NHS National Services Scotland

Recommended protocol for testing for *Clostridium difficile* and subsequent culture

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Introduction

This consensus guidance has been produced by a group of microbiologists representing the Scottish Microbiology & Virology Network (SMVN), the Scottish *Salmonella*, *Shigella* and *Clostridium difficile* reference laboratory (SSSCDRL) and Health Protection Scotland (HPS) in consultation with the full SMVN membership. It is intended for use by microbiology laboratories in NHS Scotland and supersedes the previous recommended protocol published in December 2012.

Background

The current gold standard for *C. difficile* toxin testing is a well-performed cell-culture cytotoxicity assay. However, this is not available to every laboratory in Scotland, is not straightforward to establish or maintain, and has an in-built delay of up to 3 days before results are available.

Following recommendations first published in 2009, this led to widespread adoption by Scottish diagnostic laboratories of more rapid toxin immunoassay testing as the basis for diagnosis of *C. difficile* infection (CDI) as part of a two-step algorithm. Further guidance was issued in 2012 following publication of an evaluation of algorithms for the diagnosis of CDI.[1] (See Reporting to HPS for mandatory national surveillance).

This updated guidance follows the publication in 2016 of the new European Society of Clinical Microbiology and Infectious Disease (ESCMID) diagnostic guidance for CDI.[2] The European guidance summarises the most recent available evidence published since 2009, particularly in light of new diagnostic tests that have become available. This Scottish guidance will be revised on an ongoing basis to take account of further diagnostic developments.

Recommendations (see also Appendix for frequently asked questions)

Sample selection

- Diarrhoeal stool samples from patients aged 3 years or older should be tested for CDI.[2] Note that only CDI in cases aged 15 years and above should be reported to HPS for mandatory national surveillance purposes (see Reporting to HPS for mandatory national surveillance).

- Testing of diarrhoeal stool samples from children under the age of 3 should be by clinician's request only.[2]

- Formed stool should not be tested for CDI. In the case of paralytic ileus, a rectal swab may be taken for testing.[2]

- Guidance for obtaining faecal specimens from patients with diarrhoea may be accessed from: http://www.hps.scot.nhs.uk/haiic/sshaip/guidelinedetail.aspx?id=40364

Diarrhoea is defined as the passage of 3 or more loose or liquid stools in a 24 hour period, or more frequently than is normal for the individual, and with no other underlying cause.[3] For mild disease, diarrhoea is usually the only symptom. However, severe CDI is not always associated with diarrhoea, e.g. in the case of ileus.

CDI can occur in young children and infants. However, interpretation of positive results in children less than 3 years of age is problematic, and testing in this age group should be limited to samples with a clinician's request only.[2]

Sample storage and transportation

Samples should be transported to the laboratory promptly and stored at 4 °C prior to testing. When toxin testing has been completed the faecal sample should be frozen at -20 °C for at least 3 months in order to allow culture at a later time for typing if required.

Testing protocol

- Test diarrhoeal stool samples using a sensitive screening test (GDH EIA **or** PCR test) (see Figure 1: Testing Algorithm 1). As with any other test, laboratories will have to satisfy themselves that any specific assay chosen as part of the algorithm is of an acceptable quality and performance standard.

- Report samples which are screen-negative at this point, e.g. "*C. difficile* test negative". These samples do not require further testing.

- Test screen-positive diarrhoeal samples for the presence of *C. difficile* toxin on the same sample using toxin A/B EIA. Report samples which are positive in this step, e.g. "*C. difficile* toxin positive". Report stool samples which are positive in **both** the screening test **and** the confirmatory toxin test according to the mandatory surveillance protocol for CDI (see also Reporting to HPS for mandatory national surveillance).[3]

- Any *C. difficile* toxin immunoassay being used (i.e. EIA or membrane assay) should be one of the better performing assays.[2]

- Report screen-positive results which are not confirmed by toxin testing as equivocal, e.g. "Equivocal result: *C. difficile* screening test positive but *C. difficile* toxin could not be detected in this sample. Advise repeat sample if patient remains symptomatic."

- Diagnosis of CDI is based on **both** the clinical presentation and the results of any laboratory tests; i.e., laboratory test results should not be interpreted without reference to clinical features. Issuing interpretative comments with reports may aid clinicians in understanding the significance of results. The example report texts above are only suggestions. Decision for treatment for CDI is a clinical decision and may exceptionally be justified even if all laboratory tests are negative.[2]

- Samples with a negative confirmatory test result may optionally be tested using toxigenic culture or PCR (if not already performed) to determine the presence of a toxigenic *C. difficile* strain.[2]

- When using a membrane assay, which combines GDH and Toxin A/B tests (see Figure 2: Testing Algorithm 2), samples with either both positive, both negative, or GDH positive toxin negative results can be reported as above. Where there is a negative GDH but a positive toxin test the sample should be retested, as this is an invalid result.[2]

- Laboratory CDI testing using a two-step algorithm should be available 7 days a week.[4]

At the present time, no single test or combination of tests should be considered infallible in establishing or excluding the diagnosis of CDI, and the clinical condition of the patient should always be considered when making management and treatment choices.

The use of an initial sensitive screening test increases the Negative Predictive Value of the algorithm. The use of a confirmatory test (on the same faecal sample), as part of the diagnostic algorithm, increases the accuracy of toxin-positive results. This algorithm was found to have the best clinical utility in the largest diagnostic algorithm study that has been performed to date,[1] and is supported in the current ESCMID guidance.[2] Only algorithms that include a toxin test provide an acceptably high specificity in comparison with the gold standard of a well-performed cell-culture cytotoxicity test. Although toxigenic culture or PCR may optionally be used, the interpretation of the results is not straightforward and will require careful clinical evaluation of the patient.[2]

Some samples which are positive in the initial screening test will fail to confirm in the subsequent toxin assay. This may be due to the following:

- Toxin is absent (true-negative toxin test). This may be due to the presence of *C. difficile* which are non-toxigenic, cross-reaction with the GDH of other organisms, or not expressing the toxin gene.
- Toxin concentration is below limit of detection (false-negative toxin test).
- Toxin concentration yields a result within manufacturers indeterminate range (indeterminate toxin test).
- Occasionally the screening test may be positive in the absence of viable *C. difficile* organisms (false-positive screening test).

Repeat Testing

Repeated testing after a first confirmed positive sample during the same diarrhoeal episode is not recommended.

Repeated testing after a first negative sample during the same diarrhoeal episode may be useful in selected cases with ongoing high clinical suspicion.

A test of cure is not recommended.

Clearance testing

Clearance testing is not recommended. Individuals can remain toxin positive for some weeks after symptoms have settled.

Repeat testing in confirmed positive cases should only be undertaken where symptoms have recurred after initial successful treatment.

Referral to SSSCDRL

Stool samples should be cultured for *C. difficile* and isolates referred to SSSCDRL in line with existing guidance. Isolates should be sent to SSSCDRL in Robertson's meat broth. Recovery from this medium is more reliable than from swabs.

SSSCDRL website: http://www.nhsggc.org.uk/about-us/professional-support-sites/microbiology/scottish-microbiology-reference-laboratories/scottish-salmonella-shigella-c-difficile-reference-laboratory/

Reporting to HPS for mandatory national surveillance

Only those diarrhoea cases aged 15 years and above which have tested positive on both the screening and confirmatory toxin test, and that meet the following case definition, should be reported to HPS for mandatory surveillance purposes.

A case of CDI is someone in whose stool *C. difficile* toxin has been identified at the same time as they have experienced diarrhoea not attributable to any other cause, or from cases whose stool *C. difficile* has been cultured at the same time as they have been diagnosed with PMC (pseudomembranous colitis).

Figure 1: Testing Algorithm 1

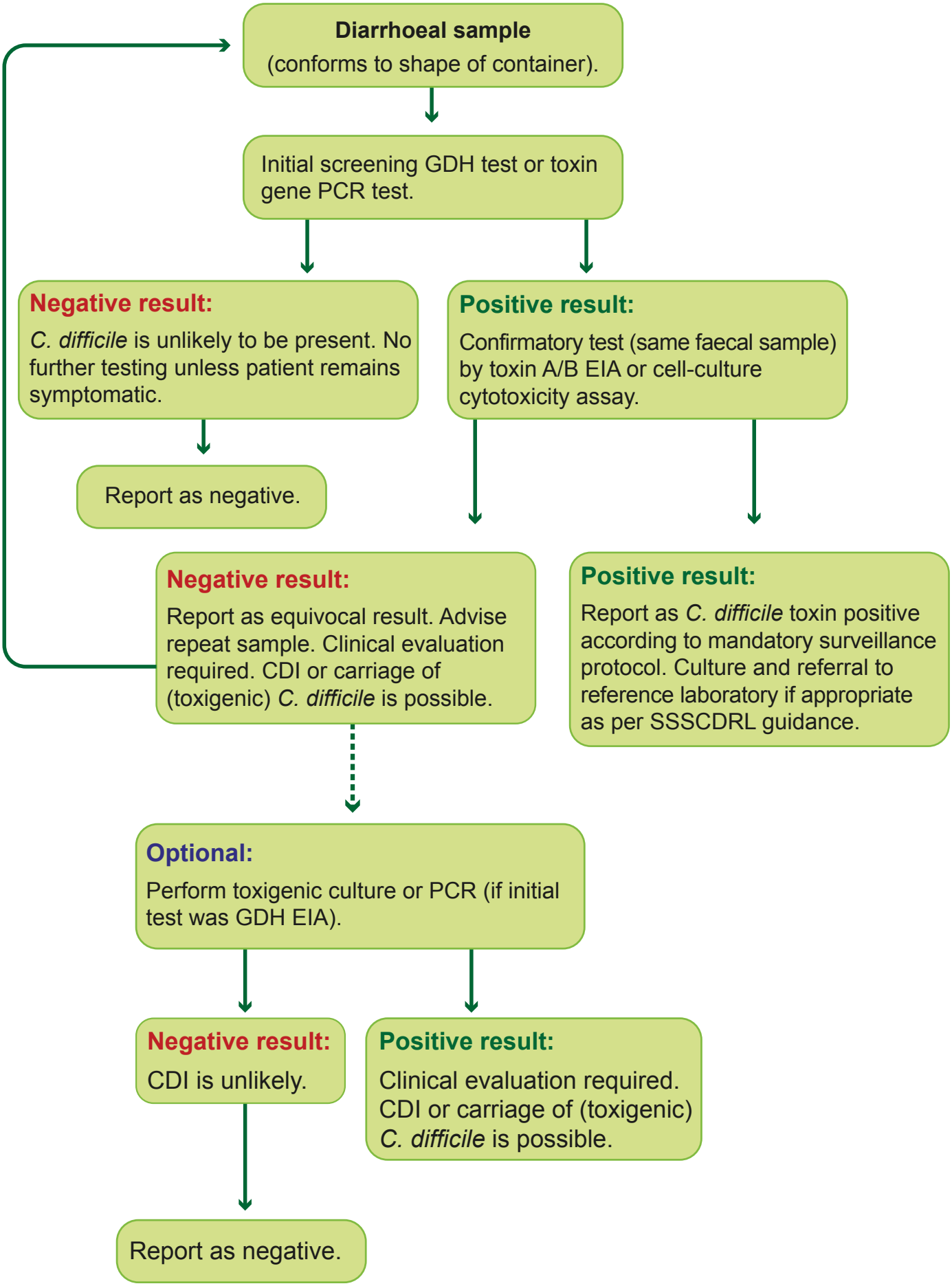
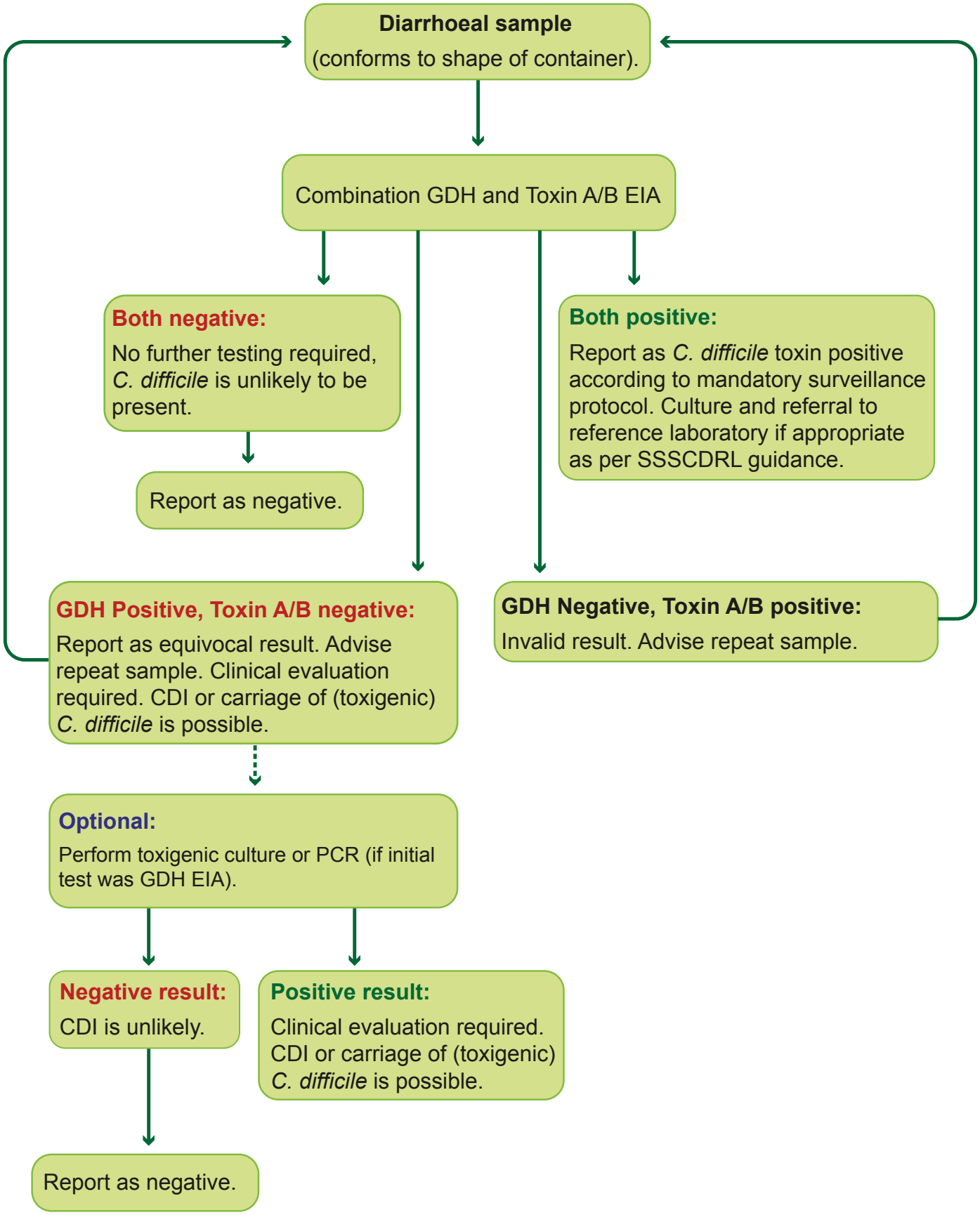


Figure 2: Testing Algorithm 2



APPENDIX

Frequently asked questions for users of the recommended protocol for testing for *Clostridium difficile* and subsequent culture

1. What is new in this version of the protocol?

This version of the protocol includes some new recommendations relating to the sample selection (recommendation to test all diarrhoeal stools in patients aged 3 and above, and testing in cases of paralytic ileus); the testing protocol (optional testing for samples with a negative confirmatory test, and use of membrane assays which combine GDH and Toxin A/B tests); and the algorithms (a separate algorithm for membrane assays).

2. What is the rationale for the recommendation to test aged 3+?

High rates of asymptomatic colonisation in infants of both toxigenic and non-toxigenic *C. difficile* have been reported. While clinical disease does rarely develop in this age group, interpretation of positive results is problematic. Therefore, the recommendation is limited to those aged 3+, with samples from children under 3 years requiring a clinician request. Current available evidence in Scotland suggests that the prevalence in children is low. HPS will monitor the situation for a period of one year to assess the true burden, which will in turn shape future developments. The extra cases that are diagnosed will not count against current LDP standards.

3. Which tests should I use?

The choice of specific tests and technologies within the framework of the algorithm will be determined by local factors. The example testing algorithms are intended as a guide and must be adapted to local circumstances. As with any other test, laboratories will have to satisfy themselves that any specific assay chosen as part of the algorithm is of an acceptable quality and performance standard.

4. Why is there a new algorithm for membrane-based assays?

Previously there was no supporting evidence covering these types of assays; however, this has been addressed in the recent ESCMID guidance.

5. Reporting: When should a result be reported to the requester, and what should be reported?

A report, oral or written, should not be issued until both tests of the algorithm have been performed.

It is good practice for laboratories to inform ward staff orally of any positive result, and it is good practice for written reports to provide both the test result and its interpretation.

6. Will reporting after performing both algorithm tests prolong the turn-around time?

The technologies involved lend themselves to rapid testing, and all labs in Scotland should already have been using a two-step algorithm, so this should not introduce further delay beyond what was already in place. Releasing a result on the basis of the initial screen would potentially result in overtreatment, overuse of antibiotics, and possible increase risk of CDI as a result.

7. If reports are not issued before the results of both algorithm tests are available, does this conflict with the rationale that using a more sensitive test first would allow earlier treatment and implementation of additional IPC measures?

The issue of IPC management would be for those patients who were toxin-negative after the second test is performed (especially those who have already had a previous recent stool sample that was toxin-negative), who are unlikely to have significant CDI, but have diarrhoea due to another cause and may nonetheless be shedding *C. difficile* in their stool. Clearly if they have significant ongoing diarrhoea this may cause potential issues in terms of environmental contamination. The management of such patients is not clear cut. It has been suggested that nursing such patients in single rooms to reduce environmental contamination may prevent infection of other patients. However, it is regarded as good practice to place patients who present a cross-infection risk, e.g. diarrhoea, in single rooms in any case.

There are no studies to date that have demonstrated that there is a reduction in infection rates associated with such a practice. Antibiotic treatment, if the patient is not considered to have CDI, could theoretically increase their risk of developing CDI. Such patients will require careful evaluation on a case by case basis by the IPCT and the clinicians involved in their care.

8. How should I interpret discrepant results?

Where the first test is positive and the second is negative then the result should be reported as equivocal. A clinical assessment should be undertaken and if the patient remains symptomatic a further sample should be submitted for testing. Even if toxin is not detected in the stool sample, *C. difficile* may be present in the sample and the patient could be a potential *C. difficile* excretor. This may be the case, even if ongoing diarrhoeal symptoms are thought to be due to another cause. Any patient with continuing undiagnosed diarrhoea will require clinical review with regards the requirement for therapeutic or supportive interventions, and infection control risk assessment with regards to potential for nosocomial transmission of enteric pathogens.

9. What further testing is required if a patient has persistent diarrhoea and the confirmatory toxin test is negative?

If a second sample yields a further equivocal result, CDI is considerably less likely to account for the patient symptoms, but a very small proportion of results may be false negatives. Patients should be carefully re-assessed clinically. Where a patient has persistent diarrhoea and CDI is considered a possibility due to associated risk factors up to 2 further samples should be submitted at least 48 hours apart. In individual cases microbiologists may consider the use of adjunctive tests, e.g. culture for *C. difficile*, the use of toxigenic culture to confirm that subsequently isolated strains of *C. difficile* are toxigenic, and the use of PCR testing for toxin genes if this was not used as an initial screen. However, the interpretation of the clinical significance of these further tests in stool samples that are persistently toxin negative will still require very careful clinical assessment.[1] It may exceptionally be clinically justified to treat a patient for CDI despite negative test results. In these cases, treatment should not be withheld on the basis of laboratory tests alone.[2]

10. When and for how long is isolation necessary?

Any patient with unexplained diarrhoea should be quickly assessed and placed in the most appropriate care setting, i.e. a single room with en suite facilities (or with a commode allocated for their sole use), unless there is clear clinical reason not to do so (e.g. it is unsafe for the patient to be isolated). Contact precautions should be followed.

A positive test result should not be awaited before placing the patient in isolation.

Patients should remain in isolation until they have been symptom-free for at least 48 hours. A negative CDI result is not in itself sufficient to discontinue isolation.

11. Should clearance testing be performed?

Clearance testing is not recommended. Individuals can remain toxin positive for some weeks after symptoms have settled.

Repeat testing in confirmed positive cases should only be undertaken where symptoms have recurred after initial successful treatment.

12. When should CDI testing be available?

Laboratories need to make testing available 7 days a week (including public holidays).

13. What about using GDH followed by PCR testing for *C. difficile*?

PCR kits are included in the guidance, i.e. GDH (or PCR) followed by toxin test. A testing algorithm comprising GDH followed by PCR is not supported by the latest research.

14. Is it acceptable to use a cytotoxin test instead of a sensitive toxin EIA?

Yes, it is acceptable to use a neutralised cell cytotoxin test instead of a sensitive toxin EIA as part of the recommended two-stage algorithm. In Department of Health (DoH) evaluations, the cytotoxin test was more sensitive than the toxin EIAs.[1] Clearly, the cytotoxin assay yields slower results than the toxin EIA, and this needs to be accounted for when making management and infection prevention decisions regarding suspected CDI cases.

15. What stools should be tested for CDI?

If a patient has diarrhoea that is not clearly attributable to an underlying condition then it is necessary to determine if this is due to *C. difficile*. The stool sample must take the shape of the container. All diarrhoeal samples from patients aged ≥3 years should be tested as a minimum.

In suspected cases of 'silent CDI' such as ileus, toxic megacolon or pseudomembranous colitis without diarrhoea, other diagnostic procedures such as colonoscopy, white cell count, serum creatinine and abdominal computerised tomography (CT) scanning may be required, potentially with referral to a gastroenterologist or gastrointestinal surgeon. A rectal swab may be taken for testing by (toxigenic) culture, PCR or GDH EIA.

16. Will a comparison of all commercially available kits be available?

The researchers in the recent DoH study did not assess all commercially available kits.[1] However, a larger number of kits were assessed previously and published as a CEP evaluation and in a peer-reviewed journal (Planche et al., Lancet Infect Dis. 2008;8: 777-84). At that time, this was the largest study of its kind. The DoH study recruited more than 20 times more patients (in order to be able to accurately distinguish between tests and combinations), and so had to reduce the number of tests examined.

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