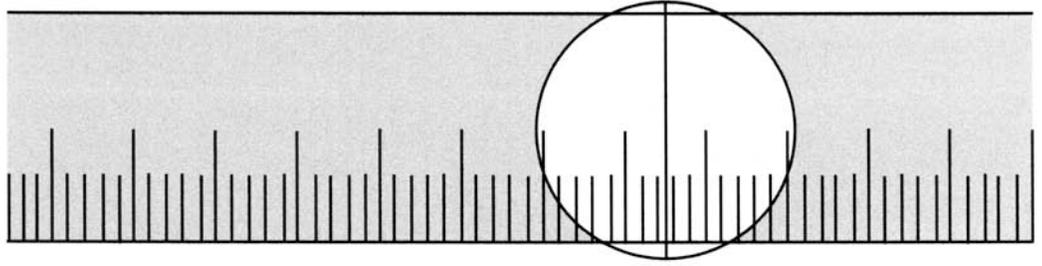


LAB NEWS



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Update on *Clostridium difficile* Two-step Test Algorithm

In November 2007, the Virology Laboratory instituted a new *C. difficile* test algorithm:

1) test for bacterial antigen by ELISA; 2) if bacterial antigen is positive, test for cytotoxin in cell culture. If bacterial antigen is negative, the result is reported the same day and cytotoxin is not done. The purpose of this new protocol was to shorten the time to reporting negative results. Previously, only the cell culture cytotoxin test was used and negatives were not reported until 48 hrs.

In the past, it has been common practice to submit 3 stools for *C. difficile* testing. To assess the value of testing more than one stool, we reviewed our records and found that when the first stool was negative, a second stool was positive in only 12 of 1096 patients. Thus, for 99% of patients, one stool was sufficient.

Recently, the diagnostic accuracy of the new protocol was questioned (*see case details on page 2*).

Investigation of the problem cases revealed that:

- a) bacterial antigen and/or cytotoxin may be rising, and if suspicion remains high, a second stool should be tested;
- b) the bacterial antigen test appears to become positive first, before the cytotoxin;
- c) some problems can be attributed to poor quality samples being submitted (stool mixed with lubricant or urine, or of insufficient volume).
- d) toxin could be degraded, and thus falsely negative, due to proteolytic enzymes or other contaminants in stool, as well as delays in inoculation into cell culture*.
- e) profuse watery diarrhea might dilute the toxin and could give falsely negative results.

**Note: Stools received after 1 PM are not tested until the following day and it is possible (though not proven) that low levels of toxin could be lost. The Virology Laboratory plans to study this further.*

Recommendations for clinicians:

1. **Submit stools to arrive in the laboratory by 1 PM** to make the same day ELISA test run
2. **Submit good quality samples** (sufficient volume, not mixed with urine or lubricants)
3. **Submit a second stool when disease is severe or progressing.**
4. **Call and ask the lab to do the toxin assay directly for severe, cytotoxin-negative cases**
5. **Notify the laboratory immediately** when you are concerned that tests are falsely negative

Plan for the laboratory:

1. Comment in the computer when a sample appears to be suboptimal.
2. Do a study to determine whether cytotoxin is lost when inoculation into cell culture is delayed.

For Questions: Call Dr. Landry, 688-3475, beeper 1-888-631-0112, or David Ferguson, 688-3524.

CASE DETAILS

Case #1 A young child post-liver transplant, was positive for bacterial antigen only, however, disease worsened when therapy was stopped, and improved when therapy was restarted. Unfortunately, a second sample was not submitted for repeat testing, thus etiology could not be confirmed.

Case #2 An elderly woman, post-abdominal surgery, on antibiotics, with past history of *C. difficile* disease, presented with profuse diarrhea. Her stool was negative for bacterial antigen, so the cytotoxin test was not done. Treatment was stopped, and patient worsened. A second stool was sent 3.5 hrs later for rotavirus testing, not *C. difficile*; this stored sample was tested retrospectively as shown below. These initial samples were clear and mucous-like. Three days later a third sample (brown like stool) was sent that was now high positive for *C. difficile*.

Clostridium difficile Bacteria and Toxin Test Results for Case 2

Sample	Date/time rec'd	Bacterial antigen ELISA (O.D.)	Cytotoxin	Sample
1	2/3/08 @Noon	Negative (O.D. 0.006)	Not done	Not available
2	2/3/08 @3:30 PM*	Borderline Positive (O.D. 0.080)	Negative	Clear, mucous-like
3	2/6/08	High positive (O.D. >3.00)	High positive	Brown stool

*Sample 2 was submitted for rotavirus and was not originally tested for *C. difficile*

Summary of case 2: Suboptimal samples and rising antigen and toxin:

1. The samples on 2/3/08 were suboptimal. Apparently a rectal tube was inserted with lubricant. The sample available for examination did not look like stool (clear, mucous-like).
2. Sample 2, arriving in the lab 3.5 hrs after sample 1, was borderline positive for antigen but cytotoxin negative.
3. Sample 3, collected 3 days later was a very high positive for both antigen and cytotoxin (positive at 4 hrs of incubation at 10^{-3} dilution)

Case #3 A patient with profuse diarrhea had a sample submitted from the ED that was liquid yet had some particular stool mixed in. This sample was *C. difficile* bacterial antigen positive (O.D. 1.5). However, a stool submitted upon arrival on the floor an hour later was negative. The negative stool had no particulate matter and was frothy like urine (perhaps recovered from a bed pan containing urine mixed with diarrheal stool?). This appeared to be another example of poor sample quality yielding false negative results. Of note, initial stool was cytotoxin-negative and norovirus-positive.

Case #4 An elderly man, recently treated with antibiotics, was admitted from a nursing home with fever, leukocytosis, abdominal pain and diarrhea. *C. difficile* antigen was a strong positive (O.D. 2.937), but cytotoxin was negative. The patient was treated with IV Flagyl and po Vancomycin without clear improvement. A second stool 3 days later while on therapy showed a low positive antigen (O.D. 1.2), and again a negative cytotoxin. A sigmoidoscopy at that time showed pseudomembranous colitis. However, the patient did not respond to treatment. Of note, since his samples were received at 4 PM in both cases, the cytotoxin test was not performed until the next day. Could the toxin have degraded? This possibility needs to be studied.

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