Procedure for the detection of *Clostridium difficile* toxin A+B in faeces

**Pathology Laboratory Manager**

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1.0 INTRODUCTION

Antibiotic-Associated Diarrhoea, Pseudomembranous Colitis, and *Clostridium difficile*

Clinical Manifestations

1.1 *Clostridium difficile* is a major nosocomial pathogen that causes a spectrum of intestinal disease from uncomplicated antibiotic-associated diarrhoea to severe, possibly fatal, antibiotic-associated colitis. Diarrhoea has come to be accepted as a natural accompaniment of treatment with many antibiotics and although this diarrhoea usually causes only minor concern, it can evolve into a life-threatening enterocolitis.

1.2 Many antibiotics have been associated with diarrhoea and with pseudomembranous colitis, including ampicillin/ amoxicillin, cephalosporins, clindamycin, and more recently quinolones (ciprofloxacin). Patients treated with clindamycin have a higher incidence of *C difficile* disease, but most cases are found in patients treated with other antibiotics because of the more widespread use of these agents. Occasionally, antineoplastic agents that alter the normal intestinal flora may also induce pseudomembranous colitis, with methotrexate most commonly implicated.

1.3 Chemotherapy-associated *C difficile* disease may not be easily recognized due either to an absence of antibiotic therapy or due to the frequent concomitant use of antibiotics, obscuring true incidence of chemotherapy-associated *C difficile* disease.

1.4 Clinical symptoms of *C difficile* infection (CDI) vary widely from mild diarrhoea to severe abdominal pain accompanied by fever (typically >101°F) and severe weakness. Diarrhoea is watery and usually non-bloody, but approximately 5 to 10% of patients have bloody diarrhoea. Faecal material typically contains excess mucus, and pus or blood may also be noted.

1.9 Pathology involves only the colon where there may be disruption of brush border membranes followed by extensive damage to the mucosa. The disease may progress to a pseudomembranous colitis, possibly including intestinal perforation and toxic megacolon. There is a leukocytic infiltrate into the lamina propria accompanied by elaboration of a mixture of fibrin, mucus, and leukocytes, which can form gray, white, or yellow patches on the mucosa. These areas are called pseudomembranes; hence the common term pseudomembranous colitis.

1.10 Pseudomembranes usually develop after 2-10 days of antibiotic treatment, but they may appear 1-2 weeks after all antibiotic therapy has stopped. Mortality varies, but may be as high as 10% in patients with pseudomembranous colitis. The ultimate cause of death often is difficult to determine, as most patients show a non specific deterioration over a period of weeks.
1.7 The incidence of pseudomembranous colitis has been diminishing in recent years, most likely due to early diagnosis of the disease and prompt antimicrobial therapy. However, *C difficile* is now considered a major cause of diarrhoea in hospitals and nursing homes. In most instances, once a patient develops antibiotic-associated diarrhoea and *C difficile* organisms and/or toxin is detected in the stool, appropriate antimicrobial therapy is initiated and the symptoms are not allowed to progress to the formation of colonic pseudomembranes. Thus, in recent years the terms "*C difficile* diarrhoea" and "*C difficile* infection" have come to be associated with a spectrum of diseases, including pseudomembranous colitis as well as diarrhoea and colitis in the absence of pseudomembranes. The common factors in all of these diseases are the presence of diarrhoea associated with antibiotic therapy and the recovery of *C difficile* organisms and/or toxin from the stool.

**Etiologic Agent**

1.8 *C difficile* is a slender, gram-positive bacillus that produces large, oval, subterminal spores. It is an anaerobe, and some strains are extremely sensitive to oxygen. *C difficile* is non-haemolytic and does not produce lecithinase or lipase reactions on egg yolk agar. The products of fermentation are many and complex and include acetic, butyric, isovaleric, valeric, isobutyric, and isocaproic acids; however, only small amounts of each are produced.

**Pathogenesis**

2.2 *C difficile* infection is caused by the overgrowth of the organism in the intestinal tract, primarily in the colon. The organism appears unable to compete successfully in the normal intestinal ecosystem, but can compete when normal flora are disturbed by antibiotics, allowing overgrowth of *C difficile*.

2.3 This organism then replicates and secretes two toxins. **Toxin A** is an enterotoxin that causes fluid accumulation in the bowel and is a weak cytotoxin for most mammalian cells; **toxin B** is a potent cytotoxin. Nearly all toxigenic strains produce both toxins A and B. Highly toxigenic strains produce high levels of both toxins, while weakly toxigenic strains produce low levels of both toxins. The production of these two toxins causes the characteristic mucosal damage consisting of plaque-like lesions leading to the formation of a pseudomembrane. Not all strains of *C.difficile* produce toxin and therefore not all cause illness.

2.4 Results from *in vitro* studies using cultured intestinal epithelial cells have indicated that toxin A causes necrosis, increased intestinal permeability, and inhibition of protein synthesis. Toxin A somehow affects phospholipase A2, resulting in the production of several arachidonic acid metabolites including prostaglandins and leukotrienes. Although
the exact mechanism of endocytosis is unclear, both toxins A and B are internalized by host cells, resulting in alterations in the actin-containing cytoskeleton.

2.5 Both toxins A and B have effects on leukocytes that include alterations in actin cytoskeletal microfilaments, and induction of tumour necrosis factor, interleukin 1, and interleukin 6. These latter effects contribute to the inflammatory response associated with \textit{C difficile} infection. Both toxins A and B kill experimental animals, and both probably are involved in the pathology of disease. Toxin production causes diarrhoea that may progress to pseudomembranous colitis, where the characteristic pseudomembranes are largely limited to the colon. In the intestinal tract, toxin A damages villous tips and brush border membranes, and may result complete in erosion of the mucosa. This tissue damage causes a viscous haemorrhagic fluid response. In contrast, toxin B does not have noticeable enterotoxic activity, but it is lethal when injected into experimental animals.

1.13 Thus, it seems reasonable to speculate that, in humans, toxin B exerts its pathogenic effect following dissemination through a damaged gut wall to extraintestinal organs. It has been speculated that infants harbouring high levels of intestinal toxins A and B are at risk for the systemic toxicity of toxin B if their intestinal barrier is compromised.

**Epidemiology**

1.14 \textit{C difficile} has been associated with outbreaks in hospitals and in extended care facilities for the elderly and represents an important cause of hospital-acquired infection. It can be isolated from soil, hospital environments and both human and animal faeces. It is rarely found in the flora of normal adults but up to 50\% of infants may be colonised in the first few months although disease is rarely present at this age.

1.15 \textit{C difficile} is a member of the normal intestinal flora of <3\% of adults. The organism can be acquired as a nosocomial pathogen and a variable incidence of disease is noted in hospitals and nursing homes. This seems to be due in part to environmental contamination with \textit{C difficile} spores, and in part to different patient populations in various institutions. Patients over 65 years of age seem to be at particular risk of acquiring \textit{C difficile} infection.

1.16 Patients with \textit{C difficile} diarrhoea excrete large numbers of \textit{C difficile} spores, and epidemiological studies have shown that the organism can reside on environmental surfaces as well as on the hands of health care workers. Healthy adults do not carry significant numbers of the organism in their intestinal tracts, but healthy infants may have large numbers of these organisms in their faeces. Most studies report a high carriage rate of approximately 50\% in neonates, although some studies report a carriage rate of 0 to 6\%, likely due to differences in environmental exposure to the organism. The toxins also are present in these infants' stools, and the same amounts of toxins are associated with disease in adults. The toxins typically have no adverse effect in infants, but confound the diagnosis of \textit{C difficile} infection (CDI). There is circumstantial evidence supporting the theory that infants do not develop disease because they lack specific intestinal receptors.
for *C difficile* toxins. In recent years, *C difficile* as also emerged as one of the causes of chronic diarrhoea in AIDS patients.

1.0 In common with several other developed countries, the number of reported cases of *C difficile* infections (CDI) in England and Wales has increased dramatically during the past decade. There are also concerns that *C difficile* infection (CDI) is more likely than previously to affect younger age groups. Alongside these developments, the proportion of *C difficile* infections (CDI) in England caused by ribotype 027 strain has increased substantially in the last few years. This particular ribotype 027 has also been implicated in outbreaks in WWL.

This strain has been characterised as a hypertoxin producer, and has been associated with *C difficile* infection outbreaks in which greater than average severity of *C difficile* infection (CDI) and mortality, together with high attack rates in the elderly and unusual numbers of cases in younger people, were reported.

**Diagnosis**

1.18 It is often difficult to distinguish *C difficile* disease from other intestinal diseases, including ulcerative colitis and Crohn's disease. Diagnosis of *C difficile* infection includes the presence of diarrhoea associated with antibiotic therapy in the preceding 4 to 6 weeks, and the recovery of *C difficile* organisms and/or toxin from the stool. However, the isolation of toxigenic *C difficile* from patients is often not a definitive diagnosis because other enteric pathogens are usually not excluded. Many cases of severe diarrhoea are caused by other enteric pathogens such as *Campylobacter* spp, *Salmonella* spp, *Shigella* spp, toxigenic strains of *Escherichia coli*, etc. Moreover, antimicrobial therapy increases the likelihood of isolating *C difficile* from the faecal flora:

1.19 *C. difficile* can be isolated from the faeces of approximately 20 to 40% of asymptomatic hospitalized patients who are receiving antimicrobial therapy. Despite these caveats, *C difficile* is likely responsible for 25% of cases of antibiotic-associated diarrhoea and colitis. Other microorganisms causing antibiotic–associated diarrhoea are *Candida albicans*, *Staphylococcus aureus*, *Klebsiella oxytoca*. Diagnosis of pseudomembranous colitis requires demonstration of pseudomembranes by colonoscopy, and *C difficile* can be isolated from the stools of almost all patients with this disease.

1.20 A selective agar medium may be used for the isolation of *C difficile* from stool, however toxin detection is normally used for diagnosis. Although the most appropriate test for toxin detection remains controversial, a cellular cytotoxicity test remains the "gold standard" unfortunately this test is time-consuming and cumbersome. Enzyme-linked immunoassays can be used to detect both toxin A and toxin B, and these tests are useful for diagnosis of *C difficile* infection.

1.21 **Typing of isolates** of *C difficile* is sometimes useful in the investigation of multiple cases of infection. Typing methods that have been used include bacteriophage/bacteriocin typing and serotyping. PCR ribotyping is gaining acceptance as an internationally
recognised method and within England a PCR ribotyping network (CDRNE) has been
established by the HPA for use where there is an increase in frequency of CDI, or
increased severity, complication, recurrence or death rate associated with CDI.
The Anaerobe Reference Laboratory in Cardiff currently provides the same service for
Wales and the rest of the UK and performs the typing for the DH/HPA surveillance
scheme In England. Ribotyping and other more refined, molecular methods of strain
differentiation are performed at the Centre for Infection. Other methods include cell
surface protein profiles and other DNA-based methods of analysis.

1.22 Within England there is now a *C. difficile* Ribotyping Network (CDRNE) consisting of
six laboratories (in Leeds – reference centre, Birmingham, London, Manchester,
Newcastle and Southampton) which is accessed in agreement with the relevant Regional
Microbiologist. Access to this network should occur if a laboratory believes they have or
could have a problem with an increased frequency or severity of cases of *C. difficile*
infection, including increases in mortality, complications or recurrence rates. A
standardised request form has been widely circulated in electronic format, which must be
completed to access the service (see Appendix A). Further details are available via the
HPA www site.

**Control**

1.23 In many cases, symptoms resolve 1-14 days after the offending antibiotic is discontinued,
and antibiotic treatment is not needed. Vancomycin or metronidazole are the antibiotics
of choice to treat active disease. Oral vancomycin is the "gold standard," and
metronidazole is most often used to treat milder infections. Some claim that
metronidazole should be considered the drug of choice in all but the most severe cases,
based on relative cost of the two drugs and based on prevention of development of
vancomycin resistance in enteric bacteria.

1.24 *C. difficile* is susceptible to both of these antimicrobial agents, but relapses occur in 15 to
20% of patients. Some patients have had many repeated relapses. Supportive therapy is
needed to compensate for the often severe fluid and electrolyte loss. Health care workers
caring for patients infected with *C. difficile* should wear gloves and strictly adhere to
proper hand washing procedures.

1.25 The Anaerobe Reference Laboratory is monitoring antimicrobial susceptibilities of all
isolates submitted under the DH/HPA surveillance scheme using the E test method for
MIC determination to eight antibiotics. It is important that regular testing is done by all
CDRNE laboratories* to screen for any emerging resistance to the drugs of choice for
treatment, namely metronidazole and vancomycin.

CDRNE* - *C. difficile* Ribotyping Network (CDRNE)
2.0 PRINCIPLE OF THE TOXIN DETECTION EIA TEST

2.1 *Clostridium difficile* TOX A/B II is an enzyme immunoassay for the detection of toxins A and B produced by toxigenic strains of *Clostridium difficile*. It can be used to detect toxins A and B from faeces specimens as an aid in the diagnosis of *Clostridium difficile* infection.

1.0 The test uses antibodies to *Clostridium difficile* toxins A and B. The microtitre wells contain immobilised affinity-purified polyclonal goat antibody against toxins A and B.

2.0 The detecting antibody consists of a mixture of toxin A monoclonal mouse antibody conjugated to horseradish peroxidase.

3.0 If toxins A and B are present in the specimen they will bind to the detecting antibody and to the immobilised polyclonal antibody during the incubation phase.

4.0 The washing steps remove any unbound material and following the addition of substrate, a colour is detected due to the enzyme-antibody-antigen complexes formed in the presence of toxin.

3.0 MATERIALS

3.1 *Clostridium difficile* TOX A/B II kit, manufactured by Tech Lab (Bioconnections, Leeds)

The kit contains:-

(iii) Diluent
(iv) Conjugate
(v) Substrate
(vi) Positive Control
(vii) Wash Buffer Concentrate
(viii) Stop solution (1M sulphuric acid: caution, corrosive)
(ix) Microassay plate
(x) Applicator sticks

4.2 Wash bottle
4.3 Timer
4.4 Discard container
4.5 Distilled water
4.6 Vortex mixer
4.7 Refrigerator (2-8°C)
4.8 Incubator (35°C – 37°C)
4.0 STORAGE AND SHELF LIFE

4.1 The expiration dates for each component of the kit are listed on the individual labels.

5.3 The kit containing the reagents with designated shelf life should be stored in a refrigerator between 2°C and 8°C.

5.4 All reagents must be allowed to equilibrate to room temperature before use and after removal from the refrigerator.

5.5 Return the kit and reagents to the refrigerator as soon as possible after use.

5.0 PRECAUTIONS

5.1 Scrupulous hygiene precautions must be taken in order to avoid gastro-intestinal infections from these and other enteric pathogens found in faeces.

5.2 Thorough hand-washing with soap and warm running water and drying is a very important precaution in preventing infection with enteric pathogens.

5.6 Ensure that your hands are kept away from the mouth.

5.4 Observe routine laboratory precautions as stated in the laboratory safety policy.

5.5 Wear disposable vinyl or latex gloves as appropriate when handling clinical material.

1.0 The stop solution contains 1M sulphuric acid. Wash with water immediately if contact occurs.

2.0 Use faeces specimens within 24 hours of collection to obtain optimal results. Specimens should be frozen (-20°C or lower) if not processed within 24 hours in order to reduce the degradation of the toxins.

3.0 All materials and reagents used are for in vitro use only – not for internal or external use on humans.

Please Note: All specimens for the detection of C. difficile toxins should now be processed on the day of receipt or within 24 hours of collection. National guidelines stipulate that samples should be obtained and tested within 18 hours of the onset of symptoms. Samples received overnight will be processed the following day.
COSHH / RISK ASSESSMENT OF WORK ACTIVITY
For use with micro-organisms or micro-organisms and chemicals

Department: Microbiology Department, Royal Albert Edward Infirmary

Authorised Personnel: Biomedical Scientists, (all grades)
Trainee Biomedical Scientists (under supervision)

Procedure: Procedure for the detection of *C. difficile* toxins A & B from faeces

### HAZARDS

<table>
<thead>
<tr>
<th>MICRO-ORGANISMS</th>
<th>ACDP CATEGORY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potentially any enteric pathogen, including <em>Salmonella</em> sp., <em>Shigella</em> sp., <em>Campylobacter</em>, <em>E.coli</em> 0157, <em>Clostridium difficile</em></td>
<td>2</td>
</tr>
</tbody>
</table>

### CHEMICALS USED

<table>
<thead>
<tr>
<th>CHEMICALS USED</th>
<th>NATURE OF HAZARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stop reagent (1M sulphuric acid)</td>
<td>Refer to COSHH data sheet B48 Corrosive</td>
</tr>
<tr>
<td><em>C. difficile</em> TOXA/B kit</td>
<td>Refer to manufacturers datasheet</td>
</tr>
<tr>
<td>TOX A/B Quik Chek</td>
<td>Refer to manufacturers datasheet</td>
</tr>
</tbody>
</table>

### CONTROL MEASURES

Routinely, process using Containment level II precautions.

**Scrupulous** hygiene precautions must be taken in order to avoid gastro-intestinal infection from pathogens associated with faeces specimens.

**Thorough** hand washing with soap in warm running water and drying is a very important precaution in preventing infection with gastrointestinal pathogens.

Ensure that gloves are worn and that your hands are kept away from your mouth.

All clinical specimens and bacteriological cultures may contain potentially infective organisms.

### OTHER HAZARDS (Specify: eg. Electrical)

Take care when handling the stop reagent – avoid skin contact with this acidic reagent.

There are no other specific hazards associated with this procedure.

### DISPOSAL METHOD(S)

All materials used to be placed in autoclave bag and sterilised by autoclaving prior to onward transportation to incinerator.
6. CRITERIA FOR TOXIN TESTING

Acceptance

6.1 Unformed stools that take the shape of the container from all patients of 65 years of age and over from hospital or community who have not been diagnosed with *C. difficile* infection in the preceding four weeks (for surveillance purposes).

6.2 Unformed stools from patients age 2 to 64 years:
- On request;
- With a history of antibiotic and/or chemotherapy exposure;
- Patients with suspected or confirmed pseudomembranous colitis.

6.3 Unformed stools from patients who tested CDT Negative (see 6.10). More than one test per patient may be required if the first test is negative but where there is a strong clinical suspicion of CDI.

6.4 As indicated by a Consultant Microbiologist.

Rejection

6.5 Formed stools.

6.6 Multiple stool samples sent on the same day.

6.7 Stools from children <2 year old.

6.8 Stool samples submitted to check for clearance.

Retesting: Criteria for repeating Clostridium difficile toxin tests on samples from the same patient (The 28 days rule)

6.9 Do not retest for *C difficile* toxin positive cases if patients are still symptomatic within a period of 28 days unless symptoms resolve and then recur and there is a need to confirm recurrent CDT.

6.10 If the first toxin test is negative but there is a strong clinical suspicion of *C difficile* infection, then a second sample should be tested 24 hours later.

6.11 Consider re-testing a sample if the patient has received *C difficile* - specific treatment with metronidazole or vancomycin but remains symptomatic with ongoing diarrhoea (possibly due to post-infective irritable bowel syndrome) and is considered for immunoglobulin treatment.
7.0 SPECIMEN COLLECTION

1.1 Faeces samples should be collected in the standard laboratory containers provided and submitted to the laboratory as soon as possible.

1.2 Upon receipt in the laboratory, the sample must be diluted in the kit diluent as soon as possible.

1.3 Specimens should be stored between 2°C and 8°C until testing. However, if the samples are not to be tested within 24 hours they must be stored at −20°C. Delaying processing should be avoided unless exceptional circumstances dictate otherwise.

1.4 In all cases samples should ideally be tested within 24 hours of collection. If insufficient samples are received to carry out a batch of EIA tests (less than about 8 samples) the Tox A/B Quik Chek test may be used.

1.5 Freezing and thawing of the specimen may result in loss of activity due to degradation of the toxins. Avoid repeated freezing and thawing of samples.

1.6 All *C. difficile* toxin positive faecal samples (or an aliquot of the sample) should be kept frozen at −20°C so that it could be submitted to Leeds CDRNE laboratory for culture, as part of an outbreak investigation, to recover isolates for typing. The duration of storage should be 12 months after which samples should be discarded (discard in the corresponding month the following year).

8.0 PRELIMINARY PREPARATIONS

6.10 Set up one dilution tube for each specimen to be tested. Add 200:1 diluent to each tube. Label the tube directly on the side.

6.11 For unformed (but not liquid) stools, use an applicator stick to transfer the faecal material to the tube (transfer an amount equal to about 3mm diameter).

6.12 For liquid stools, use a plastic pipette to transfer 50:1 specimen to tube, ensuring the liquid specimen is evenly suspended before transferring by vortexing prior to dilution to ensure even distribution of *C difficile* toxin.

6.13 Shake the tubes vigorously (vortex mixer) for 10 seconds and store in the refrigerator between 2°C and 8°C until the test is to be carried out.

6.14 All reagents must be at room temperature prior to use.

6.15 Prepare 1X wash Solution by diluting the 20X wash buffer concentrate (50 ml of concentrate in 950 ml of distilled water). Store any unused 1X wash solution between 2°C and 8°C.)
6.16 Determine the number of assay wells to be used and return the unused wells to the refrigerator (re-sealed and stored in the plastic bag provided in the kit).
9.0 ASSAY PROCEDURE (EIA)

1.0 Add 1 drop (50:1) of conjugate (red cap) to each well. Ensure the bottle is held vertically when adding the drops. Use 1 well for each stool sample, 1 well for the positive control and 1 well for the negative control (i.e. diluent).

2.0 Transfer 100:1 (2 drops using a transfer pipette from the accessory kit) of diluted specimen to the assay well.

3.0 Add 1 drop (50:1) of positive control (black cap) to the positive control well and 50:1 of the negative control (i.e. diluent) to the negative control well.

4.0 Cut the adhesive plastic sheet to the size necessary to cover the wells, cover the wells and incubate them for 50 minutes at 35°C (+/- 2°C).

5.0 Shake out the contents of the assay wells into a discard jar.

6.0 Wash each well using the 1X Wash Solution in a squirt bottle with a fine tip nozzle, directing the wash solution to the bottom of each well with force. Fill the wells and shake the wash solution out of the well into the discard container.

7.0 Slap the inverted plate on a dry paper towel and repeat steps 8.5 – 8.7 four times using a dry paper towel each time. If any particulate material is seen in the wells, continue washing until all the particulate matter is removed.

8.0 After washing, completely remove any residual liquid in the wells by striking the plate once again onto a dry paper towel until no liquid comes out. Dispose of paper towels and specimen containers carefully, using the discard containers provided.

9.0 Add 2 drops (100:1) of substrate (blue cap) to each well. Gently tap the wells to mix the substrate. Incubate the wells at room temperature for 10 minutes, gently tapping the wells after 5 minutes.

9.1 Add 1 drop of stop solution (yellow cap) to each well. Caution: Corrosive acidic solution, avoid skin contact. The addition of the stop solution converts the blue colour to a yellow colour that may be quantitated by measuring the optical density at 450 nm on a microplate Elisa reader, blanked against air.

2.0 The test may also be read visually in good light against a white background. Read within 10 minutes after adding the stop solution.
10.0 QUALITY CONTROL and INTERPRETATION OF RESULTS

10.1 A positive and negative control must be run with each batch of test specimens.

10.2 The positive and negative controls must fall within their respective ranges for the test to be regarded as valid.

   (k) **Positive** – must be visible **yellow** colour
   (l) **Negative** – must be visually clear.

10.3 If read on a spectrophotometer, the OD at 450nm must be:

   Positive - $\geq 0.500$
   Negative - $< 0.080$

1.0 Record the control results on the appropriate worksheet in the QA record file.

10.4 A sample that yields a weak positive result (i.e. $< 0.200$) and is adjacent to a strong positive must be repeated to ensure that the result is not due to carryover.

2.0 A positive result indicates that *Clostridium difficile* toxin A and/or toxin B are present in the faeces specimen.

3.0 On Masterlab report a positive test as **TOX+** and a negative result as **TOX-**.

1.0 LIMITATIONS

1.0 *Clostridium difficile* TOX A/B II is used to detect *Clostridium difficile* toxin in faecal specimens. The test confirms the presence of toxin in faeces and this information should be taken under consideration by the clinician in the light of the clinical history of the patient.

2.0 The toxins produced by *Clostridium difficile* are not stable once the faeces sample has been collected and can degrade rapidly. Optimal results are obtained with those specimens that are less than 24 hours old. Most specimens can be stored between 2°C and 8°C for 48 hours before significant degradation of the toxin is noted but ideally samples should be tested within 24 hours.

3.0 Some specimens may give weak reactions that may be due to a number of factors. These include the presence of a weakly toxigenic strain, low levels of toxin production in vivo or the presence of binding substances or inactivating enzymes in the faeces specimen.

   Under these conditions, the specimen should be re-tested or a fresh specimen should be obtained and tested.
4.0 Some toxigenic isolates of *Clostridium sordellii* produce toxins that are similar in their properties to those of *C. difficile*. These isolates however, have not been detected in patients with antibiotic-associated diarrhoea and colitis.

5.0 Stool samples that have been preserved in formaldehyde cannot be used for this procedure. A fresh sample must be obtained.

6.0 The performance characteristics of the *C. difficile* TOX A/B II test have not been thoroughly established in the paediatric population.
2.0 RAPID QUIK CHEK METHOD

12.1 This rapid method may be used for urgent requests performed outside routine working hours (i.e. nights and weekends) or when there are insufficient samples for an EIA batch.

1.0 Follow steps as stated below.
13.0 REPORTING OF RESULTS

13.1 On completing the ELISA test, all \textit{C difficile} results are entered in the Labcentre system by the BMS on the Enteric bench and the Duty Consultant Microbiologist is informed of any positive results.

13.2 Once the results are entered in the computer system, they also become available on the Infection Control ghost queue (InfCon) and the Infection Control Nurse has the duty to inform the ward of any positive patients.

13.3 This system operates Monday to Friday. At weekends and on Bank Holidays, it is the duty of the BMS working on the Enteric bench to phone any positive \textit{C difficile} toxin results to the wards.

13.4 Positive \textit{C difficile} toxin tests are reported weekly via CoSurv to the Health Protection Agency and also entered on MESS (Microbiology Secretary has the full name of this).
C difficile Specimen Request form.

Leeds, Birmingham, London, Manchester, Newcastle, Southampton

<table>
<thead>
<tr>
<th>SURNAME</th>
<th>INITIAL(s)</th>
<th>DoB</th>
</tr>
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</table>

<table>
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<tr>
<th>Hospital no or Lab no. or NHS no.</th>
<th>Laboratory/hospital</th>
</tr>
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**Why have you submitted this sample?**
- Because of severity of symptoms in this patient
- Because of severity of symptoms in other patients
- Because of cluster(s) of cases
- Because of unexplained increase in C. difficile rate
- Other reason: please state here

**Antibiotic History:**
- This must be completed
- Please tick all that apply:
  - Number of antibiotics in 30 days before onset of this episode of C. difficile infection
    - 0
    - 1
    - 2
    - 3
    - 4
    - 5
    - 6
    - >6
  - In the last one month:
    - Cephalosporin
    - Fluoroquinolone
    - Amox/ampicillin
    - Piptazol (Tazocin)
    - Co-amoxiclav
    - Macrolide
    - Trimethoprim
    - Metronidazole (not for C. difficile)
    - Vancomycin (not for C. difficile)
    - Other
    - Metronidazole for C. difficile
    - Vancomycin for C. difficile

**FOLLOW UP (complete now if data available please). Date of follow up ........................**

- Was the patient admitted to an intensive care unit due to C. difficile?  □ Yes □ No □ Unknown
- Did the patient die within 30 days of the onset of C. difficile infection?  □ Yes □ No □ Unknown
- If yes, was C. difficile a cause or contributory factor in the death?  □ Yes □ No □ Unknown

- Did the patient have:
  - Toxic megacolon?  □ Yes □ No □ Unknown
  - Pseudomembranous colitis (PMC)?  □ Yes □ No □ Unknown
  - Any surgical procedures as a consequence of the C. difficile infection?  □ Yes □ No □ Unknown

**Any other information you wish to record here:**

Thank you.
13.0 REFERENCES

1.0 Clostridium difficile TOX A/B II. Manufacturers Product Instructions: Techlab.

2.0 TOX A/B Quik-Chek. Manufacturers Product Instructions: Techlab.


1.0 National Clostridium difficile Standards Group Report to the Department of Health; Feb 2003.


