategy to diagnose *C. difficile* infection in a clinical laboratory suggest that *C. difficile* toxin EIAs are not suitable as standalone tests for the diagnosis of CDI in an endemic situation, due to the low prevalence rate that gives rise to low positive predictive values of diagnostic tests (Crobach et al., 2009). Various authors recommend a two-step method, whereby an initial highly sensitive and rapid screening test presumably detects all positive cases and is followed up by a second assay that identifies the true positive samples amongst all of the positive results detected during the screening test (Cohen et al., 2010; Crobach et al., 2009; De Silva, 2012; Surawicz et al., 2013).

The enzyme GDH is produced by all *Clostridium* species, including toxigenic and non-toxigenic strains of *C. difficile*, making it a good marker for the presence of *C. difficile* in stools. This is the basis for rapid detection methods using EIA. The negative predictive value (NPV) of the GDH test is comparable among populations having different *C. difficile* prevalence, thus making it a potential candidate for inclusion in a diagnostic algorithm for CDI (Crobach et al., 2009). A negative result would reliably exclude the presence of the organism in faeces. A positive GDH test has a very strong concordance with a positive culture but is not indicative of toxin production (Shetty et al., 2011). Positive GDH EIA requires further confirmatory testing as *C. difficile* infection is a toxin-mediated disease (De Silva, 2012).

Real-time PCR (RT-PCR) is unsuitable as a standalone test, as the lower 95% confidence interval for sensitivity is roughly 80–85% and that for specificity is about 93% (Planche & Wilcox, 2011). In settings of low prevalence, like in an endemic situation where the prevalence of CDI is expected to range between 5 and 10%, the PPV would be unacceptably low (Crobach et al., 2009). Thus, a highly specific test like RT-PCR in settings of low prevalence would lead to a high rate of false positive results. A false positive diagnosis could lead to the management and treatment of non-infected patients alongside true positive cases which, in turn, could potentially result in cross-infection of patients, thereby increasing the prevalence rate. Unnecessary treatment of CDI could be another outcome, potentially including the withdrawal of CDI-inciting antimicrobials that may be required to treat a concurrent infection. In addition, RT-PCR is significantly more expensive than EIA.

Therefore, an updated testing algorithm to conform to the recent guidelines is proposed (Figure 2). The current hospital guideline for the management of CDI would be updated to include guidance on test request submission, sample collection and result interpretation to complement the new testing algorithm, while discouraging repeat testing to ensure an efficient utilisation of laboratory resources. This approach must be accompanied by training to increase awareness of CDI, especially in view of recent insights into CDI epidemiology.

A number of limitations were present in this study. The type of stool sample submitted for testing was not recorded. Ideally, testing should be performed only on liquid specimens, thereby excluding testing on formed stool samples. Unfortunately, the number of tests carried out on formed stool samples is unknown. Moreover, data regarding the number of repeat stool tests performed until a positive test result was obtained following an initial negative result was not recorded. In addition, the time interval between the first negative test result and subsequent positive results was not documented.

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6 Conflicts of Interest

The authors report no conflicts of interest.

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