Technical Brief

Clostridium difficile Stool PCR

Background Information

Clostridium difficile-associated diarrhea is most commonly recognized as the cause of nosocomial illnesses including antibiotic-associated diarrhea, antibiotic-associated colitis and pseudomembranous colitis, the latter of which could result in toxic megacolon if not treated appropriately. It is the primary cause of hospital-associated colitis in patients who receive antibiotics, chemotherapeutic agents, or other drugs that can alter the normal gastrointestinal flora.

The incidence of C. difficile-related disease in hospitalized patients is dependent upon the specific patient population with patients older than 65 years of age affected most often. It is estimated that more than 2 million individuals in U.S. hospitals between 1993 and 2005 had C. difficile infection. Hospital discharges with C. difficile infection in 2001 were reported at approximately 149,000 cases as compared with > 300,000 cases in 2005 (1). Non-hospitalized patients also could be diagnosed with C. difficile disease, although this occurs less frequently than in hospitalized patients.

Prior to the introduction of PCR assays, an enzyme immunoassay (EIA) was utilized for detection of Toxin A and Toxin B, the two toxins produced by C. difficile in the stool. The sensitivity of the EIA assay has been demonstrated to be 61-94%, depending upon the assay and gold standard for comparison used (2-4) C. difficile PCR has been reported to detect up to 35% more C. difficile-positive specimens than are detected with EIA assays (5). Sensitivity of the BD GeneOhm™ (BD Diagnostics, Franklin Lakes, NJ) assay has been determined to be ≥91% vs. the EIA assay with a specificity of 95% and negative predictive value of 99%. As compared with toxigenic culture, probably the most sensitive assay available, the BD GeneOhm assay demonstrated an 84% sensitivity, 98% specificity and 97% predictive value negative in one study. The EIA assay compared with toxigenic culture had a sensitivity of 67% (6). Toxigenic culture, although considered the gold standard for laboratory diagnosis, usually requires 5-7 days for completion. Use of the more sensitive PCR assay should provide rapid results for better patient care and eliminate the need for multiple screening and/or confirmatory assays.

Clinical Indications

Hospitalized and non-hospitalized patients who develop diarrhea and for which the diagnosis of C. difficile-associated diarrhea is indicated should have stool submitted for detection of toxin producing C. difficile. The most common clinical definition of C. difficile infection is that of diarrhea that develops in a patient who is currently taking or has recently taken antibiotics, who has had > 3 watery stools per day. Other symptoms may include fever, abdominal pain, cramping, nausea, and loss of appetite.

Pseudomembranous colitis is defined as the presence of plaque formation on colonic membranes and is considered pathognomonic for C. difficile infection in the appropriate clinical setting.

Making a laboratory diagnosis of C. difficile disease in the infant (< 3 years) can be difficult even though cases of serious C. difficile infections have been described in infants (7,8). Infants can carry toxin-producing C. difficile organisms in their GI tract without having the disease (9). Submitting stools from infants younger than 1 year for C. difficile testing is not recommended. Testing for C. difficile between the ages of 1 and 3 may be warranted in selected cases provided that clinicians understand the limited predictive value of a positive test (10).

Reporting and Interpretation

Results are reported as:

- C. difficile toxin detected by PCR
- C. difficile toxin not detected by PCR

Limitations of the Test

Only non-formed stools should be submitted for C. difficile PCR. Diarrhea is the predominant symptom of C. difficile disease, and samples should be representative of that condition. The GenOhm PCR product lists as a limitation in their package insert that only soft or liquid stools are acceptable. Diapers are not acceptable specimens for C. difficile testing.

Infants younger than 3 years old may carry toxigenic C. difficile as part of their normal GI flora; testing on infants is not recommended. Stool from infants < 1 year old will not be accepted for C. difficile testing.

Because the sensitivity of the PCR assays is much higher than most other C. difficile diagnostic tests, only one liquid or soft stool sample is required for the diagnosis of an episode of C. difficile disease.

Neither PCR nor other diagnostic tests are appropriate to use as a “test of cure” of C. difficile. The cure of C. difficile is defined as a clinical response to treatment (11). C. difficile and its toxins may persist in stool after the resolution of symptoms and is of uncertain clinical significance. Antibiotics may not eliminate C. difficile carriage (12).
Methodology

The C. difficile PCR assay is a real-time in vitro diagnostic test for the direct, qualitative detection of C. difficile toxin B gene in specimens from patients suspected of having C. difficile-associated disease. The test is performed directly on the liquid (non-formed) stool, utilizing polymerase chain reaction (PCR) for amplification of specific targets and fluorogenic target-specific hybridization probes for the detection of the amplified DNA. The BD GeneOhm assay used at Cleveland Clinic utilizes the Smart Cycler real-time PCR instrument for amplification and detection of the toxin of C. difficile.

References


