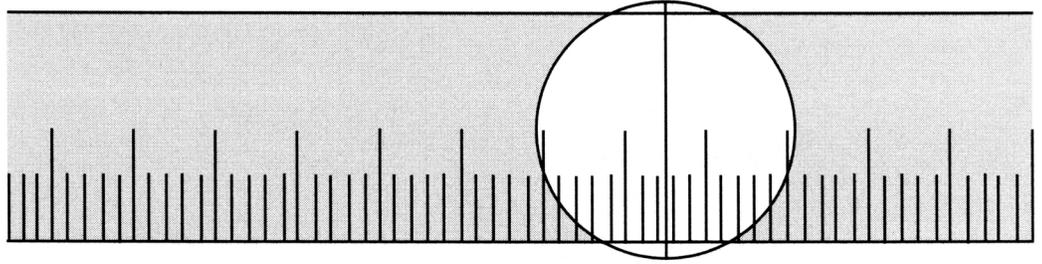


# LAB NEWS



---

From the Department of Laboratory Medicine - Yale-New Haven Hospital Medical Center

## Clinical Virology Laboratory Newsletter

---

Vol. 16 (3)

Nov. 2007

### ***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

1. Poutanen SM and Simor AE. Clostridium difficile-associated diarrhea in adults. CMAJ 171:51-58, 2004.
2. Shim JK et al. Primary symptomless colonization by *Clostridium difficile* and decreased risk of subsequent diarrhea. Lancet 1998; 351:633-6.
3. Landry ML, Topal J, Ferguson D, Giudetti D, and Tang Y. Evaluation of Biosite Triage *Clostridium difficile* Panel for rapid detection of *Clostridium difficile* in stool samples. J Clin Microbiol 39:1855-1858, 2001.
4. Ticehurst JR et al. Effective detection of toxigenic *Clostridium difficile* by a two-step algorithm including tests for antigen and cytotoxin. J Clin Microbiol 44:1145-1149, 2006.
5. Borek AP, et al. Frequency of sample submission for optimal utilization of the cell culture cytotoxicity assay for detection of *Clostridium difficile* toxin. J Clin Microbiol 43:2994-2995, 2005.
6. Mohan SS, et al. Lack of value of repeat stool testing for *Clostridium difficile* toxin. Am J Med 119:356.e7-356.e8, 2006.

**Questions or comments:** Call Marie L. Landry, M.D., Laboratory Director, at 688-3475, or David Ferguson, Laboratory Manager, Clinical Virology Laboratory at 688-3524.