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

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Scottish Microbiology and Virology Network

Scottish *Salmonella*, *Shigella*, and *Clostridium difficile* Reference laboratory

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Health Protection Scotland
Meridian Court
5 Cadogan Street
GLASGOW
G2 6QE
Tel: +44 (0) 141 300 1100
Email: NSS.HPSEnquiries@nhs.net

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 and Health Protection Scotland 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 2009.

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. 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).

It is now well recognised that *C. difficile* toxin immunoassays currently available on the market do not have particularly good sensitivity and specificity[1] [2]. This is one of the reasons why all guidance related to the diagnosis of CDI stresses the importance of clinical symptoms consistent with CDI in association with a toxin positive result.

The evaluation report on *C. difficile* toxin detection assays (CEP08054) published in February 2009 by NHS Purchasing and Supplies Agency[3] reported that there is no single assay that is clearly superior in terms of both sensitivity and specificity. However five assays (*Remel Xpect, Techlab Tox A/B, Quik Chek, Premier Toxin A+B, Vidas C. difficile Toxin A&B and Techlab Toxin A/B II*) appeared to be superior to the other four tested in that study. A recent much larger study of CDI diagnosis in England further highlighted the potential discrepancies in performance of two different *C. difficile* toxin assays evaluated in the course of that work. A summary of the main findings of this study are available in Annex A of the updated Department of Health guidance on the diagnosis of CDI[4]. Nonetheless even with the best performing kits a proportion of true positives will be missed and, perhaps of greater concern, there is a potential to generate false-positive results (especially in low prevalence settings).

In most endemic acute hospital settings the true positive prevalence is likely to be less than 10 percent of all specimens tested. The substantial reductions in CDI that have been achieved in Scotland in recent years, increased testing within the community setting, and the extension of the National surveillance programme for CDI to cover all those aged 15 and over, means toxin testing is now carried out in groups of patients where the prevalence of CDI may be lower still. Thus there is a genuine risk of false-positive test results with the potential for inappropriate management.

This concern was highlighted to the service in 2009[1] and the Scottish Microbiology Forum (SMF), with the support of SSSCDRL and HPS, issued guidance on testing protocols and the use of a diagnostic algorithm to increase the accuracy of CDI diagnosis in December 2009. Available evidence indicates that this guidance has been widely adopted in Scotland. The current document updates this guidance in the light of further studies that have been performed in the intervening period, with particular reference to the very large evaluation of the use of diagnostic algorithms for the diagnosis of CDI that was recently completed in England[4].

Recommendations

This is consensus guidance produced by SMVN representatives and the SSSCDRL and supported by HPS.

1. Any *C. difficile* toxin immunoassay (i.e. EIA or membrane assay) being used should be one of the better performing assays[3 4].
2. Diarrhoeal stool samples should be tested by an initial sensitive screening test (GDH test **or** toxin B gene PCR test). 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.
3. Those samples which are screen-negative do not require further testing, and can be reported at this point e.g. "*C. difficile* screening test negative".
4. Initial screen-positive faecal samples should be tested for the presence of *C. difficile* toxin on the same sample. Samples which are also positive in this toxin assay can then be reported accordingly e.g. "*C. difficile* toxin positive". Only those stool samples which are positive in **both** the initial screening test **and** the subsequent toxin test are eligible for reporting under mandatory surveillance of CDI.
5. 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 concentration is below limit of detection (false-negative toxin test).
   - Toxin concentration yields a result within manufacturers indeterminate range (indeterminate toxin test).
   - Toxin is absent (true-negative toxin test). This may be due to the presence of *C. difficile* which are non-toxigenic or not expressing the toxin gene.
   - Occasionally the screening test may be positive in the absence of viable *C. difficile* organisms (false-positive screening test).

   These discrepant results should then be reported 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".

6. The use of an initial sensitive screening test will increase the Negative Predictive Value of the algorithm. The use of a confirmatory test (on the same faecal sample), as part of the diagnostic algorithm, will increase 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. In this study, only algorithms that included a toxin test provided an acceptably high specificity in comparison with the gold standard of a well-performed cell-culture cytotoxicity test. This guidance is compatible with current ESCMID guidance[5]. The guidance will be revised on an ongoing basis to take account of further diagnostic developments.
7. All of these assays will fail to detect some true toxin positive samples. If there remains a strong clinical suspicion of CDI then a repeat faecal sample should be sent and tested, and the need for empirical treatment considered.
8. 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.

Suggested testing protocol (see algorithm below)

Initial sensitive screening test – GDH test **or** toxin B gene PCR test. Samples which are screen-negative do not require further testing. Negative results at this stage can be issued as final reports. However, all of these assays will fail to detect some true positive samples. If there remains a strong clinical suspicion of CDI then a repeat faecal sample should be sent and tested, and the need for empirical treatment considered.

Confirmatory test – Performed on the same faecal sample for all specimens testing positive on the initial screen – Toxin immunoassay (using one of the better performing tests as indicated above) or cell-culture cytotoxicity assay.

The choice of specific tests and technologies within the framework of the algorithm will be determined by local factors. The example testing algorithm is 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.

Interpretation of 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.

Persistent diarrhoea when toxin negative

If a second sample yields a further equivocal result, CDI is 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[6].

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.

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[7]. Recovery from this medium is more reliable than from swabs.

Group members

Prof J Coia	SSSCDRL
Dr B Jones	SMVN
Dr M Lockhart	SMVN
Mr H Pankhania	SMVN
Dr C Wiuff	HPS
Dr J Cowden	HPS
Mr A Banks	HPS

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[1] HPS Weekly Report Newsletter Volume 43 Number 13 Year 2009
[2] Questions and answers about the laboratory diagnosis of *Clostridium difficile* infection (CDI). Available at: http://www.hps.scot.nhs.uk/haiic/sshaip/guidelinedetail.aspx?id=40852
[3] NHS Purchasing and Supplies Agency, Centre for Evidence based Purchasing. *Clostridium difficile* toxin detection assays. Evaluation report CEP08054, 2009. Wilcox MH, Eastwood KA. Available at: http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_127720
[4] Updated guidance on the diagnosis and reporting of *Clostridium difficile*. Available at: http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_132927
[5] Crobach MJT, Dekkers OM, Wilcox MH, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): Data review and recommendations for diagnosing *Clostridium difficile*-infection (CDI). *Clinical Microbiology and Infection* 2009;**15**:1053-1066.
[6] Coia JE, Eastaway A, Wiuff C. The diagnosis of *C. difficile* infection (CDI) - two steps forwards? *Journal of Infection* 2011 Nov;63(5):398-9.
[7] Services available from Scottish *C. difficile* Reference Service. Available at: http://www.ssrl.scot.nhs.uk/cdiffservices.asp

Testing Algorithm