Clostridium difficile toxin: A Two-step Protocol for Faster, yet Sensitive, Results

Clostridium difficile is an important nosocomial pathogen (1). Once infected with C. difficile, a patient can become an asymptomatic carrier or, if infected with a toxigenic strain, can develop C. difficile associated diarrhea (CDAD). Risk factors for CDAD include disruption of normal enteric flora by antibiotics, surgery or other trauma. However, it is important to note that only 20% of antibiotic associated diarrhea is actually due to C. difficile.

CDAD can range from mild, watery diarrhea, to life-threatening pseudomembranous enterocolitis. First line treatment is oral metronidazole. Oral vancomycin is reserved for patients with contraindications, intolerance or failure to respond to metronidazole (1,2).

Diagnostic Methods: Diagnosis of CDAD is based primarily on detection of C. difficile toxin A and/or B in stool, using either tissue culture or an immunoassay (1). For description of methods and test rationale, see page 2.

New two-step test protocol at YNHH beginning November 28, 2007:

To provide a more rapid turnaround time for negative stool samples, while not sacrificing sensitivity and specificity of the cytotoxin assay, the Virology Laboratory will adopt a new two-step test protocol (3,4).

Step 1- C. difficile bacterial antigen ELISA: All stools will be tested by ELISA for the presence of the C. difficile bacteria. If negative, C. difficile can be excluded as a cause for the diarrhea.

Step 2- Cytotoxin neutralization assay: If positive for bacterial antigen, the laboratory will then test the stool using the tissue culture assay for C. difficile toxin.

It is expected that only 2/3 of stools harboring the bacteria will be positive for toxin. Treatment should be based on a positive toxin result.

Positive bacterial antigen with a negative toxin indicates colonization only and should not be treated (1,2).

Test Ordering:

1. Submit one stool sample to the Virology Laboratory and order C. difficile antigen test. All samples received by noon will be tested the same day by ELISA, with results reported later that afternoon. Note: Samples received in the laboratory after 12 noon will not be tested until the next day.

2. If positive for C. difficile bacterial antigen, the Virology Laboratory will automatically test the stool for cytotoxin, using tissue culture. Positive cytotoxin results will be reported at 4, 24 and 48 hrs.

NOTE: Submit one stool only per episode of diarrhea. The old practice of submitting 3 stools increases cost with no benefit, especially if the tissue culture cytotoxin assay is used (5,6). Recent studies in our lab have shown that a second stool detected a positive missed by the first stool in only 8 of 1008 patients tested. Thus, a single stool detected 99.2% of positives.
Description of Test Methods

1. **Cytotoxin neutralization assay in tissue culture** detects the toxin B in stool filtrate and is considered the "gold standard" due to its high specificity and sensitivity. It requires cell culture facilities and expertise, thus it is best performed in a virology laboratory. Although positives at YNHH are reported at 4 hrs (30%), 24 hrs (50%), and 48 hrs (20%), all negatives (ultimately about 80% of samples) are not reported until 48 hrs after sample receipt. Until now, this has been the sole test used at Yale New Haven Hospital.

2. **Toxin A or A+B ELISAs** are the most commonly used tests in other hospitals, since they are technically simple and results are reported the same day as sample receipt. However, sensitivity and specificity are 65-90% and 98-99% respectively compared to the cytotoxin assay. As a result, this test has not been offered at YNHH.

3. **Stool culture to isolate *C. difficile*, with subsequent cytotoxin assay or PCR to detect the toxin gene** may be the most sensitive approach, but is labor intensive and requires at least 3-4 days for a result. This methodology is most useful for strain typing and investigation of outbreaks.

4. **C. difficile bacterial antigen ELISA** is a new test that detects the presence of the *C. difficile* bacteria itself, but not the diarrhea-causing toxin. Therefore, asymptomatic *C. difficile* colonization is also detected. This test can be used in a two-step protocol as described (3,4).

References

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