



Marshfield Labs™

A division of Marshfield Clinic

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

Marshfield Labs is pleased to announce that effective February 8, 2010, we will switch to a Real Time PCR in vitro diagnostic test 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.

Less than 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 in individuals (6.9-7.6/100K population) with few if any classical risk factors. The CDC, 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. 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. 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 this test:

This test detects but does not differentiate the NAP1 (Ribotype 027) 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 Information:

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 -20oC for storage after 48 hours.

TAT: Performed daily

CPT Code: 87493