AMILTON REGIONAL LABORATORY MEDICINE PROGRAM

LAB CONNECTIONS

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IN THIS ISSUE: Molecular methods that detect the genes encoding *C. difficile* toxin could offer a rapid and sensitive alternative to traditional *C. difficile testing.* Dr. Padman Jayaratne, HRLMP microbiologist, describes this test and its limitations.

WHAT'S NEW?: In July, Pediatric Pathology welcomed Dr. Jefferson Terry to the team at McMaster Children's Hospital. This summer, the team's work area underwent a huge facelift which will facilitate teaching and research, and increase efficiencies.

A New Real-Time PCR Method for Detection of Toxigenic *Clostridium difficile* at the HRLMP

Clostridium difficile is considered to be one of the most important causes of health care-associated infections and is the leading cause of nosocomial diarrhea in adults. *C. difficile* infections are also emerging in the community and in animals used for food (1). The symptoms of *Clostridium difficile* infection (CDI) can range from mild, self-limiting diarrhea to pseudomembranous colitis and fulminant toxic megacolon. Since 2001, there has been a substantial increase in the number of cases and an escalating rate of serious disease with a four-fold increase in mortality (1). This appears to be largely explained by the emergence of the NAP-1 strain that has unregulated toxin production. The NAP-1 strain is now implicated in causing outbreaks, more serious disease and infections that are more likely to relapse. Therefore, rapid and accurate detection and reporting of toxigenic *C. difficile* is essential for improving patient outcomes and minimizing hospital-acquired disease.

The traditional gold standard for *C. difficile* diagnosis is a cytotoxin assay that detects the cell cytotoxicity of toxins in fecal eluate. This is a time-consuming test that has been largely replaced by toxin A and B detecting enzyme immunoassays and membrane assays. The major concern with these commercial assays is the lack of sensitivity and their unacceptably low (<50% in some circumstances) Positive Predictive Value (PPV). Several recent studies in the UK, Europe and the USA have shown that laboratory testing based on EIA detection of *C. difficile* toxins may miss up to 50% of positive patients (2). Unfortunately, these assays are still used in 95% of laboratories to detect *C. difficile* toxins A and B (2). The use of commercial toxin detection kits can result in significant costs as missed positives may result in transmission of the organism with the additional cost of new infections, increased length of stay and patient morbidity and mortality. Therefore, healthcare facilities should consider moving towards the use of more sensitive methods for quickly and reliably identifying patients with CDI. For clinical laboratories, rapid tests that approach the sensitivity and specificity of toxigenic culture include PCR tests or

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two-step testing paradigms that use rapid antigen assays with confirmatory testing by PCR (2). Real-Time PCR methods that detect toxin gene targets have been described to detect toxigenic *C. difficile* strains (3). Currently, there are a number of rapid PCR-based commercial assays available to clinical laboratories for diagnosing CDI (4). Unfortunately, these commercial diagnostic tests are expensive and not cost-effective for routine laboratory use.

With this in mind, the Molecular Microbiology Section of the HRLMP developed a novel Real-Time PCR method for the detection of toxin genes present in toxigenic strains of *C. difficile* associated with CDI. This novel method targets the *tcdC* negative regulator gene, which is a surrogate marker for the genes that code for toxin A and toxin B for amplification and real-time detection. In addition, the current method also amplifies and detects the binary toxin gene (*cdtA*) in a multiplex format. Detection of the *cdtA* gene with the *tcdC* gene in this new method presumptively identifies the NAP-1 strain providing useful information for molecular epidemiology and for infection control. The test performance characteristics of the new method were comparable to all PCR-based commercial assays tested and showed the following values: Sensitivity (95.2%), Specificity (99.8%), PPV (97.6), and NPV (99.8%).

The method has been validated and was first introduced into the routine laboratory at the St. Joseph's Healthcare site of HRLMP in November 2010 to combat an outbreak. In December 2010, testing was expanded to all HHS sites. Since then, testing has been offered through Lab Reference Centre to other external healthcare facilities. In June 2011, we undertook *C. difficile* testing for Life Labs. At present, the test is performed twice daily (morning and afternoon), seven days a week. The average turn-around-time for the test from specimen processing to final resulting is under 4 hours and is comparable with other commercial PCR assays. The limit of detection (LOD) of this method was estimated to be 130 genome equivalents, giving a detection sensitivity of 10^3 CFU/ml of stools. Since the introduction into the routine laboratory, the Molecular Microbiology lab has performed over 4,000 tests.

Molecular methods that detect the genes encoding *C. difficile* toxin could offer a rapid and sensitive alternative to traditional *C. difficile testing*; however, a positive result is not synonymous with the presence of the toxin. This methodology might identify *C. difficile* strains that carry the gene but do not produce the toxin. Therefore, clinicians should interpret the positive results with care, taking the clinical scenario into consideration. Furthermore, toxigenic strains can be carried asymptomatically by up to 50% of elderly patients who are residents of a long-term care or nursing home facility (5). Therefore, testing should only be performed in patients with diarrhea and there is no role for performing a test of cure.

References:

1. **Rupnik, M., M.H. Wilcox, and D.N. Gerding**. *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. 2009. Nature Rev. Microbiol. **7**:526-536.
2. **Ticehurst JR, Aird DZ, Dam LM, Borek AP, Hargrove JT, and Carroll KC**. Effective detection of toxigenic *Clostridium difficile* by a two-step algorithm tests for antigen and cytotoxin. J. Clin. Microbiol. 2006. **44**:1145-1149.
3. **Peterson LR, Manson RU, Paule SM, Hacek DM, Robicsek A, Thomsom RB, and Kaul KL.** Detection of toxigenic *Clostridium difficile* in stool samples by real-time polymerase chain reaction for the diagnosis of C. difficile-associated diarrhea. Clin. Infect. Dis. 2007. **45**: 1152-1160.
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5. **Riggs, M.,et. al.** Asymptomatic carriers are a potential source for transmission of epidemic and nonepidemic *Clostridium difficile* strains among long-term care facility residents. Clin. Infect. Dis. **45**:992-998.

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QUALITY SNAPSHOT:

HRLMP meets Accreditation Canada's Standards

For the first time, the laboratories in the Hamilton Regional Laboratory Medicine Program (HRLMP) were an area of focus by the hospital accreditation assessors in the recent Accreditation Canada surveys at Hamilton Health Sciences and St. Joseph's Healthcare, Hamilton.

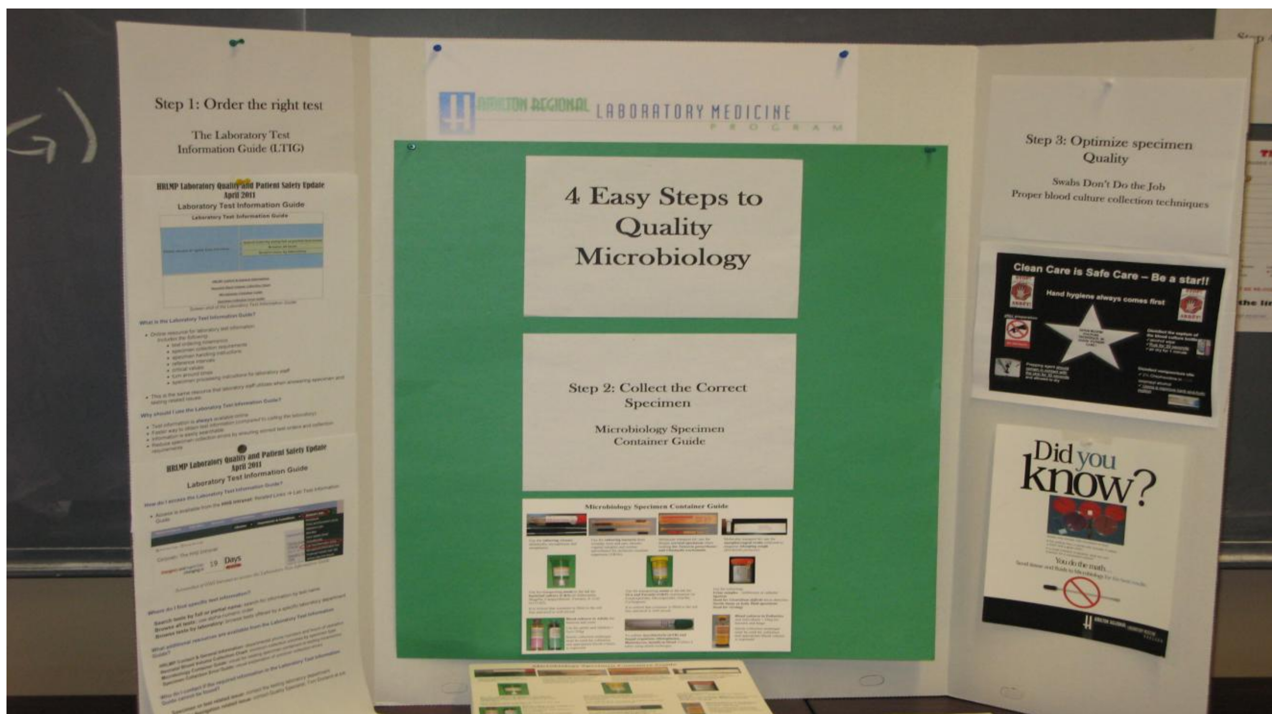
This was a very positive experience for both the assessors and our laboratory staff. The ongoing readiness, maintenance and continual improvement of our Quality Management System and our accreditation status with Ontario Laboratory Accreditation placed us in a good position to demonstrate that we meet the requirements of Accreditation Canada. In separate assessments, both organizations met each of the 176 criteria set out for Laboratories and Transfusion Medicine.

It is good for the laboratory to be reminded of the contributions we make to patient care and for care givers in the hospitals to know that the laboratories meet their standards. Thank you to all staff who participated in the accreditation and congratulations to all HRLMP staff on this success!

Cathie McCallum, Quality Manager, HRLMP

From Microbiology:

Laboratory technologists presented at the MUMC annual review week from May 9 -13. The station was titled 4 steps to Quality Microbiology. These steps are: Order the correct test, use the appropriate specimen container, ensure specimens are of optimal quality and label your specimens properly. The station was visited by more than 200 nurses and allied health staff members. Educational tools were provided for them to take back to their clinical areas.



EDUCATION:

Congratulations to Dr Kika Veljkovic, a postdoctoral fellow in clinical chemistry who received a travel award to attend the American Association of Clinical Chemistry meeting in Atlanta, Georgia. Four of seventy student posters were chosen for the oral presentation and Kika's was in this select group.

Training Programs:

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