LABORATORY TRENDS

A Report from the BCCDC Public Health Laboratory

Inside this Issue

LABORATORY NEWS 2
- Accreditation visit from DAP
- MALDI acquisition

SPOTLIGHT 3
- Human infection with horsehair worm
- How to decontaminate a CL3 lab

SURVEILLANCE 5
- Carbapenemase Producing Organisms

OUTBREAKS 6
- Legionella outbreak
- Gastrointestinal outbreaks
- Respiratory outbreaks

Issues: www.bccdc.ca/publichealthlab
Accreditation visit from the Diagnostic Accreditation Program

As part of the 4-year accreditation cycle of Accredited Laboratory Medicine facilities, auditors from the Diagnostic Accreditation Program (DAP) visited the BCCDC Public Health Laboratory (PHL) on October 2nd for a 3-day audit.

The assessment focused on evaluating conformance to DAP standards for laboratory medicine, encompassing their medical, safety, general, administrative and information technology check lists as well specific laboratory standards for the public health laboratory as well as for toxicology and tumor marker testing.

Assessors included microbiology laboratory specialists and leaders from health authority partners (IHA, IH, FHA), a pathologist as well as DAP team representatives (manager and accreditation assessment officers).

While we await an official report detailing our performance, it was relayed that a compliance rate of 94.6% was attained and accreditation status achieved. This accomplishment is significant as the new DAP standards now includes over 2000 check list items. Preparing for these audits and maintaining adherence to these standards takes considerable and ongoing investments in time through the dedication and teamwork from staff at all levels.

Acquisition of the MALDI-TOF MS

The BCCDC PHL is now in possession of a Bruker MALDI-TOF MS (matrix-assisted laser desorption ionization-time of flight mass spectrometry) Biotypser. This instrument is used for the rapid diagnostic identification of microorganisms and is increasingly seen in both clinical and reference laboratories.

For reliable identification, internal reference libraries of each MALDI-TOF MS instrument must contain the spectrum (unique protein fingerprint) of the organism. Operators must also apply the correct library (clinical or research) if more than one reference library is available.

In addition to the standard library, the BCCDC PHL has also implemented the mycology, mycobacteriology and bioterrorism libraries. Once operationalized, frontline laboratories who also have compatible MALDI instruments will be able to send spectra instead of isolates to the Public Health Advanced Bacteriology & Mycology Program to confirm cases of potential Risk Group 3 (RG3)/Security Sensitive Biological Agents. This will reduce the risk of possible laboratory-acquired infections when forwarding these isolates. The nature of the work of the PHL with RG3 organisms will also provide opportunities to add to the available spectra in the library to expand the organisms that can be accurately detected and to reduce the likelihood of misidentification. The plan is to establish a curated database of BC strains validated against results from our 16S sequencing and to further explore subtyping capabilities with the MALDI.

Following staff training on the instrument, studies will be performed to evaluate the potential role of MALDI for botulinum toxin detection.
Identification of a free-living horsehair worm

Recently, the BCCDC PHL Parasitology Program identified a *Gordius* spp. worm, commonly known as a horsehair or Gordian worm (they often tie themselves in knots). The patient had vomited into the toilet bowl and then noticed the Gordian worm. It is not conclusive whether the worm had been expelled by the patient or if the worm was already in the toilet water (water supply is stream water).

The genus *Gordius* belongs to the phylum Nematomorpha and its morphology is similar to nematode worms that can be parasitic to humans. There are four stages in the life of a horsehair worm: the egg, the pre-parasitic larva that hatches from the egg, the parasitic larva that develops within an invertebrate (its host), and the free-living aquatic adult. The adults mate in water and then the females lay long gelatinous strings of eggs. Depending on the water temperature, the eggs will hatch anywhere from 2 weeks to 3 months. Although not completely understood, it is believed that within 24 hours of hatching, a protective covering forms around the pre-parasitic larva or cyst. If the cyst is consumed by a suitable arthropod (grasshoppers, crickets, cockroaches, and some beetles), the protective covering dissolves and the released parasitic larva bores through the gut wall and into the body cavity of the arthropod host. There, it digests and absorbs the surrounding tissue. When mature, the adult leaves the host arthropod to start the entire cycle again.

The horsehair worm's morphology is similar to nematodes but they are much longer (50 to 100 mm long) and very thin (1-3mm) (Figure 1). Gordian worms are not parasitic to humans, livestock, or pets and pose no public health threat. However, if humans, livestock, or pets accidentally ingest infected arthropods or the free-living worms, this may lead to some mild discomfort of the intestinal tract, but infection never occurs.

To date there have been few reports of hairworm infections in humans globally. Most cases have been reported in Japan with six human cases of *Gordius* spp. reported in 1966 alone. In these cases, worms were vomited, shed in feces or expelled from the anus. Morphological examination can identify the worms to the genus level, while 18S RNA sequencing can confirm species identification.

*Figure 1.* Recently retrieved horsehair worm under 0.35x magnification.
How to decontaminate a CL3 lab

Due to the nature of the work at the public health laboratory, decontamination is a routine requirement and essential to ensuring adequate biosafety in a quality assured environment. Containment Level 3 (CL3) suites are decontaminated periodically for routine maintenance, after a major program change, and during a CL3 spill event.

CL3 suites are decontaminated using a combination of surface cleaning as well as either formaldehyde gas or vaporized hydrogen peroxide. The procedure is as follows:

1. Prior to gas decontamination, surfaces of the suite, change room and shower are cleaned using an appropriate disinfectant (Figure 2A).
2. Removable materials are autoclaved or gas decontaminated and removed from the suite.
3. The suite is prepared for gas decontamination by opening equipment doors, drawers and cupboards and positioning for optimal gas circulation.
4. Mechanical preparations are made to seal HVAC inputs/outputs, turn off equipment, and sealing valves and ports.
5. Paired biological (BIs) and chemical indicators (CIs) are prepared and placed for validation of the decontamination (Figure 2B).
6. Gas decontamination (2 runs for a total of 10 hours 20 mins) (Figure 2C) followed by hydrogen peroxide level testing (Figure 2D) and venting.

To monitor the efficacy of a sterilization process, physical monitoring as well as the placement of BIs and CIs are important. For BIs, spore strips of *Bacillus subtillis* are placed on several locations in the suite and then incubated at 55°C for 7 days post decontamination (Figure 2E) and then checked for growth after being placed in media. CIs provide a crude assessment of vapor distribution with visual comparison of the degree of color change on the strips after exposure to hydrogen peroxide.

To complete the decontamination program, preventative maintenance of the sterilizer is crucial as is accurate and complete record keeping of the process. This process is led by our Biosafety, Biohazard, Biosecurity Containment Program, with support and assistance from other programs at the BCCDC PHL as well as BCCDC Facilities (Brookfield LePage Johnson Controls Workplace Services Inc).

---

**Figure 2.** Components of the decontamination process including A: surface cleaning/disinfection and preparation, B: biological and chemical indicator preparation, C: preparation/installation of the gas sterilization system, D: Biosafety Officer John Tansey tests hydrogen peroxide levels post decontamination, E: incubation of biological indicators to determine success of the procedure.
Carbapenemase producing organisms

The latest counts for cases of carbapenem-resistant Enterobacteriaceae in BC can be found in Figure 3 (updated from our February 2017 issue). The BCCDC PHL Public Health Advanced Bacteriology/Mycology Program provides molecular and genotypic testing of suspect isolates submitted from other microbiology laboratories and health care facilities in the province for carbapenemase genes. To date, there have been 682 patients with carbapenem-resistant organisms: 434 harboured the New Delhi Metallo-β-lactamase (NDM) gene, 96 cases with OXA-48 carbapenemase and 59 cases with the Klebsiella pneumoniae carbapenem (KPC) β-lactamase gene; some patients had multiple resistance factors including 44 patients with NDM and OXA-48 carbapenemase, six cases with NDM and KPC genes, and one other case with the KPC β-lactamase gene as well as a the Verona integron-encoded metallo-β-lactamase (VIM) gene. Forty-two cases with the Serratia marcescens enzyme (SME) resistance gene have also been identified as well as five cases of mcr-1 (mobilized colistin resistance) to date including one case with co-resistance with a NDM gene.

Carbapenemase Producing Organisms (CPO) were added to the reportable conditions list in December of 2016. New cases of CPOs are also reported to the Provincial Infection Control Network of BC (PICNet) who monitor trends in CPOs as part of the provincial surveillance program. Surveillance for this program initially concentrated on testing for patients at acute care facilities with previous care in countries where CPOs are more common but has since expanded to screening patients who are residents of retirement communities where CPO spread is suspected. Recent outbreaks at two community care facilities have underscored the need for additional testing at the community level.

The Public Health Advanced Bacteriology/Mycology Program routinely sequences CPO-positive isolates to look at relatedness between strains. This is currently performed on the Illumina MiSeq platform but requires specialized bioinformatics expertise to characterize sequence types and plasmids. Recently, the introduction of sequences using Oxford Nanopore MinION technology is being used to complement MiSeq data, particularly data when there are new plasmids that Illumina data have difficulty resolving.

It is through the collaborative work of acute care hospitals, infection prevention and control colleagues, PICNet and the BCCDC PHL that supports early identification for mitigating the spread of CPOs in the province.

**Figure 3.** Carbapenem-resistant Enterobacteriaceae detected since 2010, Public Health Advanced Bacteriology & Mycology Program, BCPHMRL. *Counts include 44 cases with NDM and OXA-48, six cases with NDM and KPC, one patient with KPC and VIM, and one with NDM and mcr-1.
Legionella pneumophila outbreak

Following reports of patients hospitalized in intensive care units with Legionella, an outbreak of Legionella pneumophila was identified in August in the Fraser Health Authority.

Legionella is a gram-negative bacterium that naturally occurs in soil and fresh water. When water sources such as air conditioning units, hot tubs, cooling tanks and water heaters are contaminated with Legionella, susceptible individuals who come into contact with droplets containing the bacterium are at risk of developing either Pontiac fever (milder form of Legionellosis) or Legionnaire’s disease, a more severe, atypical pneumonia-type illness. L. pneumophila accounts for an estimated 80% of cases of Legionella.

Recommendations for testing symptomatic patients include Legionella urine antigen testing for community out patients while lower respiratory samples for culture or nucleic acid testing and urine for Legionella urine antigen testing are suggested for in-patients.

Multiple laboratories at the BCCDC PHL are involved in the clinical testing for this outbreak including the Public Health Advanced Bacteriology/Mycology (culture), Zoonotic Diseases & Emerging Pathogens (urine antigen testing), and Virology (polymerase chain reaction, PCR) Programs. The Environmental Microbiology Program also provides Legionella PCR and culture for water samples from the affected area. As summarized in the October 2017 issue, the PCR screening assay for Legionella in water samples detects Legionella spp., L. pneumophila, and L. pneumophila serogroup 1. PCR positives are then further cultured to identify and/or confirm the species of Legionella. The National Microbiology Laboratory is also supporting this investigation by providing whole genome sequencing of clinical and environmental samples.
Gastrointestinal outbreaks

From January to September there were 168 gastrointestinal (GI) outbreaks investigated by the BCCDC PHL (Figure 4). Outbreaks were investigated from 86 (51%) LTC facilities, 45 (27%) daycares/schools, 19 (11%) restaurants, nine (5%) hospitals, eight events (5%) and one (1%) other facility type. Samples were received from 69% of these outbreaks with norovirus detected in 70 (60%) (51 from LTC facilities, nine from restaurants (including six linked to oysters), three from private events, two from daycares/schools, two from hospitals, from an event where oysters were served and from another facility type. Sapovirus was detected from samples from three wings of a LTC facility, another LTC facility as well as a daycare/school. Astrovirus was detected from samples from two daycare outbreaks as well as a LTC facility outbreak, rotavirus was detected two LTC facilities as well as at a daycare/school, and Campylobacter was detected from a restaurant outbreak. Adenovirus was detected at a daycare/school and Salmonella Brandenburg was detected from a clinical sample associated with a food service establishment in August.

Unless the outbreak is associated with a daycare, the algorithm for GI outbreak testing includes screening for norovirus NAT first and testing is then reflexed to the GI panel if norovirus is not detected. The GI panel can detect sapovirus, adenovirus, rotavirus and astrovirus. For daycare outbreaks, norovirus NAT and the GI panel are run concurrently. When no viruses are detected, stool samples are forwarded to the Public Health Advanced Bacteriology & Mycology and Parasitology Programs for further assessment.

Other Enteric outbreaks

The Public Health Advanced Bacteriology & Mycology Program participates in outbreak investigations of enteric disease. A multi-provincial effort to investigate a cluster of Salmonella Infantis has been ongoing since individuals first became ill in June. A total of 50 confirmed cases to date have been reported predominantly by BC (42 cases) while the remaining cases reside in Alberta, Manitoba, Quebec and Saskatchewan. The food safety investigation has identified English cucumbers as the likely source of the outbreak. Collaboration continues between the Canadian Food Inspection Agency, affected provincial public health partners and the Public Health Agency of Canada to investigate this outbreak.
Respiratory outbreaks

From January to September there were 293 influenza-like illness (ILI) outbreaks investigated by the Virology Program of BCCDC PHL. Specimens from these outbreaks were submitted from 279 (95%) LTC facilities and 14 (5%) hospitals. The number of outbreaks is consistent with the average weekly submissions from the past five years during this interseasonal period (Figure 5).

Of the 41 outbreaks investigated in May-September, entero/rhino virus was detected in 23 facilities (56%) while parainfluenza virus was detected in samples from eight other facilities (31%) and 3 (7%) facilities had human metapneumovirus detected in submitted samples. Two facilities had dual infections of entero/rhino virus and parainfluenza virus detected.

Figure 5. Influenza-like illness outbreaks investigated in 2017 to date, Virology Program, BCCDC PHL. Note that some outbreaks are not reflected here if they are awaiting subtyping.
The Public Health Laboratory at the BC Centre for Disease Control (BCCDC) provides consultative, interpretative testing and analyses for clinical and environmental infectious diseases in partnership with other microbiology laboratories and public health workers across the province and nationally. The BCCDC PHL is the provincial communicable disease detection, fingerprinting and molecular epidemiology centre providing advanced and specialized services along with international defined laboratory core functions. The Provincial Toxicology Centre conducts toxicology testing and analysis for clinical patients, including therapeutic drug monitoring, drug screening tests and forensic toxicology analyses for the BC Coroners Service.

This report may be freely distributed to your colleagues. If you would like more specific information or would like to include any figures for other reporting purposes, please contact us.

Editor: Yin Chang  
Contact: yin.chang@bccdc.ca  
Website: www.bccdc.ca/publichealthlab

Co-Editors:

**Biosafety, Biosecurity, Biohazard Containment**
Program Head: Neil Chin  
Assistant Biosafety Officer: John Tansey

**Environmental Microbiology**
Program Head and Environmental Microbiologist: Dr. Paul Levett  
Team Lead: Frankie Tsang

**Molecular Microbiology & Genomics**
Program Head and Medical Microbiologist: Dr. Linda Hoang & Dr. Mel Krajden  
Team Lead: Brian Auk

**Parasitology**
Program Head and Clinical Microbiologist: Dr. Muhammad Morshed  
Team Lead: Navdeep Chahil

**Pre-Analytical, Central Processing & Receiving**
Laboratory Manager: Quantine Wong  
Site Supervisor: Brian Auk

**Public Health Advanced Bacteriology/Mycology**
Program Head and Medical Microbiologist: Dr. Linda Hoang  
Team Lead: Ana Paccagnella

Medical Microbiologist Locum for all Programs: Dr. Pat Doyle

**Public Health High Volume Serology**
Program Head and Medical Microbiologist (Viral Serology): Dr. Mel Krajden  
Clinical Microbiologist: Dr. Paul Levett  
Program Head and Clinical Microbiologist (Syphilis and H. pylori): Dr. Muhammad Morshed  
Team Lead: Annie Mak

**Laboratory Support Services**
Program Head and Medical Microbiologist: Dr. Linda Hoang & Dr. Mel Krajden  
Team Lead: Dr. Mabel Rodrigues

**Provincial Toxicology Centre**
Scientific Director: Dr. Sergei Likhodi  
Associate Scientific Director: Dr. Aaron Shapiro  
Team Lead: Dennis Friesen

**TB/Mycobacteriology**
Program Head and Medical Microbiologist: Dr. Inna Sekirov  
Team Lead: Dr. Mabel Rodrigues

**Virology**
Program Head and Clinical Microbiologist: Dr. Paul Levett  
Team Lead: Brian Auk

**Zoonotic Diseases and Emerging Pathogens**
Program Head and Clinical Microbiologist: Dr. Muhammad Morshed  
Team Lead: Navdeep Chahil