

Real-time PCR for Detection of *Clostridium difficile* Infections

Setting and History

Clostridium difficile (*C. difficile*) is a Gram-positive, spore-forming anaerobic bacillus that was first linked to disease, specifically pseudomembranous colitis (PMC), over 30 years ago.¹ *C. difficile*-associated diarrhea (CDAD) is an antibiotic associated diarrhea that has come to be essentially synonymous with PMC, although there are other etiologies for pseudomembrane formation. *C. difficile* has two forms, an active, infectious form that cannot survive in the environment for prolonged periods, and an inactive spore that can survive in the environment for extended lengths of time. Spores do not directly cause infection. However, when ingested they can transform into the active form and hence cause infection. *C. difficile* spores are found in a vast array of environments including hospitals, nursing homes, extended care facilities, and nurseries. They have been found on almost every surface imaginable in the patient's room and of course on unwashed hands and stethoscopes. The diarrhea is caused by an overgrowth of *C. difficile* almost always subsequent to recent antibiotic use. The main culprits are well known to be clindamycin and broad-spectrum cephalosporins, however almost any antibiotic may be found responsible at times.

The diarrhea is secondary to the bacterium producing toxin A (tcdA) and/or toxin B (tcdB). The majority of pathogenic strains are toxin A+/toxin B+ with some strains toxin-A-/toxin-B+. CDAD has also seen an explosive increase in both its frequency and severity during the past decade here in the United States thought to be mainly due to an epidemic strain of *C. difficile* (NAP1, ribotype 027, REA type BI).^{2,3} Importantly, this strain has demonstrated increased toxin production, disseminates easily in hospital environments and has a higher mortality rate.

The diagnosis of CDAD had usually been made based on the clinical history of recent antibiotic usage followed by diarrhea in conjunction with confirmatory laboratory tests. The laboratory diagnosis of CDAD was made with culture, cyto-

toxic assays, or toxin-detection immunoassays. Anaerobic culture of stools is more sensitive than the toxin-detection assays, however it is also more time consuming, labor intensive and requires confirmation of the toxigenicity of the isolate via a second method (often the cytotoxic assay). The toxin-detection immunoassays directly detect toxin A and/or toxin B and are available in multiple enzyme immunoassays (EIAs) currently on the market. While the EIA method is both relatively rapid and simple compared to the other methods, it is also much less sensitive than culture of the organism with some sensitivities reportedly as low as 48%.³⁻⁵ Culture, of course, is time consuming and the diagnosis of CDAD must be made in as rapid a manner as possible in order to direct prompt therapy and subsequently reduce hospital length of stay.

Real-time PCR Diagnosis of CDAD

A commercially available multiplex real-time PCR assay is now being utilized here in our Bronson Laboratory (Cepheid Xpert *C. difficile* assay) for the diagnosis of CDAD. The sensitivity has been reported at 93.5-97.1% with a specificity of 94.0-96.3%.^{4,5} This PCR molecular technique detects the toxin B gene. The above sensitivity and specificity are a major improvement over previous methods and eliminate the need for ordering repeat testing (previously as many as three times). All of the test cartridges are self-contained thus eliminating cross contamination.

Specimen Collection, Interpretation and Treatment

The assay must be performed on fresh stool (either liquid or soft) collected in a clean, dry container. Testing will not be performed on any formed stools. A positive PCR result is indicative of the presence of *C. difficile* and toxin B production. A negative result indicates the absence of *C. difficile* infection. Rare false-negatives may occur due to *C. difficile* present in quantities below the limit of detection or inhibition of PCR. Due to the greatly improved sensitivity

and specificity, only one stool specimen during a patient's admission will be accepted for *C. difficile* testing. Occasionally patients may be asymptomatic carriers of *C. difficile* and clinical correlation is needed in their management. The assay detects, but does not differentiate, the NAP1 strain from other toxigenic strains of *C. difficile*. Treatment of CDAD usually involves discontinuing the offending antibiotic and administering oral metronidazole or vancomycin.

Anticipated Start Date

It is anticipated that Bronson will begin exclusively using the real-time PCR for *C. difficile* on February 1, 2011.

References:

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3. Sloan LM, Duresko BJ, Gustafson DR, et al. Comparison of Real-Time PCR for Detection of the tcdC Gene with Four Toxin Immunoassays and Culture in Diagnosis of *Clostridium difficile* Infection. *J. Clin. Microbiol.* 2008;46:1996-2001.
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6. Mayo Medical Laboratories, Online Test Catalog, Unit Code 83124: *Clostridium difficile* Toxin, Molecular Detection, PCR, Mayo Clinic, Rochester, MN

— Jeff Hinson, DO

First Laboratory in the State to Adopt Green Alternative Bronson Histology Department Uses Xylene-Free Tissue Processing

In July of 2008, the histology department at Bronson Methodist Hospital began using a Peloris Rapid Tissue processor. Rapid tissue processing allows us to optimize our processing schedule for increased throughput of various tissues depending on the type of tissue and thickness of sections. The department has also realized valuable gains with the Peloris instrument's reagent management system that tracks reagent quality and then instructs the user to change a reagent based on this determination.

With the Peloris instrument, processing runs are performed during the day compared with conventional processing that batches all tissue into overnight runs. Surgical tissue is sorted throughout the day into batches for 2-, 4-, 8- and 12-hour processing cycles based on specimen evaluation during gross examination. Surgical tissue specimens eligible for the 2- and 4-hour processing cycles can be processed during the day offering improved turnaround time and even same-day reporting of results.

In April 2010, we installed a second Peloris instrument, which afforded us the opportunity to consider conventional versus xylene-free tissue processing. We would be pioneers in the endeavor to use xylene-free processing with the Peloris instrument in Michigan.

A Brief Explanation of Tissue Processing

In order to prepare tissue samples for microscopic interpretation, the tissue is processed to allow infiltration by wax for sectioning onto microscope slides for staining. In conventional tissue processing, water is removed from the tissue by dehydration through submersion in ethanol followed by a hydrophobic clearing agent (xylene) to remove the alcohol and enable infiltration of the paraffin wax.

Xylene is used as a clearing agent in conventional tissue processing because it is miscible with both alcohol and wax. Xylene is a hazardous chemical recognized as a potential carcinogen. Xylene is also hazardous to the environment and requires special hazardous waste disposal procedures incurring additional costs for the department.

Xylene-free Tissue Processing

In xylene-free processing, Isopropyl alcohol acts as the dehydrant and is also miscible with wax, enabling the tissue to be infiltrated with wax for sectioning. Following installation and validation of the second Peloris with xylene-free protocols, we performed parallel xylene-free and conventional processing on routine histology samples. The Peloris instruments were used interchangeably over the course of four months to provide adequate time and sampling to determine which type of processing was the best fit for our department. In general, there were very few remarkable differences in the tissue outcomes and instrument utilization. There was no impact on turnaround time by going to xylene-free processing. In some cases, tissues processed by the xylene-free protocols were superior in both microtomy and staining quality. Ultimately, the benefits of reduced exposure to xylene for our staff and reduction of xylene waste are the motivating factors for converting to xylene-free tissue processing in our histology department. Xylene-free processing on the Peloris instrument provides a safer environment for staff, reduced reagent and disposal costs, and equivalent, if not superior, processing results.

— Virginia Rupert, MBA CT (ASCP)
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Improved Testing Methodology for Testosterone

Effective December 15, 2010, the Bronson Laboratory has converted the Testosterone assay to the Elecsys® Testosterone II assay, which has improved precision at the low end of the reporting range and excellent correlation with liquid chromatography tandem mass spectroscopy. With this change, there are additional age specific ranges and test units have changed from ng/ml to ng/dl.

NEW RANGES

Adult Males:

18-49 years = 249-836 ng/dl
≥50 years = 193-740 ng/dl

Adult Females:

18-49 years = 8.4-48.1 ng/dl
≥50 years = 2.9-40.8 ng/dl

Children: The reference range for children is based upon the Tanner Stage. For individuals younger than 18 years of age, the tables shown below are attached to the results:

Male ng/dl ng/dl			Female ng/dl ng/dl		
Stage	Min	Max	Stage	Min	Max
1	<2.5	<2.5	1	<2.5	6.1
2	<2.5	432	2	<2.5	10.4
3	64.9	778	3	<2.5	23.7
4	180	763	4	<2.5	26.8
5	188	882	5	4.6	38.3

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