



Laboratory *News*

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The Changing Nature of *Clostridium difficile* Disease and Laboratory Diagnosis

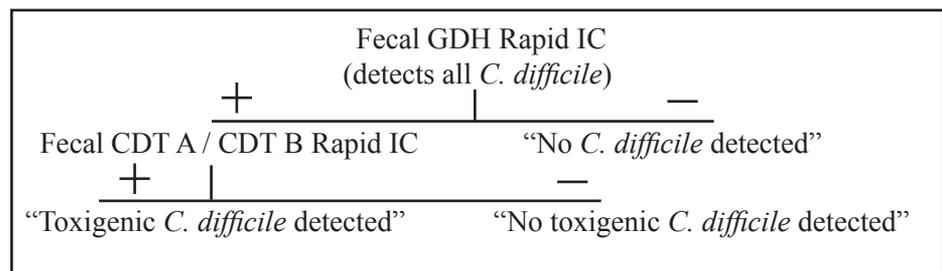
Thomas J. Novicki, PhD and Thomas R. Fritsche, MD, PhD

On March 9, 2009, Marshfield Labs will switch to a two-step algorithm for laboratory diagnosis of *Clostridium difficile*-associated disease (CDAD). (See Figure 1.) This algorithm will use a new rapid immunochromatography (IC) method that is as sensitive, and more specific, than the current toxin enzyme immunoassay (EIA) test. Additionally, the new method is superior in terms of rapid turnaround time.

Not all *C. difficile* strains are toxigenic. Therefore, all fecal specimens submitted for *C. difficile* toxin study will first be screened for glutamate dehydrogenase (GDH) antigen, common to all toxigenic and non-toxigenic *C. difficile* strains, using rapid IC. Specimens negative for GDH can predictably be called negative for *C. difficile*. In the second step, GDH positive specimens will be tested for *Clostridium difficile* toxins CDT A and CDT B, again by rapid IC. While somewhat complex, this approach offers better performance compared to standard EIA assays. The use of the rapid test will also allow flexibility in laboratory staff utilization and decreased materials costs.

Reports will either state “No *C. difficile* detected”, “No toxigenic *C. difficile* detected” or “Toxigenic *C. difficile* detected”. No *C. difficile* detected and No toxigenic *C. difficile* detected may clinically be considered to have the same meaning. In keeping with current recommendations, only unformed specimens, i.e., taking the shape of the container at room temperature, will now be routinely accepted. Patient charges for this test and ordering information will remain unchanged. (See sidebar on page 2.)

Figure 1: Two-Step Algorithm for CDAD Laboratory Diagnosis



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Ordering Information

Test Name:

Clostridium difficile Toxin Assay

Test Code:

CTX

CPT Code:

87324

Specimen:Fresh, unpreserved, unformed*
feces in leak-proof container

(*takes shape of container at room temp.)

Turnaround time:

1 Day

Where and When Performed:

Performed at Marshfield Center:

- Patients at St. Joseph's
Hospital Marshfield: 7 days/wk
- Other patients: M-F

Background

Clostridium difficile is a Gram positive, anaerobic, spore forming bacillus that has the ability to produce two enterotoxins. These toxins, CDT A and CDT B, cause profuse watery diarrhea, colitis, and sometimes more serious effects such as toxic megacolon, bowel obstruction, and death. Other symptoms often include fever, nausea/vomiting, and abdominal bloating. Notably, some *C. difficile* strains are non-toxigenic, and are not thought to cause CDAD. In fact, non-toxigenic strains may provide protection against toxigenic *C. difficile* infection through competition in the gut. Some isolates also produce another enterotoxin, Binary Toxin, whose contribution to CDAD is unclear.

C. difficile is spread by the ingestion of its spores. These spores, which are difficult to eradicate, allow *C. difficile* to persist in the environment. Upon ingestion, spores pass unharmed through the acid environment of the stomach to the colon where they germinate. *C. difficile* cells colonize the intestinal crypts, causing inflammation and cell death. Cellular debris and mucus build up to form plaques, which then grow and coalesce into the pseudomembranes that are classically associated with CDAD colitis. Of interest, while < 3% of otherwise healthy adults carry *C. difficile*, 15-70% of all neonates carry toxigenic *C. difficile* with no apparent ill effects. The reasons for this are unknown; lack of the intestinal receptor for *C. difficile* toxins in the juvenile gut has been postulated, but other factors are also likely to play a role. In adults, risk factors for the development of CDAD include: recent antibiotic exposure, increasing age, recent stay in a hospital or long term care facility, AIDS. Proton pump inhibitor anti-ulcer medications may also predispose individuals towards CDAD. Cephalosporins, penicillins and clindamycin were the first antibiotics associated with CDAD, but most classes of antibiotics have now been linked to CDAD to a greater or lesser extent. Fluoroquinolone use has recently emerged as a major drug-induced cause of CDAD with the development of fluoroquinolone resistant strains of *C. difficile*.

The epidemiology of CDAD has changed in recent years. In the 1970's, *C. difficile* came to be recognized as a major cause of nosocomial (hospital related) antibiotic-associated diarrhea (ADD). *C. difficile* is also associated with 50-75% of antibiotic associated colitis, and >90% of pseudomembranous colitis and toxic megacolon. The annual cost of CDAD in the USA alone was estimated at >\$1B in 2003.

CDAD continues to be a major infection control problem for hospitals and long term care facilities. In 2006, the CDC reported that the rates of CDAD-related discharge diagnoses from US hospitals had increased from 31/100K to 61/100K between 1996 and 2003, with the greatest increase occurring between 2000 and 2003. At the same time, several studies independently identified a new strain of *C. difficile* in Canada and the USA that had the unusual (for *C. difficile*) ability to cause institutional outbreaks with apparent greater levels of morbidity and

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mortality. This strain, variously referred to as North American Pulsed Field Gel Electrophoresis Type 1 (NAP1), B1, or O27, has now been identified in hospitals worldwide. The reasons for the increased virulence of NAP1 is not conclusively known, however several findings give hints. NAP1 *C. difficile* strains produce higher levels *in vitro* of CDT A and CDT B earlier in its growth phase than do other strains. The increased toxin production is most likely due to a mutation in the *tdcC* gene, which negatively regulates toxin production. While not validated in individuals with NAP1-associated CDAD, higher amounts of CDT A or CDT B are consistent with an increase in the severity of disease. NAP1 strains also produce Binary Toxin, which may contribute to severity. Finally, NAP1 strains also produce higher levels of spores than do non-NAP1 strains.

Concurrent with the emergence of NAP1 in hospitals was the appearance of CDAD in the community (community associated CDAD, or CA-CDAD). Once a rare occurrence, CA-CDAD is being identified with greater frequency in individuals (6.9-7.6/100K population) with few if any classical risk factors. The Centers for Disease Control, in two reports from 2005 and 2006, identified the following characteristics of CA-CDAD individuals:

- Lack of antibiotic exposure
- Lack of recent hospitalization
- Young age
- Bloody diarrhea

Although the appearance of CA-CDAD corresponded with the emergence of NAP1 in hospital populations, CA-CDAD has not been found to be entirely due to NAP1; a mixture of NAP1, NAP1-related, and non-NAP1 strains have been identified. Laboratory detection of the NAP1 strain and Binary Toxin is not yet generally available, but tests are in development.

Laboratory-based studies for the detection of *C. difficile* and its toxins, while still considered a cornerstone of CDAD diagnosis, is another area that is undergoing change. The first tests developed relied on cell culture to detect CDT B, either directly in feces (fecal CTX), or from fecal isolates of *C. difficile* (toxigenic culture). Toxigenic culture, with a sensitivity approaching 100%, is still considered by many to be the gold standard for diagnosis. However, these tests proved cumbersome, requiring virology lab cell culture facilities and having a long (2-7 days) turnaround time. The 1980's saw a rapid transition to EIA tests for CDAD diagnosis, particularly in North America. While also detecting toxin directly in feces, EIA offers the advantages of relative ease of use and rapid turnaround time (< 1 day). However, a major shortcoming that persists to this day is that EIA is significantly less sensitive than either fecal CTX or toxigenic culture. In-house developed and validated PCR assays for the detection of toxigenic *C. difficile* in feces have been used in some reference laboratories for a number of years. While these PCR assays were not standardized, their use has suggested that molecular assays for *C. difficile* rival toxigenic culture in performance and may actually be more sensitive than culture-based tests.

Selected References

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Interpretive Questions

**Contact Thomas Novicki, PhD
or Thomas Fritsche, MD, PhD at
800-222-5835, or extension
1-6300.**

One FDA-cleared test has recently become available and others are under development. Marshfield Labs is looking closely at these assays, which in addition to increased sensitivity offer turnaround times rivaling toxin EIA methods.

The use of GDH and toxin testing, in one published study, was found to be 96% sensitive compared to fecal CTX, while conventional toxin EIA was only 38% sensitive. In our in-house pilot study of 110 consecutive fecal specimens submitted for *C. difficile* toxin testing, the GDH and CDT A/CDT B rapid IC algorithm was found to be as sensitive, and more specific, than our current toxin EIA, when compared to fecal CTX. One limitation of this study is its small sample size; studies are ongoing. 