

Questions and answers about the laboratory diagnosis of *Clostridium difficile* infection (CDI)

The NHS Centre for Evidence based Purchasing (CEP) has published the results of an evaluation of the performance of commercial kits for the detection of *C. difficile* toxins (<http://www.pasa.nhs.uk/pasa/Doc.aspx?Path=%5bMN%5d%5bSP%5d/NHSprocurement/CEP/CEP08054.pdf>). The evaluation represents the largest single study of *C. difficile* toxin detection kits. None of the nine assays tested had a particularly high sensitivity. Also, the poor positive predictive values of the kits, especially in the context of widespread testing, raises doubts about their appropriateness when used as single tests for the laboratory detection of *C. difficile* toxins. The results of the CEP evaluation are consistent with those of a recently published review of (smaller) studies that have examined the performance of *C. difficile* toxin detection kits (Planche *et al.*, *Lancet Infect Dis* 2008;8:777-84).

The following Questions and Answers are in response to the results of the CEP evaluation.

How does the diagnosis of CDI affect how patients are cared for?

The minority of patients (about 10-20%) with hospital acquired diarrhoea have CDI. There are no robust clinical features that rule in or rule out a diagnosis of CDI. Patients with hospital acquired diarrhoea should be isolated to prevent transmission of norovirus, *C. difficile* or other transmissible pathogens. Patients should also be considered for treatment of CDI before the results of tests are available, particularly if symptoms/signs indicate severe infection.

How is *C. difficile* detected in the laboratory?

The most common tests (EIAs) detect the *C. difficile* toxin not the bacterium; cytotoxin testing also detects *C. difficile* toxins, but does this using an *in-vitro* cell sheet assay as the detection method. Alternative approaches include culture (which is relatively slow and requires supplementary testing to confirm that an isolate is a toxin producer) and detection of an antigen (e.g. the enzyme glutamate dehydrogenase) or a gene (e.g. the toxin B gene).

When is it not appropriate to submit faecal samples for the laboratory detection of *C. difficile*?

Only diarrhoeal samples (i.e. unformed stools) should be tested for the presence of *C. difficile* toxins. If there is a clear explanation (not related to infection) for the onset of diarrhoea (for example, laxative use to overcome constipation) then it is not helpful to submit faecal samples for the detection of *C. difficile*/toxins. If diarrhoea continues or the patient has other signs or symptoms consistent with CDI then it is appropriate to submit faecal samples for the laboratory detection of *C. difficile* toxin. It is not recommended to routinely test faecal samples from children aged less than 2 years. It should be noted that occasional patients with severe CDI may have little or no diarrhoea.

What are the implications of an incorrect laboratory test?

A false negative result may result in a patient being thought to be free of CDI. This patient may then receive sub-optimum care, and may not be appropriately isolated, thus increasing the risk of transmission of *C. difficile*. The consequences of false positive are perhaps less well appreciated. These patients may subsequently have necessary antibiotics curtailed or

changed, and receive unnecessary treatment for CDI. Such patients might be nursed in wards or bays together with genuine cases, putting them at increased risk of acquiring true CDI.

How has the present uncertainty about the diagnosis of CDI arisen?

Almost all laboratory tests for the detection of a bacterium or its products can sometimes give false positive or false negative results. However, recently there has been a large rise in the number of *C. difficile* tests being performed. Such widespread testing increases the chance of false positive results (as the great majority of patients tested for CDI do not have this infection).

Are some toxin detection kits better than others?

It is apparent from the available published data on the performance of commercial toxin detection kits, that there may be significant differences in the sensitivity and specificity of these kits. If using these, laboratories should ensure that they are using one of the better performing kits. In the CEP evaluation no single assay was 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*) appear to be superior to the other four. However, even equally good kits can produce very different results. More sensitive tests tend to be less specific and vice versa.

Are toxin detection kits sufficiently accurate to be used as single tests for the detection of *C. difficile* toxins?

The currently available kits for detection of *C. difficile* toxins have variable performance. Recent studies show that the currently available kits may miss about 1 in 5 to 1 in 10 cases of CDI and will falsely identify cases as positive when they are not. The poor positive predictive values of toxin detection kits, especially in the context of widespread testing, and the possibility of missing true positives mean there are limitations to using these as single tests for the laboratory diagnosis of CDI.

How should the results from toxin detection kits be interpreted?

In general the kits are better at ruling out the presence of *C. difficile* toxins (i.e. they have a high negative predictive value). However, the kits generally have sub-optimal positive predictive values, and thus positive results may sometimes actually be false positives. Some tests miss more positive results than others; for example, approximately 1 in 5 cases of CDI. In general, more sensitive tests that find as many positive cases as possible also tend to produce more false positive cases. The converse is also true; so, tests with good positive predictive values tend to miss cases of CDI. This means that caution should be applied to the interpretation of toxin detection results generated using currently available kits, particularly if the clinical details are not consistent with the test result.

Are there particular situations when a test has a low positive predictive value and will yield more incorrect results?

In settings where the disease is rare (i.e. low prevalence) then tests with a low positive predictive value will more often yield false positive results. In the case of CDI, as this is less common in the community setting, particular care should be taken in interpreting positive results on samples from patients who are not hospitalised. When CDI is uncommon or unlikely then caution should be exercised in interpreting test results, and consideration should be given to altering the testing strategy.

What is the best single test for the detection of *C. difficile* toxins?

Well-performed cell-culture cytotoxicity assays are still regarded as the “gold standard” for diagnostic testing. However, these tests have a disadvantage in terms of speed, which may require the use of another rapid assay as an initial screen, particularly in outbreak or endemic settings.

Is the cytotoxin test a practicable alternative for routine microbiology laboratories?

Cytotoxin testing used to be the most commonly performed test for the laboratory diagnosis of CDI. As laboratories have moved away from carrying out cell culture based methods, this has partly hastened the switch from cytotoxin testing, which requires the maintenance of a cell line. However, it is not difficult to maintain a continuous cell line, and thus this approach could still be an option for some laboratories. There is also the possibility of sending faecal samples to another laboratory, as these may be stored at 4°C for several days before re-testing.

Should laboratories that are currently using EIA kits alter their practice?

Currently available kits for the detection of toxins are generally better at excluding the presence of toxins. However, caution is required in the interpretation of toxin-positive results to ensure that these are consistent with the clinical presentation. The issuing of interpretive comments with reports may aid clinicians in interpreting results. The use of a confirmatory test, as part of a diagnostic algorithm, will increase the accuracy of toxin-positive results.

Should laboratories change to using two step protocols (one test followed by another test) for the diagnosis of CDI?

The use of a confirmatory or second test, as part of a diagnostic algorithm, will increase the accuracy of toxin-positive results. However, the choice of first and second test is unclear at present. If the first test lacks sensitivity and only positives are checked by a second test then the overall testing strategy will still miss true positives. The use of the “gold standard” cell cytotoxicity assay is the most accurate test, although there may be practical considerations in the introduction of this assay. Provisional positive results could be issued whilst awaiting confirmation by a cytotoxicity assay. There is also the possibility of performing confirmatory cytotoxicity assays in another laboratory as faecal samples may be stored at 4°C for several days before re-testing.

What is the role of membrane-based immunoassay kits?

There is no evidence the performance of this kind of assay is any better than that of other assays. These assays are quick to perform, and lend themselves to ad hoc testing without the need to batch specimens. This makes them suitable for situations where a result is

required rapidly, or sample volumes are low. However in comparison with well-based assays, their specificity tends to be higher, with a concomitant relative decrease in sensitivity.

Are alternative methods to detect *C. difficile*, such as assays for GDH or PCR for toxin B gene more accurate than toxin assays?

Currently available alternative test methods, such as GDH or toxin B PCR tests, may have a role as part of a diagnostic algorithm for the diagnosis of CDI. However, from the information currently available, as standalone tests, their performance is not significantly more accurate than some of the toxin assays.

How can we compare the accuracy of different assays for the laboratory diagnosis of CDI?

There are now a number of studies which have compared, either directly or by meta-analysis of published studies, the accuracy of many of the currently available tests. However, information on some of the newer tests that do not directly detect *C. difficile* toxins is less widely available.

Should laboratories redefine cut-off levels that define positive results for *C. difficile* toxin detection kits?

Manufacturers' instructions should always be followed. We do not currently recommend redefining cut off values, without research using extensive comparisons to reference standards.

Should multiple repeat samples be submitted for testing

No. If a negative test for the presence of *C. difficile* toxins has been obtained, it is appropriate to consider one repeat test if there remains a high clinical suspicion of CDI. Otherwise, submission of multiple samples for testing for the presence of *C. difficile* toxins should be avoided as, with tests that have a sub-optimal positive predictive value, this increases the chance of obtaining false positive results.

Are further tests required to demonstrate clearance of the organism?

No. Tests of cure (i.e. clearance testing) are not clinically helpful as toxin excretion may continue even when symptoms have stopped.

Does exclusion of formed stools for testing not cause the sensitivity of toxin testing to go down?

No. There is evidence that demonstrates that restricting of testing to diarrhoeal (unformed) stools does not cause the diagnosis of CDI to be missed.

Should toxin testing be restricted to patients with known risk factors for CDI?

No. It is apparent that currently circulating strains of *C. difficile* cause CDI in individuals without conventional risk factors, including patients in the community.

Can results of toxin testing be discounted in the setting of norovirus infection?

No. Suspected or confirmed norovirus infection is not a reason for not testing for the presence of *C. difficile* toxins. However, potential interactions between the two organisms are poorly understood.

How do the results of the CEP evaluation affect the accuracy of *C. difficile* surveillance data?

Local surveillance data based upon a standardised approach to testing, using consistent methodology, reported in a timely fashion, and acted upon appropriately is likely to reflect genuine changes in the level of CDI, and is ultimately the most important tool in controlling the infection at the local level. At a national surveillance level, amalgamation of data based upon different methodologies will inevitably reduce some unwanted “noise” into the system, but this approach can still provide valuable data on whether CDI is being reduced. Changes to the method being used for the laboratory diagnosis of CDI, including the numbers of samples analysed, should be recorded and audited locally so that the implications for surveillance data can be understood.

What key questions need to be answered concerning the optimum laboratory diagnosis of CDI?

There is a clear need for research into the optimisation of the laboratory diagnosis of CDI. It is likely that a two stage algorithm will be a simple and practical way to improve diagnosis. However, there needs to be research into how this is best achieved. Guidance on the optimum way of carrying out cytotoxin testing is also required. There may be scope for re-defining cut-off values in diagnostic kits, but this requires large numbers of samples to ensure a robust outcome and is not recommended as part of routine practice. Further research is needed concerning the concordance of laboratory test results with CDI symptoms and outcome.

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4th March 2009**