August 2018

BC Centre for Disease Control
An agency of the Provincial Health Services Authority

LM LABS

LABORATORY TRENDS

A Report from the BCCDC Public Health Laboratory

Inside this Issue

cap ACCREDITED™

Issues: www.bccdc.ca/publichealthlab

Retirement of Darrel Cook

In June we bid farewell to Darrel Cook who dedicated over 30 years to service in public health. Darrel started with the BC Center for Disease Control (BCCDC) Public Health Laboratory (PHL) as Virology section head in the mid 1980s before becoming the laboratory manager in the early 2000s. From 2008-2018, Darrel served as the research projects leader for human papillomavirus (HPV) with the Cervical Cancer Screening Program. During this time, Darrel also provided scientific and technical expertise in navigating the many projects and joint studies carried out by the Clinical Trials Unit and was instrumental in leading the evaluation of primary cervical HPV testing compared with liquid-based cytology screening in the HPV FOCAL Trial. This large scale, Canadian trial recruited over 19,000 women testing them over 4 years and following them for a further 5 years. Among some of the key findings was the demonstration that HPV testing was better able to detect precancerous changes than cytology over a 48-month period, as well as being able to do so, earlier (1). These data may ultimately impact guidelines for HPV testing in the province, if not, in Canada.

Darrel's many contributions span several decades. We will miss his vast knowledge of virology and general laboratory practice. Most importantly, we will miss his easy-going nature, and how approachable and how good a mentor he was to all of us.

Reference

Ogilvie GS et al. Effect of screening with primary cervical HPV testing vs cytology testing on high-grade cervical intraepithelial neoplasia at 48 months. JAMA. 2018;320(1):43-52. doi:10.1001/jama.2018.7464. Available at: https://jamanetwork.com/journals/jama/fullarticle/2686793

Bordetella species detection using two multiplexed real-time PCR assays

Presented at the AMMI-CACMID 2018 Annual Conference (Vancouver, BC) by Martin Cheung
Cheung M, Lee TD, Azana R, Janz L, Prystajecky N, and Hoang L

Pertussis (or whooping cough) is a highly contagious respiratory disease caused by the bacterium *Bordetella* pertussis. Since 2011, cases of pertussis have been on the rise in BC (20.9 cases per 100,000 population in 2016 vs. 1.3 cases per 100,000 population in 2011) ("Reportable Disease Dashboard", BCCDC website). The increase in the number of reported cases over the last few years can be attributed to clusters in various regions across the province and large outbreaks such as experienced in the Lower Mainland in 2012 and Haida Gwaii in 2014.

The gold standard of pertussis diagnosis is culture from nasopharyngeal (NP) swabs. However, real-time polymerase chain reaction (qPCR) assays provide faster turnaround times and do not require viable bacteria for detection. In an effort to improve the real-time PCR assay used to detect B. pertussis, the BCCDC PHL Public Health Advanced Bacteriology and Mycology Program worked with Martin Cheung, medical technologist from the BCCDC PHL Molecular Microbiology and Genomics Program to redevelop the assay.

The improved method includes two qPCR assays designed to detect and differentiate between clinically relevant *Bordetella* species (*B. pertussis*, *B. parapertussis*, *B. holmesii*, *B. bronchiseptica*). Validation was performed using a panel of *Bordetella* culture isolates, other clinically relevant organisms, and clinical lysates from nasopharyngeal swabs. The assays were found to be better able to assess for PCR inhibition, reduced the likelihood of multi-target cross-reactivity and improved turnaround time. The primary assay screens for the *B. pertussis* insertion sequence 481 (IS481) and *B. parapertussis*. IS1001. However, IS481 has been found to be present in some strains of *B. bronchiseptica* and in low copy number in *B. holmesii*. IS1001 is also carried by some strains of *B. bronchiseptica*. For these reasons, a secondary, reflex assay may be required that is able to rule out cross-reaction by either *B. holmesii* or *B. bronchiseptica*.

The assays were implemented into routine clinical use in mid 2017 for *B. pertussis*. Full validation of the PCR detection of *B. parapertussis*, *B. holmesii* and *B. bronchiseptica* is ongoing due to lack of sufficient positive samples; results of positive *Bordetella* species other than *B. pertussis* will continue to be reported based on culture results only.

Reference

Reportable Disease Dashboard. (n.d.).
Retrieved from http://www.bccdc.ca

Implementation of viral multiplex NAT for investigation of gastroenteritis

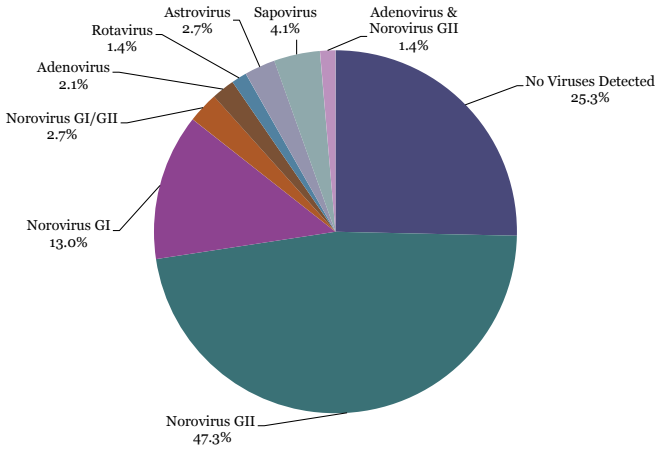
Presented at the AMMI-CACMID 2018 Annual Conference (Vancouver, BC) by Christine Tchao
Tchao C, Tsang F, Man S, Kong A, Yu D, Hird T, Auk B, and Prystajecky N.

In April 2017, the BCCDC PHL Environmental Microbiology Program implemented a new algorithm for gastroenteritis investigation. Previously only targets for noroviurs and sapovirus were tested by nucleic acid testing (NAT) while rotavirus and adenovirus were tested by enzyme immunoassay. The new two-step procedure includes an initial detection assay for the ORF1-ORF2 junction of norovirus, followed by a multiplexed panel with the further three viruses and their targets (sapovirus (polymerase and capsid junction), adenovirus (40/41 hexon), rotavirus (non-structure protein 2) in addition to astrovirus (polymerase and capsid junction). Previously as well, mainly outbreak-related samples were accepted with the exception of solid organ transplant patients and fecal transplant donors. With the new testing approach, the number of approved patient categories has expanded to include:

- gastrointestinal outbreak samples,
- transplant patients,
- hospitalized and/or emergency room patients, and,
- pediatric patients less than 6 years old.

After a year of implementing the new process, it is clear that significant savings in turnaround time from 7 days (sapovirus) and between 2-5 days (adenovirus, rotavirus) to 1-2 days were achieved with this new panel. However, with the modified mandate for testing, there was a 330% increase in test volume for non-outbreaks (288 cases vs 67 cases previously). Where previously, around 72% of gastrointestinal viral outbreaks were attributed to norovirus annually, norovirus was detected in only 63% of outbreaks between April 2017 to March 2018, with nearly 12% due to other viral pathogens (4.1% sapovirus, 3.5%, adenovirus 2.7% astrovirus and, 1.4% rotavirus) (Figure 1). Not only was there improved detection of other viruses (including astrovirus), detections of viral co-infections and multiple etiologies is also now possible. Monitoring viral etiologies for non-outbreak/sporadic cases may also provide data on trends in other populations.

Figure 1. Distribution of viral etiologies in outbreak samples from April 2017-March 2018, Environmental Microbiology Program, BCCDC PHL.



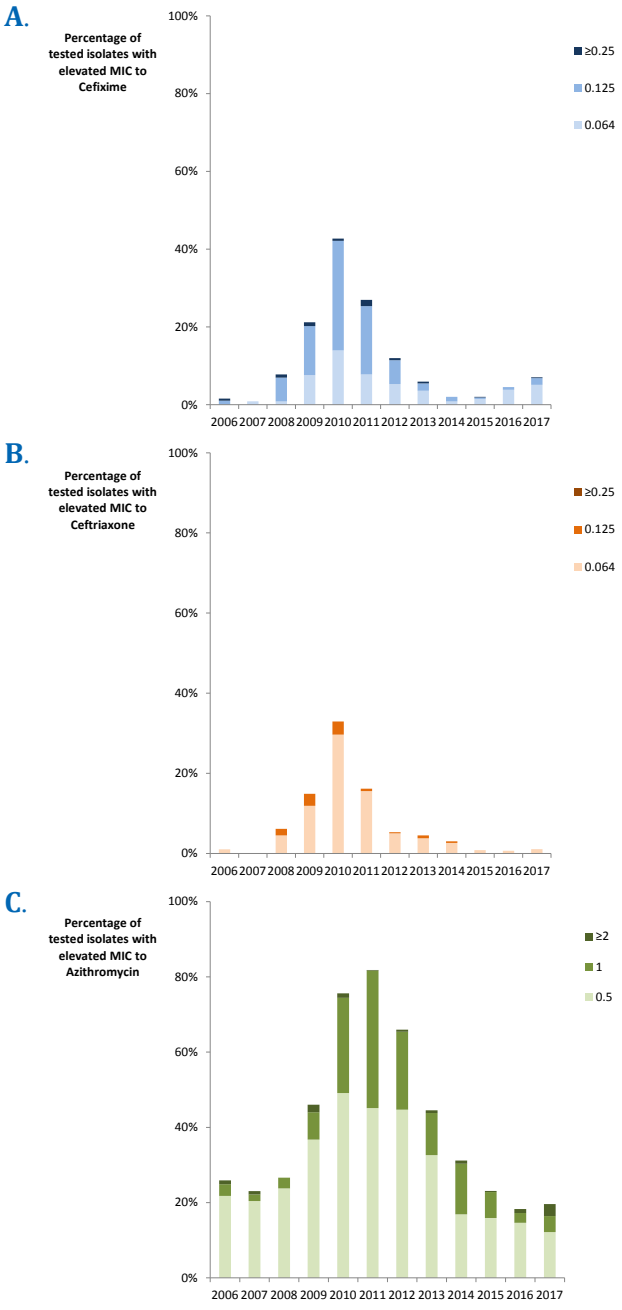
Neisseria gonorrhoeae susceptibility trends

Gonorrhea is a sexually transmitted infection caused by *Neisseria gonorrhoeae*. In BC, rates of gonorrhea increased by 70% in 2015 (74.5 cases per 100,000 population), increased again slightly in 2016 (77.7 cases per 100,000 population) and decreased in 2017 (68.5 cases in 100,000 population) (BCCDC Clinical Prevention Services). Treatment regimens for gonorrhea using recommended antibiotics can cure the infection; however, antimicrobial resistance is of increasing concern. The BCCDC PHL Public Health Advanced Bacteriology & Mycology (PHABM) Program routinely performs surveillance of *N. gonorrhoeae* to monitor for trends of antimicrobial resistance. Culture-positive isolates from the province are evaluated by E test® to assess the minimum inhibitory concentration (MIC) or the lowest concentration of antibiotic required to inhibit *N. gonorrhoeae* growth to first-line cephalosporins as well as alternative antimicrobials.

The increasing trends in the percentage of isolates with elevated MICs to cephalosporins from 2006-2010 prompted a revision in treatment guidelines at the national and provincial level. Since then, the PHABM Program has observed an overall decreasing trend of isolates with higher MICs for ceftriaxone and azithromycin (Figure 2). However, in 2016, there were elevated numbers for cefixime at the MIC breakpoints of 0.064 µg/mL (3.8%; 22/574 isolates) and 0.125 µg/mL (0.7%; 4/574 isolates) compared to previous years. In 2017, there were further observations approaching decreased susceptibility to cefixime with 5.1% (24/469) isolates at 0.064 µg/mL MIC, 1.7% (8/469) isolates at 0.125 µg/mL MIC and 1 isolate with an MIC ≥ 0.25 µg/mL. In addition, while there was little change in the total percentage of resistant (MIC ≥ 2 µg/mL) isolates to azithromycin between 2016 and 2017 (1.0% (6/574) isolates in 2017 compared to 1.0% (15/469) isolates in 2016), the number of intermediate (MIC 1 µg/mL) isolates increased from 2016 to 2017 (4.3% (20/469) isolates in 2017 compared to 2.6% (15/574) isolates in 2016).

The PHABM Program along with microbiology laboratory and public health partners will continue to monitor these trends as indicators of the resistance of circulating strains in the province and to understand if there may be threats to current treatment regimens.

Figure 2. Percentage of tested *N. gonorrhoeae* isolates with elevated minimum inhibitory concentrations (MICs) to cefixime (A), ceftriaxone (B), and azithromycin (C) from 2006- 2017, Public Health Advanced Bacteriology & Mycology Program, BCCDC PHL. MIC units are in µg/mL.



Non-toxigenic cholera

Sample of herring eggs received by the Enivronmental Microbiology Program, BCCDC PHL.

This past spring, communities on Vancouver Island were affected by a non-toxigenic *Vibrio cholerae* outbreak linked to consumption of herring eggs harvested from the marine environment. Three of the cases were laboratory–confirmed. While *Vibrio parahaemolyticus* is known to be present in coastal BC waters and can cause outbreaks when shellfish contaminated with the bacterium is consumed, V. cholerae infection is rare in Canada and is typically associated with travel to tropical countries where the infection is endemic.

This unusual cluster of cases led to the closure of the herring egg harvest sites in the affected region for the season. Many local, provincial and national partners were involved in the investigation, including Island and First Nations Health Authorities, BC Centre for Disease Control, Department of Fisheries and Oceans, Environment and Climate Change Canada, Public Health Agency of Canada and the National Microbiology Laboratory (NML). Isolates from clinical samples forwarded by Island Health and Lifelabs and environmental samples (herring eggs and marine water) collected from harvest areas by First Nations Health Authority were sent for culture and confirmed to be positive for *V. cholerae* by the BCCDC PHL Environmental Microbiology Program. Subsequent marine water samples from six targeted sites tested negative for *V. cholerae*.

V. cholerae isolates from clinical and environmental samples were forwarded to the NML for toxin testing, pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (WGS). Isolates were characterized as non O1/O139 and cholera toxin negative. PFGE results demonstrated two novel and highly-related patterns. Clustering was also supported by WGS. Clinical isolates were found to be genetically related to some environmental isolates.

As this event is rare in Canada, there is only a small *V. cholerae* database to compare to. International repository is also limited. V*V. cholerae* has over 139 known serogroups, only two of which, O1 and O139, are considered to be responsible for epidemic cholera. Although isolates of non-O1 and non-O139 typically do not possess genes for cholera toxin, substantive virulence may still be observed in these subtypes (1).

In the case of this event, this non-toxigenic/non-epidemic strain of *V. cholerae* is suspected to reflect the complex life cycles and interactions (associations with marine water and marine life) of this organism (2, 3) rather than being associated with human sewage or person-to-person spread. This was a first detected, locally originating Canadian cluster of foodborne non-toxigenic *V. cholerae*. It highlighted the diversity of local marine *Vibrio* species and their capacity to contaminate a high variety of food sources.

References:

1. Sharma et al. Molecular analysis of non-O1, non-O139 *Vibrio cholerae* associated with an unusual upsurge in the incidence of cholera-like disease in Calcutta, India. J Clin Microbiol. 1998; 36(3): 756-63. Available at: http://jcm.asm.org/content/36/3/756.full.pdf+html

2. Almagro-Moreno and Taylor RK. Cholera: Environmental Reservoirs and Impact on Disease Transmission. Microbiol Spectr. 2013; 1(2). doi: 10.1128/microbiolspec.OH-0003-2012. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4321695/

3. Lutz et al. Environmental reservoirs and mechanisms of persistence of *Vibrio cholerae*. Front Microbiol. 2013 Dec 16;4:375. doi: 10.3389/fmicb.2013.00375. Available at: https://www.ncbi.nlm.nih.gov/pubmed/24379807

Gastrointestinal outbreaks

From January to June there were 153 gastrointestinal (GI) outbreaks investigated by the BCCDC PHL (Figure 3). Outbreaks were investigated from 83 (54%) LTC facilities, 38 (25%) daycares/schools, 16 (10%) restaurants, nine (6%) hospitals, six events (4%) and one (1%) other facility type. Samples were received from 68% of these outbreaks with norovirus detected in 69 (66%) (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.

Norovirus sequencing provides further context to surveillance of circulating strains of the virus. As part of the ongoing norovirus outbreak assessment, the Environmental Microbiology Program of BCCDC PHL genotypes all confirmed norovirus outbreaks to monitor the molecular epidemiology of norovirus transmission across BC. Based on sequencing the capsid gene (region C), it is clear that GII.4 Sydney remains the dominant strain in the province (and worldwide), appearing in 2012 and replacing GII.4 New Orleans by 2013 (Figure 4). The new GII.17 variant that replaced GII.4 Sydney as the predominant strain in China and Japan in late 2014 also appeared in outbreaks in BC starting in 2014. However, although it continues to circulate in our population causing sporadic outbreaks, GII.17 has not displayed the epidemic potential as it has in other regions in the world.

Figure 3. Gastrointestinal outbreaks investigated in 2017, Environmental Microbiology, Public Health Advanced Bacteriology & Mycology, Parasitology and Virology Programs, BCCDC PHL. The data available are from outbreaks in which the BCCDC PHL has been notified. Some acute care microbiology laboratories are also testing for norovirus in the province and these data may not include outbreaks from all health authorities.

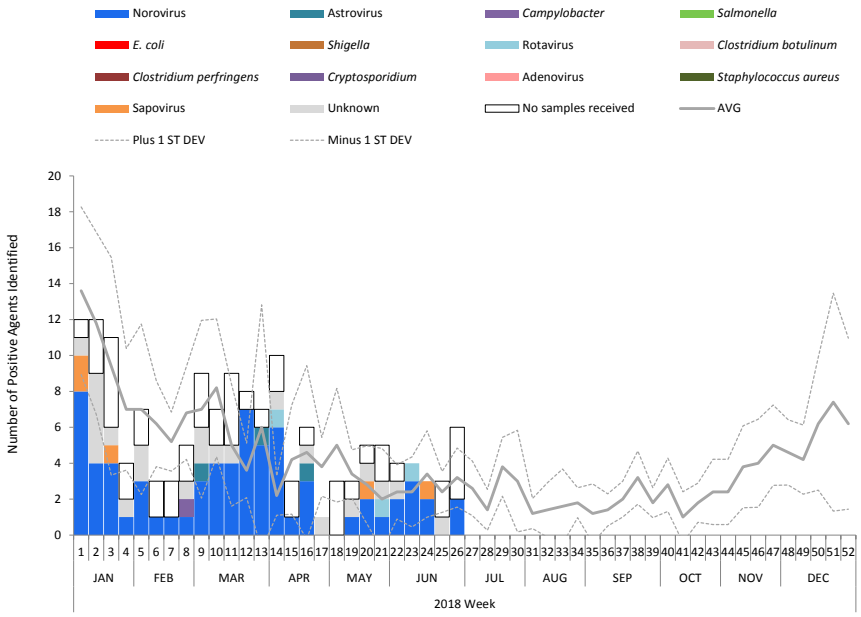
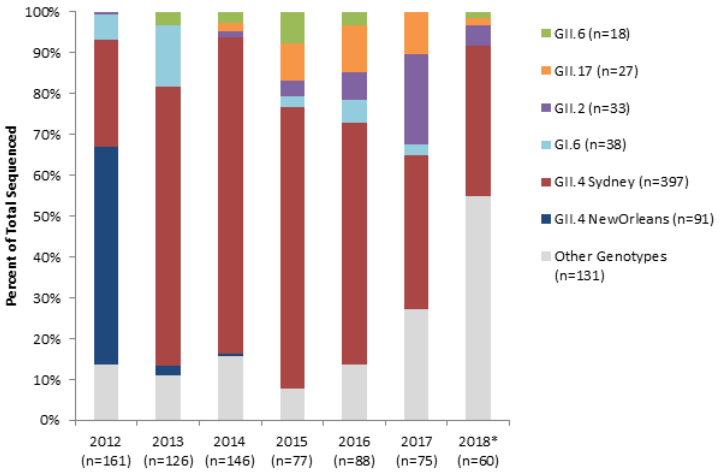


Figure 4. Region C genotyping of norovirus outbreak samples, Environmental Microbiology Program, BCCDC PHL. Note, not all norovirus-positive outbreaks are reflected here due to sequencing limitations.



*From samples up to June 12, 2018 notification

Cyclospora outbreak

BC, Ontario and Quebec is experiencing an outbreak of *Cyclospora* infection with 70 locally-acquired cases reported since April. *Cyclospora cayetanensis* is a parasite which causes a prolonged gastrointestinal infection. Symptoms include frequent watery diarrhea, anorexia, abdominal cramps and bloating, nausea and flatulence. Symptoms typically last several weeks to over a month and wax and wane in intensity. People are infected by ingesting contaminated food or water. The infection is not spread from person-to-person.

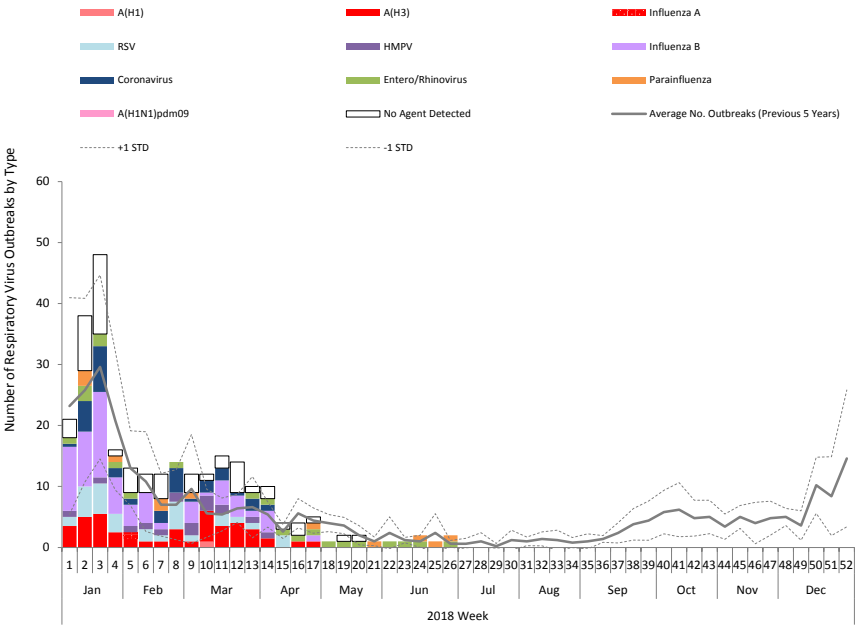
Cyclospora is not endemic in Canada. Most people are infected when visiting an endemic country in Latin America or South East Asia in the spring and early summer. When cases occur in Canadians who did not travel, an outbreak investigation is launched. Most outbreaks occur in the spring and early summer and have been linked to contaminated, imported produce such as berries or herbs.

Cyclospora infection is diagnosed by a stool ova and parasite (O&P) examination. A special request must be made for *Cyclospora* diagnosis to ensure the appropriate stain is used by the laboratory. While routine O&P requires stool in SAF (sodium acetate-acetic acid-formalin), for molecular fingerprinting to occur, **unpreserved, fresh stool** is requested to enable enhanced surveillance during local outbreaks. Investigations are currently underway to determine the source of the outbreak.

Respiratory outbreaks

From January to June there were 274 influenza-like illness (ILI) outbreaks investigated by the Virology Program of BCCDC PHL. Specimens from these outbreaks were submitted from 250 (91%) LTC facilities and 14 (5%) hospitals. As we are now at the end of the influenza season, the number of outbreaks is consistent with the average weekly submissions from the past five years during this period (Figure 5). Influenza B was detected in 59 (22%) outbreaks as well as with other mixed infections in a further 12 (4%) outbreaks. Influenza A(H3) was detected in 39 (14%) outbreaks and with other mixed infections in a further 7 (3%) outbreaks. There were two additional outbreaks where influenza A was detected (unsubtypeable and A(H1N1)pdm09). Of the 13 outbreaks investigated in May-June, entero/rhinovirus was detected in seven facilities (54%) while parainfluenza was detected in samples from four other facilities (31%).

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.



BC Centre for Disease Control
An agency of the Provincial Health Services Authority

LABORATORY TRENDS

August 2018

LM LABS

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
Public Health Lead: 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

cap ACCREDITED™