



TECHNICAL NOTICE

SOUTH BEND MEDICAL FOUNDATION

February 2010

Clostridium difficile in Feces – Changes in Testing Approach

Effective Date: February 1, 2010

Performing Department: • Microbiology

Overview:

SBMF is introducing a new approach for screening and diagnosing *Clostridium difficile*-associated disease (CDAD). This approach is more sensitive than the current Toxin A & B by Enzyme Immunoassay (EIA) and is more specific than the current *Clostridium difficile* culture without cytotoxin assay. It provides a cost-effective and timely result.

A new EIA test for *C. difficile* Glutamate Dehydrogenase (GDH) Antigen and *C. difficile* Toxins A & B will replace the previous EIA test for Toxins A & B only.

If the EIA results for GDH antigen are positive and the EIA results for toxins A & B are negative, the specimen will be further tested for *C. difficile* Toxin B gene (tcdB) using Polymerase Chain Reaction.

Clinical Significance:

After treatment with antibiotics, many patients develop gastrointestinal problems ranging from mild diarrhea to severe pseudomembranous colitis. Many cases of the milder forms of gastrointestinal illness and most of the cases of pseudomembranous colitis are caused by toxigenic strains of *Clostridium difficile*. *C. difficile* is an opportunistic anaerobic bacterium that grows in the intestine once normal flora has been altered by the antibiotic. Toxigenic strains of *C. difficile* carry the genes encoding the toxins while non-toxigenic strains do not carry the toxin genes. Disease onset is associated with the toxins that are produced by the toxigenic organism.

The clinical symptoms associated with the disease are believed to be primarily due to Toxin A, a tissue-damaging enterotoxin. *C. difficile* also produces a second toxin that is designated as Toxin B. Toxin B, which has been referred to as the cytotoxin of the organism, is the toxin detected by tissue culture assay. Toxigenic *C. difficile* strains produce both Toxin A and Toxin B, or only Toxin B.

The glutamate dehydrogenase (GDH) *C. difficile* antigen is a good marker for the organism in feces because it is produced in high amounts by all strains, toxigenic or non-toxigenic. The GDH antigen can be detected in fecal specimens by EIA. A positive result GDH confirms the presence of the organism, while a negative result indicates its absence. A positive EIA result for toxins A and B confirms the presence of toxigenic *C. difficile*.

If EIA screening tests for *C. difficile* GDH antigen and *C. difficile* toxins A & B provide discrepant results, PCR testing for *C. difficile* Toxin B gene (tcdB) is recommended to aid in establishing the diagnosis.

Method:

- Enzyme Immunoassay (EIA) for *C. difficile* GDH Antigen and Toxins A & B
- Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) for *C. difficile* Toxin B Gene (tcdB)

Use:

- Qualitative detection of *C. difficile* Glutamate Dehydrogenase (GDH) Antigen
- Qualitative detection of *C. difficile* Toxins A and/or B
- As needed, qualitative detection of *C. difficile* Toxin B Gene (tcdB)

Reference Range:

- Negative for *Clostridium difficile* Glutamate Dehydrogenase (GDH) Antigen by EIA
- Negative for *Clostridium difficile* Toxins A & B by EIA

Note: Testing for *C. difficile* Toxin B Gene (tcdB) will be performed by RT-PCR on samples with discrepant EIA results; an additional charge may apply

***Clostridium difficile* – Changes in Testing Approach (continued):**

Specimen Requirements and Collection:

- Preferred Specimen: • Feces, liquid (preferred), in sterile plastic container with tightly fitting lid
- Alternate Specimen: • Feces, unformed (semi-solid), in sterile plastic container with tightly fitting lid
- Requested Volume: • 1.0 mL liquid feces Minimum Volume: • 0.25 mL liquid feces *-or-* • 2.0 g unformed feces
- Processing: • Maintain refrigerated during storage and transport: if refrigeration is not available, place in ice slush
- Stability: • 3 days (72 hours) refrigerated (2-8°C) • Greater than 72 hours, freeze (-20°C)
- Storage/Transport: • Refrigerated (72 hours) or, if greater than 72 hours, freeze
- Rejection Criteria: • Sample not refrigerated • Sample refrigerated, but greater than 72 hours and not frozen
- Specimen Remarks: • *C. difficile* toxin is heat labile (toxin degrades without refrigeration)

Testing Schedule: • Daily • Result available within 24 hours

Order:

- ***Clostridium difficile* GDH Antigen and Toxins A & B, by EIA, Feces** Test #: **21136** CPT: • 87324 • 87449
- ***Clostridium difficile* GDH Antigen and Toxins A & B, by EIA,
with *C. difficile* Toxin B Gene, by PCR, if Indicated, Feces** Test #: **21142** CPT: • 87324 • 87449
If indicated by EIA testing for GDH Antigen and Toxins A & B, add: • 87493
- ***Clostridium difficile* Toxin B Gene, by PCR, Feces.....** Test #: **21137** CPT: • 87493

For additional information contact C. Kurtis Kim, M.D., Mary G. Stepney, Microbiology Specialist, or Nan Boston, Microbiology Manager, South Bend Medical Foundation , (574) 234-4176 or (800) 544-0925.