



L a b o r a t o r y News

VOL. 33, NO. 1 - FEBRUARY 4, 2010

New Molecular Assay for the Laboratory Diagnosis of *Clostridium difficile* Disease

Timothy S. Uphoff, Ph.D., Mary E. Stemper, M.S., Thomas J. Novicki, Ph.D. and Thomas R. Fritsche, M.D. Ph.D.

Marshfield Labs is pleased to announce that effective February 8, 2010, we will switch to a Real Time PCR in vitro diagnostic test (CDNAT) for the qualitative detection of toxigenic *Clostridium difficile* nucleic acids isolated from liquid or soft stool specimens obtained from symptomatic patients. This test targets the *C. difficile* toxin B gene (tcdB) and is intended for use to aid in the diagnosis of toxigenic *C. difficile* infections. This real time PCR method is more sensitive and specific than the current three step algorithm assays it replaces.

Testing will be performed once daily, with same day availability of results. (Results from samples received after 7am on Sunday will be available by noon on Monday.) In keeping with current recommendations, only fresh, unpreserved, unformed specimens (i.e., taking the shape of the container at room temperature) in a clean leak proof container will be routinely accepted. Specimens transported in Cary-Blair or other C & S transport media are unacceptable.

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 *C. difficile* associated disease (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

continued on page 2

INSIDE THIS ISSUE

Marshfield Labs Discontinues EGFR Testing 4

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- 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 from the actions of expressed toxins and cell death. Cellular debris and mucus build up to form plaques, which then grow and coalesce into 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 and 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 US 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 US that had the unusual (for *C. difficile*) ability to cause institutional outbreaks with apparent greater levels of morbidity and 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 are 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 *tcdC* gene, which negatively regulates toxin production. While not characterized 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

continued on page 3

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 characterization of the NAP1 strain and Binary Toxin is not yet generally available, but tests are in development.

Testing

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 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 enzyme immunoassay (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 (<1day). However, a major shortcoming that persists to this day is that EIA is significantly less sensitive than either fecal CTX or toxigenic culture. Real Time PCR assays for the detection of toxigenic *C. difficile* in feces have recently been introduced into some reference laboratories. Studies have shown that molecular assays for *C. difficile* rival toxigenic culture in performance and may actually be more sensitive than culture-based tests. The FDA approved Real Time PCR assays now implemented by Marshfield Labs have been demonstrated in clinical trials to be more sensitive than the gold-standard cytotoxin assay and culture.

Limitations of New Test

- This test detects all toxigenic strains of *C. difficile*, including NAP1 (Ribotype 027) but does not differentiate the NAP1 strain from other toxigenic strains of *C. difficile*.
- This test targets the *tcdB* gene for Toxin B production. This test will not detect the very rare strains of *C. difficile* that do not contain the *tcdB* gene.
- In keeping with current recommendations, only unformed specimens (i.e., taking the shape of the container at room temperature) will be routinely accepted. 

Test Name:

Clostridium difficile nucleic acid test

Test Code:

CDNAT

Specimen:

Fresh, unpreserved, unformed feces (takes shape of container at room temp.) in leak-proof container; Refrigerate up to 48 hours. Freeze at -20°C for storage after 48 hours.

Turn Around Time:

1 Day

Availability:

Performed at Marshfield Center once daily, with same day availability of results. Samples received after 7 a.m. on Sunday will be result by noon on Monday.

CPT:

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Interpretive Questions

Contact any of the following:

Timothy Uphoff, PhD
Thomas Novicki, PhD
Thomas Fritsche, MD, PhD

at 800-222-5835, or ext. 1-6300

Marshfield Labs Discontinues Epidermal Growth Factor Receptor (EGFR) Testing

Effective Monday, February 1st, Marshfield Labs will discontinue EGFR testing by immunohistochemistry (IHC) due to poor correlation between EGFR expression by IHC and treatment response. Recently published guidelines from the National Comprehensive Cancer Network and the American Society of Clinical Oncology recommend KRAS Mutation Analysis as part of the evaluation of metastatic colorectal cancer patients who are being considered for anti-EGFR therapy. KRAS Mutation Analysis is available through Marshfield Labs as a send-out test.

Retrospective analyses of clinical trials have consistently demonstrated that patients with metastatic colorectal carcinoma and mutant KRAS are unlikely to benefit from the addition of cetuximab or panitumumab to a chemotherapy regimen. Testing for KRAS mutation in this setting is now recommended and marks a paradigm shift in the management of these patients. Contact Marshfield Labs Histology Department with questions. 