

***Clostridium difficile* Toxin - FACT SHEET**

SAMPLE PROCESSING

A single, freshly passed fecal specimen (10 to 20 ml of watery stool) is the preferred specimen for *C. difficile* culture, antigen, and toxin assay. Only liquid or unformed stool specimens should be processed.

C. difficile toxin is unstable and will degrade at room temperature within 2 hours after collection. For optimal recovery, stool specimens should be processed within 2 hours of collection.

Specimens for toxin assay may be stored at 4° C for up to 3 days and should be frozen at -70°C, if performance of the assay is delayed. Freezing the sample at -20°C results in a dramatic loss of cytotoxin activity.

False-negative results occur when specimens are not promptly tested or refrigerated until testing can be done.

Culture alone (without subsequent testing of *C. difficile* isolates for toxin production) results in lower specificity and misdiagnosis of CDAD.

The organism is isolated only for epidemiological studies and for antimicrobial susceptibility testing when required.

DETECTIONS METHODS

Toxin detection and neutralization by a cell culture cytotoxin assay are often considered the “gold standard” due to the high sensitivity (94- 100%) and high specificity (99%) of the assay.

However, the assay is costly and technically demanding and has slow turnaround time.

The commercially available enzyme immunoassays (EIAs) generally show lower sensitivities and specificities (45 to 95% and 75 to 100%, respectively). EIAs that detect both toxins A and B are preferable since some strains of *C. difficile* produce only toxin B.

Real time PCR has been successfully used for quantitative detection of *C. difficile* toxin genes in fecal samples.

C. DIFFICILE COMMON ANTIGEN

C. difficile common antigen testing has been available for more than 15 years. It detects the antigen, which is glutamate dehydrogenase (GDH), commonly produced and retained by both toxigenic and nontoxigenic isolates.

C. difficile GDH will cross react with GDH from other anaerobes, including *Peptostreptococcus anaerobius*, *Clostridium sporogenes*, and *Clostridium botulinum*.

When positive by itself and compared to clinical diagnosis of *C. difficile* diarrhea, common antigen testing is a sensitive (97%) indicator for culture presence of *C. difficile*.

However, it is not a good indicator of potential expression of toxin. A recent publication indicates that in one centre, 62 percent of GDH positive samples tested positive for toxin directly. When GDH positive toxin negative samples were investigated further, 15% contain toxigenic isolates that are capable of toxin expression.

RESULTS INTERPRETATION

Only toxigenic isolates are associated with disease while non-toxigenic isolates may be protected by competitive exclusion. For this reason, toxin detection methods are required in the laboratory diagnosis of CDAD.

Ideally, laboratories should use methods that detect both toxin A and B (TcdA and TcdB) since atypical TcdA-/TcdB+ isolates have been documented.

A positive test for *C. difficile* toxin in symptomatic patients generally confirms the diagnosis of CDAD.

Special considerations exist for the pediatric population. Infants have a high colonization rate (up to 65%) with toxin-producing *C. difficile*. Testing performed

on stool from infants less than 12 months of age who develop diarrhea after antibiotic treatment, has a high probability of a positive result regardless of the real cause of the diarrhea. Therefore, testing for toxin-producing *C. difficile* is not recommended in infants.

A positive test for *C. difficile* common antigen confirms the presence of the organism in the sample, but does not confirm either toxigenic or nontoxigenic CDAD. Reporting a sample as toxin positive solely on the evidence of a positive test

for *C. difficile* common antigen is inaccurate and misleading.

A negative test for *C. difficile* toxin with a positive test for *C. difficile* antigen can indicate that the cause of the diarrhea is not due to *C. difficile*.

Also, this scenario may indicate a false negative result (lack of proper sample storage or low sensitivity of the method) and must be viewed in the context of a patient with symptoms and risk factors for CDAD. Testing for *C. difficile* toxin with an alternative method, such as toxigenic culture, is useful in this case.

A negative test for *C. difficile* antigen has a high negative predictive value (~95%), making this test very useful to rule out CDAD.

C. difficile testing algorithms have been recently published. The SHEA/IDSA guidelines have been summarized in the **2010 Summer issue of Connections**. A review of these algorithms “Strategies for Diagnosis and Management of *Clostridium difficile* Infections” by Dr. Robert Rennie published in the **2011-2012 Winter issue of Connections**.
