Scottish Microbiology and Virology Network
Established 2012

NHS SCOTLAND

Review of *Clostridioides difficile* diagnostic testing in NHS Scotland:

Best practice recommendations

3 May 2024

NOTE

This guideline is not intended to be construed or to serve as a standard of care. Standards of care are determined based on all clinical data available for an individual case and are subject to change as scientific knowledge and technology advance and patterns of care evolve. Adherence to guideline recommendations will not ensure a successful outcome in every case, nor should they be construed as including all proper methods of care or excluding other acceptable methods of care aimed at the same results. The ultimate judgement must be made by the appropriate healthcare professional(s) responsible for clinical decisions regarding a particular clinical procedure or treatment plan. This judgement should only be arrived at following discussion of the options with the patient, covering the diagnostic and treatment choices available. It is advised, however, that significant departures from the national guideline or any local guidelines derived from it should be fully documented in the patient's case notes at the time the relevant decision is taken.

Contents

Situation

NHS Scotland guidelines for *Clostridioides difficile* testing[1] were last published jointly by the SMVN and HPS (now PHS / ARHAI Scotland) in October 2016 with their review delayed due to the COVID-19 pandemic.

Accurate and timely diagnosis of *C. difficile* infection (CDI) is imperative to prevent transmission and reduce morbidity and mortality due to CDI. CDI is a clinical diagnosis supported by laboratory findings. Within the 2016 testing recommendations, the reporting of equivocal laboratory results hinders diagnosis of CDI.

A legacy of the COVID-19 pandemic in Scotland is the universal availability of molecular diagnostics in NHS laboratories; this should be considered as a means of reducing equivocal reporting and the 2016 recommendations updated accordingly.

Background

1. The organism / disease

C. difficile is a Gram-positive spore bearing anaerobic bacterium. Toxigenic strains can cause CDI; disease develops when the organism proliferates in the colon, commonly after antibiotic use has eliminated the normal flora. It is the most common cause of health care-associated infectious diarrhoea in developed countries and a major source of nosocomial morbidity and mortality worldwide.

C. difficile Glutamate Dehydrogenase (GDH) is an enzyme produced in large quantities by all toxigenic and non-toxigenic strains, making it an excellent (antigen) marker for the organism. GDH detection is used as a screening test.

C. difficile can release two high-molecular-weight toxins, toxin A and toxin B, which are responsible for a wide range of clinical manifestations, ranging from mild, self-limited watery diarrhoea to fulminant pseudomembranous colitis, toxic megacolon, and death.

C. difficile is a notifiable organism [Public Health etc. (Scotland) Act 2008].

2. 2016 testing guidance[1]

Diagnosis of CDI is based on <u>both</u> the clinical presentation and the results of any laboratory tests.

The testing algorithm detailed in the 2016 guidelines may be found in appendix 1 and are summarised as follows for diarrhoeal stool samples:

Step 1: Use sensitive screening test (GHD enzyme immunoassay [EIA] or PCR).
If test is negative report as negative. No further testing needed.
If test is positive, carry on to step 2.

Step 2: Confirmatory toxin A/B EIA ("better performing assay").
Report samples which are positive in this step.
Report screen-positive results which are not confirmed by toxin testing as equivocal.

Step 3: Samples with a negative confirmatory test result may optionally be tested using toxigenic culture (not available) or PCR (if not already performed) to determine the presence of a toxigenic *C. difficile* strain.[1]

In practice, NHS Scotland laboratories have relied on GDH and toxin A/B EIAs for steps one and two respectively. Optional step three is generally not carried out, meaning significant numbers of samples are reported with an equivocal result, advising repeat sampling. Toxin testing performed by cell-culture cytotoxicity assay is out-dated and no longer available in NHS Scotland. Some laboratories have moved towards toxin gene PCR testing.

Assessment

a. It is acknowledged that CDI laboratory diagnostics are complex and no single commercial test can be used as a stand-alone test for diagnosing CDI and no laboratory test can distinguish between CDI and *C. difficile* colonisation.[2]

b. The positive predictive value (PPV) of any test depends on the disease prevalence with lower CDI rates associated with lower PPVs.[2]

c. GDH tests have been shown to have good sensitivity but poor specificity, as other species of Clostridia produce GDH, leading to false positive results.[3] They are quick and easy to perform.

d. Toxin tests have been shown to have poor sensitivity but good specificity leading to false negative results.[3] They are also quick and easy to perform.

e. Nucleic acid amplification tests (NAATs) / PCR detects the presence of the toxin genes, not the toxins themselves. They are highly sensitive and specific. Some argue that their use may lead to over diagnosis of *C. difficile* by identifying carriers[4] whilst others have found a good correlation with clinical diagnosis.[5]

f. The main benefit of NAATS is realised when they are added as a third step in "GDH positive, toxin EIA negative" cases (generating an equivocal result that is unhelpful to clinicians), compensating for the lower sensitivity of toxin tests.[6] Appendix 2 shows that 4 – 5% of samples from one NHS Scotland Board were reported as "equivocal" and would benefit from PCR testing.

g. There is evidence that a significant proportion (>60%) of GDH + / Toxin – specimens do harbour toxigenic *C. difficile*, including outbreak-associated ribotypes [7-9] and that a lack of clinical suspicion accounts for under-diagnosis and mis-diagnosis.[10,11] There is also evidence that such cases account for a significant proportion of in-hospital *C. difficile* transmission.[12]

h. Of note, NAATs have traditionally been more expensive than EIAs but costs are reducing.[13] Any additional costs of this highly accurate and rapid test may be offset by the cost savings and benefits brought about by avoiding hospital acquired outbreaks of CDI.[14-16]

i. Wales introduced PCR testing as part of a 2-step algorithm for *C. difficile* in 2018; they used it to replace GDH testing at step-1.[13]

j. Large areas of England have adopted NAATs to address GDH positive, toxin negative samples meaning NHS Scotland is lagging behind the rest of the UK.[17]

k. One of the legacies of the COVID-19 pandemic, is that all NHS Scotland territorial Boards now have multiple PCR testing platforms and the expertise to use them.

Recommendations

a. NHS Scotland CDI guidance should be updated to include a three-step best practice laboratory testing / reporting algorithm. This will address GDH positive, toxin negative discordant / equivocal results.

b. The principle GDH screening and confirmatory toxin EIAs will remain the mainstay of testing

c. Toxin gene PCR testing will be used on GDH positive, toxin negative samples and results will be reported as follows (previously optional, now recommended):

3 step testing algorithm:

Step 1: **Use sensitive screening test (GHD EIA) or PCR for toxin gene(s)**
If test is negative report as negative. No further testing needed.
If test is positive, carry on to step 2.

Step 2: **Confirmatory toxin A/B EIA.**
If toxin positive, report as "Toxin-producing *C. difficile* detected"
If toxin EIA negative, proceed to step 3.

Step 3: **NAAT for toxin gene(s) *(not required if carried out at step 1)***
If negative, report as "*C. difficile* screening test positive. No evidence of toxin production".
If positive, report as "*C. difficile* screening test positive, Toxin gene(s) detected".

Test results (to be considered in tandem with clinical presentation)			**Report as: (to be decided)**
GDH / (PCR) **negative**			*C. difficile* screening test negative.
GDH / (PCR) **positive**	Toxin **positive**		Toxin-producing *C. difficile* detected.
GDH **positive**	Toxin **negative**	PCR **negative**	*C. difficile* screening test positive. No evidence of toxin production.
GDH / (PCR) **positive**	Toxin **negative**	PCR **positive**	*C. difficile* screening test positive. Toxin gene(s) detected.

d. Local Boards may wish to add additional, interpretive comments to reports.

e. NHS Board diagnostics laboratories are responsible for liaising with PHS to Share data with ECOSS, as appropriate and as required.

f. NHS Scotland Health Board laboratories will be audited for compliance of this guideline within one year of publication.

References

1. Scottish Microbiology & Virology Network, Scottish *C. difficile* Reference Service and Health Protection Scotland. Recommended protocol for testing for *Clostridium difficile* and subsequent culture. Health Protection Scotland 2016.
2. Crobach MJ, Planche T, Eckert C, Barbut F, Terveer EM, Dekkers OM, Wilcox MH, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for Clostridium difficile infection. *Clin Microbiol Infect*. 2016 22 Suppl 4:S63-81.
3. Boly FJ, Reske KA, Kwon JH, The Role of Diagnostic Stewardship in *Clostridioides difficile* Testing: Challenges and Opportunities. *Curr Infect Dis Rep*, 2020 Mar;22(3):7
4. Lee HS, Plechot K, Gohil S, Le J. *Clostridium difficile*: Diagnosis and the Consequence of Over Diagnosis. *Infect Dis Ther* (2021) 10:687–69
5. Berry N, Sewell B, Jafri S, Puli C, Vagia S, Lewis AM, Davies D, Rees E, Ch'ng CL. Real-time polymerase chain reaction correlates well with clinical diagnosis of *Clostridium difficile* infection*. J Hosp Infect*. 2014 Jun;87(2):109-14
6. Gateau C, Couturier J, Coia J, Barbut F. How to: diagnose infection caused by *Clostridium difficile*. *Clin Microbiol Infect*. 2018 May;24(5):463-468
7. Orendi JM, Monnery DJ, Manzoor S, Hawkey PM. A two-stage algorithm for *Clostridium difficile* including PCR: can we replace the toxin EIA? *J Hosp Infect*. 2012 80(1): 82-4.
8. Akamatsu Y, Morishita S, Chikumi H, Okamoto R, Okada K, Kitaura T, et al. Evaluation of antigen-positive toxin-negative enzyme immunoassay results for the diagnosis of toxigenic *Clostridium difficile* infection. *J Med Invest*. (2018) 65:131–5.
9. Anwar F, Clark M, Lindsey J, Claus-Walker R, Mansoor A, Nguyen E, Billy J, Lainhart W, Shehab K, Viswanathan VK and Vedantam G (2023) Prevalence of diagnostically-discrepant *Clostridioides difficile*

   clinical specimens: insights from longitudinal surveillance*. Front. Med*. 10:1238159
10. Reigadas E, Alcalá L, Marín M, Burillo A, Muñoz P, Bouza E. Missed diagnosis of *Clostridium difficile* infection; a prospective evaluation of unselected stool samples*. J Infect*. (2015) 70:264–72.
11. Skally M, Bennett K, Burns K, Brennan R, Finn C, O'Connell K, et al. A decade of *Clostridioides difficile* infection: a constant challenge to maintain the status quo. *J Hosp Infect*. (2023) 135:59–66.
12. Mawer DPC, Eyre DW, Griffiths D, Fawley WN, Martin JSH, Quan TP, et al. Contribution to Clostridium difficile transmission of symptomatic patients with toxigenic strains who are fecal toxin negative. Clin Infect Dis. (2017) 64:1163–70.

13. Perry MD, White PL, Morris TE. Impact of the introduction of nucleic acid amplification testing *on Clostridioides difficile* detection and ribotype distribution in Wales. *Anaerobe* (2021); 67:102313

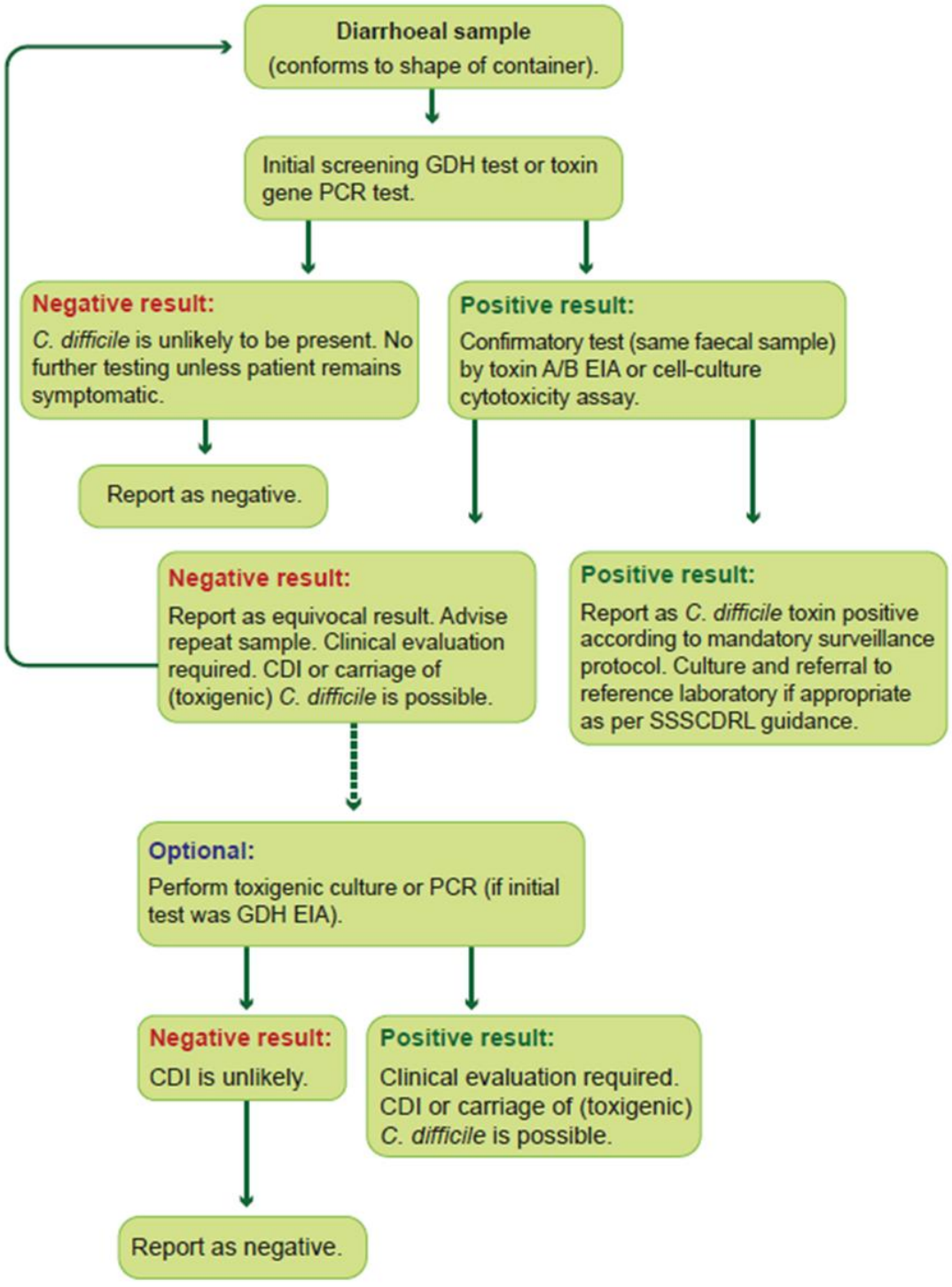
14. Napierala M, Munson E, Skonieczny P, Rodriguez S, Riederer N, Land G, Luzinski M, Block D, Hryciuk JE. Impact of toxigenic *Clostridium difficile* polymerase chain reaction testing on the clinical microbiology laboratory and inpatient epidemiology. *Diagn Microbiol Infect Dis*. 2013 76(4):534-8

15. Kato H, Hagihara M, Asai N, Shibata Y, Yamagishi Y, Iwamoto T, Mikamo H. 'A systematic review and meta-analysis of decontamination methods to prevent hospital environmental contamination and transmission of *Clostridioides difficile*.' *Anaerobe* 2022: 73, 102,478

16. Culbreath K, Ager E, Nemeyer RJ, Kerr A, Gilligan PH. Evolution of testing algorithms at a university hospital for detection of *Clostridium difficile* infections. *J Clin Microbiol*. 2012 Sep;50(9):3073-6.

17. Personal communication, Peter Hawkey 2023.

Appendix 1
2016 HPS / SMVN Recommended protocol for testing *C. difficile* and subsequent culture: Testing algorithm



Appendix 2
NHS Scotland example *C. difficile* test results by category

NHS Grampian

	2021-22	**2022-23**
Total number of GDH tests	7,907	9,002
Number of GDH positives	565	545
Number of toxin (A+B) positives	160	161
Reported *C. difficile* positive	**160 (2%)**	**161 (1.8%)**
Reported *C. difficile* Equivocal	**405 (5%)**	**383 (4%)**

NHS Tayside

Twelve months of test data were reviewed from February 2022 to January 2023

- A total of 12,379 specimens were tested for *C. difficile*
- 382 (3.1%) were PCR positive *C. difficile*
- An average of 36% (monthly variation between 24-45%) of those positives are toxin positive

(Caveat: this data is based on specimens, not on patients/cases)

Appendix 3
NHS Scotland laboratory protocol for testing for *Clostridioides difficile*
Based on ESCMID recommendations (updated)

Sample selection

- Diarrhoeal stool samples from patients aged 3 years or older should be tested for CDI. 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.
- Formed stool should not be tested for CDI. In the case of paralytic ileus, a rectal swab may be taken for testing.

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. 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.

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

a. Test diarrhoeal stool samples using a sensitive screening test (GDH EIA or 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.
b. Report samples which are screen-negative at this point, e.g. "*C. difficile* screening test negative".
c. These samples do not require further testing.
d. 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. "Toxin-producing *C. difficile* detected". Report stool samples which are positive in both the screening test and the confirmatory toxin test according to the mandatory surveillance protocol for CDI.

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

f. Test diarrhoeal samples that are GDH screen positive, toxin EIA negative for the presence of toxin gene(s) using a suitable NAAT, on the same sample.

   Report GDH screen positive, toxin EIA negative, PCR negative as "*C. difficile* screening test positive. No evidence of toxin production.

   Report GDH screen positive, toxin EIA negative, PCR positive as "Toxin-producing *C. difficile* detected".

g. 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.

h. Laboratory CDI testing using a three-step algorithm should be available 7 days a week. 4

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 and is supported in the current ESCMID guidance.

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.

Reporting to ARHAI Scotland for mandatory national surveillance

Only cases of diarrhoea that meet the inclusion and testing criteria set out in the ARHAI Scotland protocol for surveillance of CDI are required to be reported to ARHAI Scotland via Electronic Communication of Surveillance in Scotland (ECOSS) for mandatory surveillance.

Please see ARHAI Scotland CDI page for links to relevant protocol documents:

https://www.nss.nhs.scot/antimicrobial-resistance-and-healthcare-associated-infection/data-and-intelligence/clostridioides-difficile-infection/

Appendix 4: *C. difficile* laboratory testing / reporting algorithm 2024