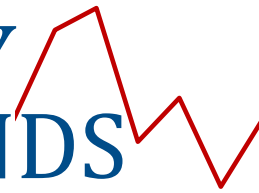


LABORATORY TRENDS



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A Report from the BCCDC Public Health Laboratory



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Programs updates at the Public Health Laboratory

The BC Centre for Disease Control Public Health Laboratory (BCCDC PHL) plays a critical role in public health diagnostic testing, monitoring and outbreak detection, which includes food and water testing and threat response. This work depends on highly skilled and talented medical, scientific, technical and operational staff working together to achieve a culture of caring and quality. The following are recent updates to some of our programs, including many familiar faces who have taken on different roles within the BCCDC PHL.



TB/Mycobacteriology

Inna Sekirov, MD, PhD, FRCPC
Program Head

Dr. Sekirov recently completed her Medical Microbiology Residency Training at UBC. Prior to that, she had obtained an MD/PhD working with Dr. Brett Finlay at UBC on the role intestinal microbiota plays in the progression of *Salmonella* gastroenteritis. Given her experience and interest in molecular diagnostics, genomics, and research we also look forward to her contributions to other sections of the laboratory.



Genomics, Bioinformatics, Advanced Molecular Diagnostics

William Hsiao, PhD
Senior Scientist Lead

Dr. Hsiao completed his doctoral studies at Simon Fraser University under Dr. Fiona Brinkman and post-doctoral fellowship under Dr. Claire Fraser. Dr. Hsiao is focused on developing bioinformatics applications and using next generation sequencing technologies to study microbial pathogens and microbial communities (microbiomes). He has co-developed a bioinformatics platform to use whole genome sequencing to facilitate public health infectious disease surveillance and outbreak investigations.



Virology

Agatha Jassem, PhD, (D)ABMM, FCCM
Program Head

Dr. Jassem completed her doctoral studies in the Department of Pathology and Laboratory Medicine at UBC. She then went on to complete the prestigious one-year CPEP-accredited fellowship at the National Institutes of Health Clinical Center and is certified in Clinical Microbiology by the American Board of Medical Microbiology and the Canadian College of Microbiologists. Dr. Jassem brings a keen mind and near infinite energy to her evolving virology role.



Medical Microbiology (Locum)

Dr. Patrick Doyle MD, MHSc, FRCPC
Medical Microbiologist

Efforts are ongoing to recruit one additional Medical Microbiologist. Dr. Patrick Doyle is doing an outstanding job supporting the BCCDC PHL during this time. He will be continuing in his role until this vacancy can be filled. Dr. Doyle was recently recognized with the Clinical Faculty Award for Career Excellence in Clinical Teaching from the UBC Faculty of Medicine. He joins other recipients honoured for excellence in teaching, research, administration, innovation and public service.



Environmental Microbiology

Natalie Prystajeky, PhD, SCCM(ENV)
Program Head

Dr. Natalie Prystajeky completed her doctoral studies in the Department of Pathology and Laboratory Medicine at UBC, studying environmental transmission of giardiasis with Dr. Judy Isaac-Renton. She is an environmental microbiologist and specializes in molecular genomics and water testing. Dr. Prystajeky will provide leadership and program development in food and water safety and One Health genomics.



Parasitology & Zoonotic Diseases & Emerging Pathogens (ZEP)

Navdeep Chahil, BSc, RT
Team Lead

Navdeep has been with the BCCDC PHL for nearly 29 years, working in the previous HIV Level 3 laboratory, ZEP Laboratory and most recently the Central Processing and Receiving Lane Level Laboratory as a Technical Coordinator. She brings leadership in the field of serological testing, expertise in the LIS environment and Lean/Improve methodologies.

eLab: New electronic guide to programs & services

Since 2007 the eLab Handbook has been the electronic platform used by the BC Children’s and Women’s (CW) Laboratory to house testing information. Building upon this infrastructure, the BCCDC PHL has collaborated with the CW Laboratory to enable eLab to support testing performed by the BCCDC PHL.

This online manual replaces the previous *Guide to Programs and Services*. You can use it to find details about sample collection, test methodology, transport, requisitions to use, test turnaround times and contacts for consultation. New information that is now available includes the days testing is performed and important cut off times. The eLab Handbook also includes contact information in case tests need to be expedited.

Through eLab we hope that BCCDC PHL testing information will be easier to find for all our clients. We want to thank the CW team for working with the BCCDC PHL such that eLab could become a shared resource. Please send in any comments/feedback you have so we may better serve you.

Accessing eLab

From inside the Provincial Health Services Authority (PHSA) network

The eLab Handbook can be accessed by entering the name “elab” into any browser on the PHSA network.

From outside the PHSA network

Users can access eLab at the following location:
<http://www.elabhandbook.info/phsa>

Searching for Tests in eLab

There are two methods to search for tests:

a) Main method - Search by Test Name

Enter all or part of a test name in the ‘Search for Test’ field and click ‘Go’ – this will search the test by the actual test name or any alternate names specified.

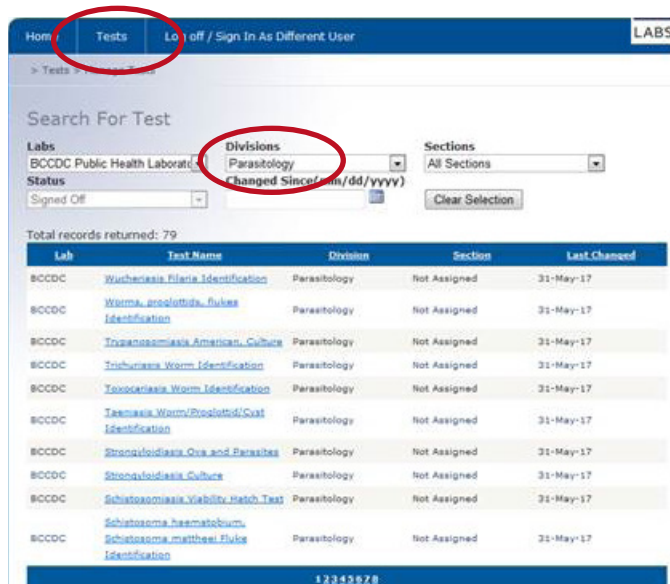
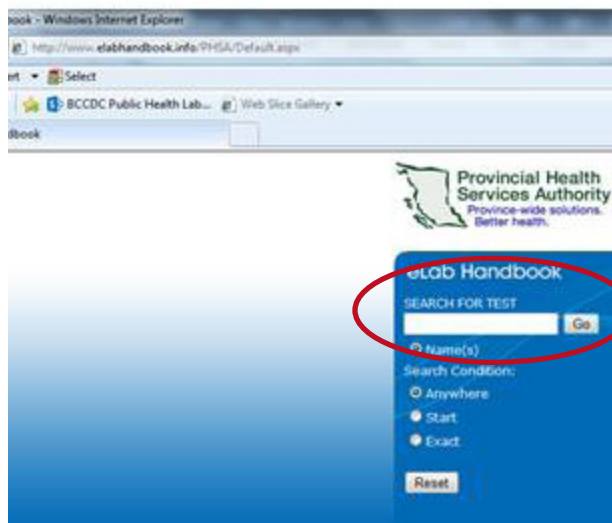
b) Advanced method - Search by Laboratory Division*

Select ‘Tests’ on the top ribbon

Select ‘BCCDC Public Health Laboratory’ under the Labs drop down and choose from the list of tests displayed. You can narrow down your search if you know which laboratory program you are interested in by choosing a laboratory under Division. The BCCDC PHL is divided into the following laboratories:

- Advanced Bacteriology/Mycology
- Environmental Microbiology
- Mycobacteriology/TB
- Parasitology
- Virology
- High Volume Serology

*** Please note that the names of the laboratory divisions may not be comprehensive of the types of tests performed.**



Case reports of public health significance

Laboratory exposure to *Francisella tularemia*

Risk Group 3 (RG3) organisms are identified as organisms in culture that pose a health threat to the patient and may be transmissible to the laboratory worker, but not a risk to the general public. Examples include *Bacillus anthracis*, *Francisella tularensis*, *Yersinia pestis* and the dimorphic fungi.

We recently had a laboratory exposure to *F. tularensis*, and have identified a number of issues and mitigation strategies that you, as front line colleagues may wish to consider.

While recovery of RG3 organisms is rare in BC, they do occur, and as laboratorians, we need to ensure that their detection is rapid and safe. While *F. tularensis* is endemic in BC, other RG3 organisms are endemic in Canada and the US. Between 2003 and present, we have identified 6 cases of tularemia in BC. Between 2007 and present, we have seen almost 20 RG3 isolates that include *Brucella* species, *F. tularensis* and *B. pseudomallei*, with *B. melitensis* being most prominent followed by *F. tularensis*.

The BCCDC PHL is part of the Canadian Laboratory Response Network (CLRN) (aka Bioterrorism Program). We have certified technologists who are able to run the CLRN assays upon request. These assays include direct multiplex qPCR for *B. anthracis*, *F. tularensis* and *Y. pestis*. As well, we also have single assays with multiple targets for each of these organisms for culture confirmation.

The following are recommendations for your laboratory to consider:

- It is important that technologists are familiar with the key phenotypic features of RG3 bacteria and fungi. This can only be achieved through education and regular review. At BCCDC PHL, we will be implementing an annual review of RG3 organisms along with the annual spill response demo. As well, we will include RG3-related questions in our annual competencies. We will be happy to share any educational material with you to assist in a similar activity at your centre.
- Ensure that the technologists are familiar with the transport requirements of RG3 isolates and follow *Transport of Dangerous Goods Regulations*.
- Ensure that an alert process is in place with the BCCDC PHL for any potential RG3 agent before sending samples for testing. RG3 organisms are also reportable to Public Health.

First *Candida auris* case identified in BC

In July, the first case of *Candida auris* in BC was confirmed by BCCDC PHL by DNA sequencing. The case was a traveler who was hospitalized in India who, upon return, was admitted to an acute care facility in the province. The patient was also infected with multiple carbapenemase producing organisms (NDM, VIM and OXA-48) along with vancomycin-resistant enterococci. The patient was managed with proper infection prevention and control measures. All the multidrug-resistant organisms identified were likely acquired in India.

C. auris, a globally-emerging multi-drug resistant yeast, was first identified in Japan in 2009 and has been identified in 17 countries on 5 continents to date. *C. auris* has been associated with invasive infections, high mortality rates, and outbreaks in healthcare settings worldwide. The organism may be difficult to identify in the laboratory and antifungal agents commonly used to treat *Candida* infections may not be effective. It can also persist in the hospital environment with potential for nosocomial transmission. The first case in Canada was identified in May 2017, in Winnipeg (1). The 64-year-old individual had recent prior history of oral surgery requiring hospitalization in India.

A [communication notice](#) from July, 2017 from the Public Health Agency of Canada provides further information, including guidance on infection prevention and control.

Reference:

1. Schwartz IS, Hammond GW. First reported case of multidrug-resistant *Candida auris* in Canada. *Can Commun Dis Rep.* 2017;43(7/8):150-3.

Correlating childhood viral exposures to allergy and asthma using novel viromic methods: a feasibility study

Respiratory tract infections caused by viruses such as influenza, respiratory syncytial virus (RSV) and rhinovirus are frequent in young children, with many primary infections acquired before the age of one. Previous studies (1, 2) suggest that respiratory infections early in life may contribute to the development of allergies and asthma in children. However, studies have focused only on correlations to clinical symptoms and the few viruses that can be detected with traditional testing methods. Therefore, the contribution of viruses to the development of allergy and asthma still remains unclear. To investigate this idea further, Dr. Agatha Jassem and colleagues were recently awarded a BCCDC Foundation Blue Sky funding award to complete a pilot project on this area of research.

Traditional molecular methods for respiratory virus detection are PCR-based, limited to a few pathogens, and only useful during time of illness. Traditional serological methods of IgG detection are not widely available for many respiratory viruses since molecular tests are now used clinically to detect active infection. This study will allow investigators to evaluate a novel method that can detect antibodies to all known human viruses to provide comprehensive assessment of past viral infections in an individual (3), thus overcoming the limitations of traditional molecular- and serology-based testing. These methods will be

applied to samples from the Canadian Healthy Infant Longitudinal Development (CHILD) cohort where clinical data on childhood allergy and asthma can be linked.

This pilot will provide preliminary data and inform a future large-scale study on the role of viral infections in the development of allergy and asthma. The study will also provide insight on the potential application of this advanced novel technology for other viral disease scenarios relevant to individual and public health.



BCCDC Foundation for Public Health

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References:

1. Balekian DS, Linnemann RW, Hasegawa K, Thadhani R, Camargo CA, Jr. 2017. Cohort Study of Severe Bronchiolitis during Infancy and Risk of Asthma by Age 5 Years. *J Allergy Clin Immunol Pract* 5:92-96.
2. Sigurs N, Aljassim F, Kjellman B, Robinson PD, Sigurbergsson F, Bjarnason R, Gustafsson PM. 2010. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. *Thorax* 65:1045-105
3. Xu GJ, Kula T, Xu Q, Li MZ, Vernon SD, Ndung'u T, Ruxrungtham K, Sanchez J, Brander C, Chung RT, O'Connor KC, Walker B, Larman HB, Elledge SJ. 2015. Viral immunology. Comprehensive serological profiling of human populations using a synthetic human virome. *Science* 348:aaa0698.

Development and evaluation of molecular screening assay for *Legionella* in water

Yu D, Tsang F, Eisler D, Tchao C, Man S, Yu V, Auk B and Prystajecy N. 2017. Association of Medical Microbiology and Infectious Disease Canada/Canadian Association for Clinical Microbiology and Infectious Diseases Annual Conference, Toronto, ON.

Legionella bacteria are a public health concern due to their ability to cause both mild (Pontiac fever) and severe (Legionnaires' disease) illnesses, as well as their ubiquity in water and soil. Environmental investigations of legionellosis cases are challenging as the organism is fastidious and slow growing in culture. Extensive environmental testing is often required due to the range of possible sources.

At the BCCDC PHL, *Legionella* detection in water was previously performed by culture of water filtrates followed by phenotypic identification and sequencing of the 16S rRNA gene. While this method isolates the organism for further testing, culture can take from 7 to 10 days and confirmation a few more days. To improve laboratory throughput, a molecular screening assay for water and swab samples was evaluated. The Benitez and Winchell (2013) assay detecting *Legionella* spp., *L. pneumophila*, and *L. pneumophila* serogroup 1 was adapted by the BCCDC PHL Molecular Microbiology & Genomics Laboratory as a panel of three singleplex PCR reactions. A fourth PCR reaction targeting the ITS2 gene of *Oncorhynchus keta* DNA (2) was validated to maximize detection sensitivity in environmental water as an exogenous internal positive control. Water samples were processed by membrane filtration for both PCR as well as culture set up as part of this study.

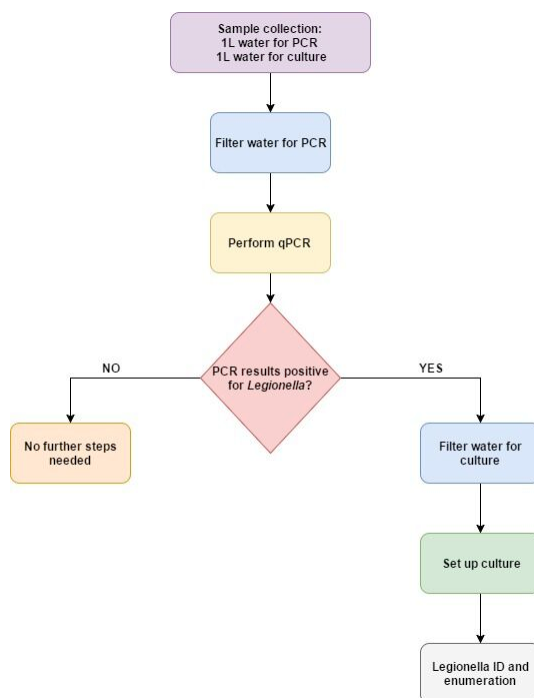
Once optimized, the assay was demonstrated to be 100% sensitive and 100% specific to their targets. Approximately 88% of bacteria were recovered from membrane filtration and there was no interference of the water matrix for the detection of *Legionella* by either PCR

or culture. The PCR panel was further able to detect non-culturable cells due to non-viable organisms. With these findings, the BCCDC PHL Environmental Microbiology Laboratory now has a quicker methodology for detecting and differentiating *Legionella* in water samples and has implemented the PCR panel using the workflow described in Figure 1.

References:

1. Benitez AJ and Winchell M. 2013. Clinical Application of a Multiplex Real-Time PCR Assay for Simultaneous Detection of *Legionella* Species, *Legionella pneumophila*, and *Legionella pneumophila* Serogroup 1. J. Clin. Microbiol. 51(1):348-351.
2. Haugland RA, Varma M, Sivaganesan M, Kely C, Peed L, and Shanks OC. 2010. Evaluation of genetic markers from the 16S rRNA gene V2 region for use in quantitative detection of selected *Bacteroidales* species and human fecal waste by qPCR. Syst. Appl. Microbiol. 33:348-357.

Figure 1. Water screening for *Legionella* at the BCCDC PHL Environmental Microbiology Laboratory.



Detection and speciation of *Plasmodium* infections

Lee T, Adie K, Lo T, Lee M, Auk B, Wong Q, Prystajeky N and Morshed M. 2017. Association of Medical Microbiology and Infectious Disease Canada/Canadian Association for Clinical Microbiology and Infectious Diseases Annual Conference, Toronto, ON.

The parasite of the genus *Plasmodium* is the infectious agent of malaria which causes an estimated 1 million deaths annually worldwide. Of the five *Plasmodium* species (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*), infections with *P. falciparum* are the most serious, requiring rapid detection and speciation.

Laboratory diagnosis of malaria is most commonly done by microscopy and rapid antigen detection kits. Limitations to these methods include the requirement of blood collection at the appropriate phase in the parasite life cycle, proper/quality controlled stains to detect the parasite and expertise in the reading of smears to identify the parasite, especially in cases of rare species or low parasitemia. Also, most commonly used kits detect only *P. falciparum*, making infection with other species dependent on microscopy interpretation. In addition to these technical challenges, one of the major differentials for Ebola virus infection is malaria. It is imperative that a rapid and safe method for testing suspect Ebola samples for malaria is available. For these reasons and to improve turn-around-time, sensitivity, and specificity, the BCCDC PHL investigated molecular methods for malarial diagnosis. The Molecular Microbiology & Genomics Laboratory worked with the Parasitology Laboratory to develop and validate three multiplexed real-time PCR assays for the Applied Biosystems 7500 FAST Taqman platform for the detection and speciation of *Plasmodium* infections.

A total of 84 clinical extracts (56 *Plasmodium* positive and 28 *Plasmodium* negative) were tested with the laboratory developed real-time PCR assays: a triplex for the detection of all *Plasmodium* species, *P. falciparum* speciation, and an internal positive control (IPC), plus two additional duplexes for speciation of *P. vivax/P. knowlesi* and *P. ovale/P. malariae*. Results were compared to microscopic diagnosis (current gold standard) and discordant results were tested by two alternative melt-curve analysis real-time PCR assays. In silico analysis was completed by a probe BLAST using the NCBI database with base pair mismatches analysed by the IDT Biophysics web tool.

Results demonstrated 95.2% analytical specificity and 100% sensitivity to *Plasmodium* positive samples and the species identified by microscopy. In total, 4 samples had discordant smear results and real-time PCR results. Upon testing with the referee melt-curve assays, three of the four discordant samples were correctly identified by this assay, including one previously unidentified mixed infection. In addition, there was no cross-reactivity identified using in silico analysis, including with Ebola virus.

The real-time PCR assays developed were found to be a reliable method for identification and speciation of *Plasmodium* infections. It will be used to supplement the gold standard of microscopic diagnosis and for quality assurance purposes.

Invasive Group A *Streptococcus*

From 2009-2017 (August 26) there have been 1708 isolates submitted for *Streptococcus pyogenes* (Group A *Streptococcus*) serotyping. There was a 39% increase in submissions in 2015 compared to the previous year and a 33% increase in 2016 compared to what was submitted in 2015 (Figure 2).

The National Microbiology Laboratory serotypes *S. pyogenes* by the M protein virulence factor (*emm* typing). There are over 100 *emm* types known and with the exception of 2016, the most frequent serotype seen in the province since 2009 has been *emm* type 1, accounting for 9-32% of all serotypes isolated (Figure 3).

In 2016, *emm* type 82 was the most frequently isolated (20%), followed by *emm* type 101 (18%) and then *emm* type 1 (11%). Type 82 was seen most frequently in adults 40-49 years old while there were fewer *emm* type 101 isolated in this age group. The majority of adults with *emm* type 1 were from those 40-49 years old and 60 years and older (Figure 4).

So far in 2017, *emm* type 1 has been the top serotype isolated (21%) followed by *emm* type 101 (12%) (Figure 5). Fifty-eight percent of *emm* type 1 isolates are from patients 40 years and older and 15% from children aged 1-4 years. Fifty-seven percent of *emm* type 101 isolates are from patients 50 years and older (Figure 5).

Figure 2. Total isolates submitted for serotyping, collected 2009-August 26, 2017, Advanced Bacteriology & Mycology Program, BCCDC PHL.

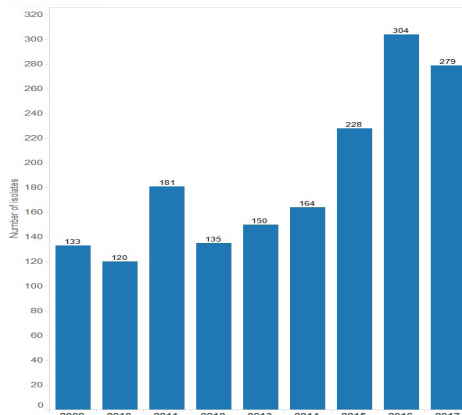


Figure 3. Percentage of top *emm* types, collected 2009-August 26, 2017. Advanced Bacteriology & Mycology Program, BCCDC PHL.

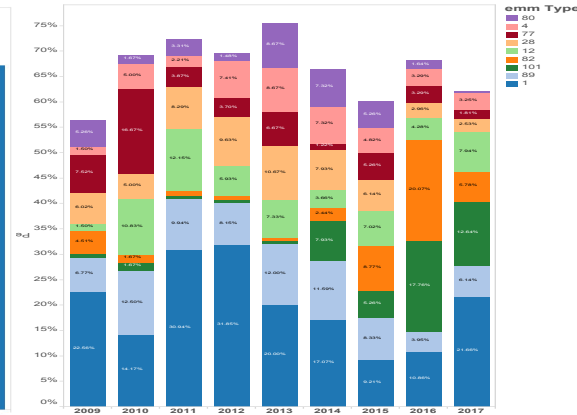


Figure 4. Number of isolates with *emm* types 82, 101 collected in 2016 from patients of various age groups, Advanced Bacteriology & Mycology Program, BCCDC PHL.

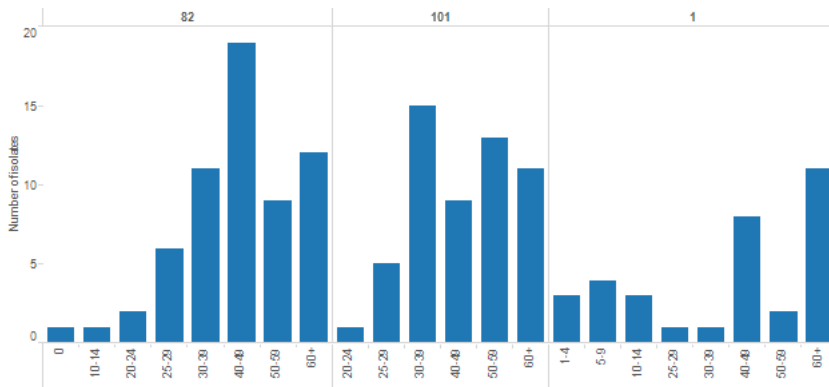
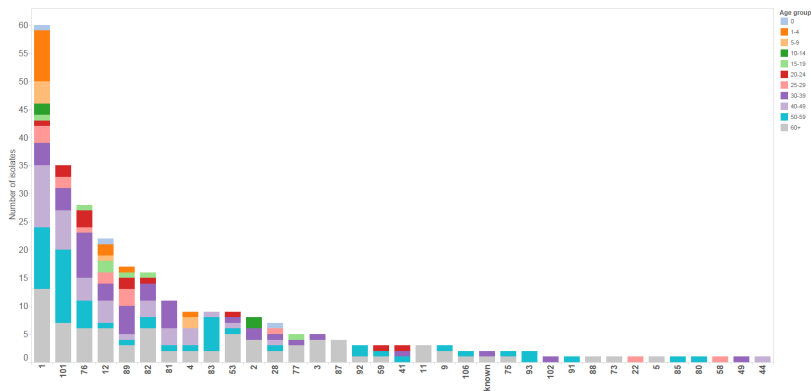


Figure 5. 2017 *emm* types and patient age groups, Advanced Bacteriology & Mycology Program, BCCDC PHL.



Respiratory surveillance

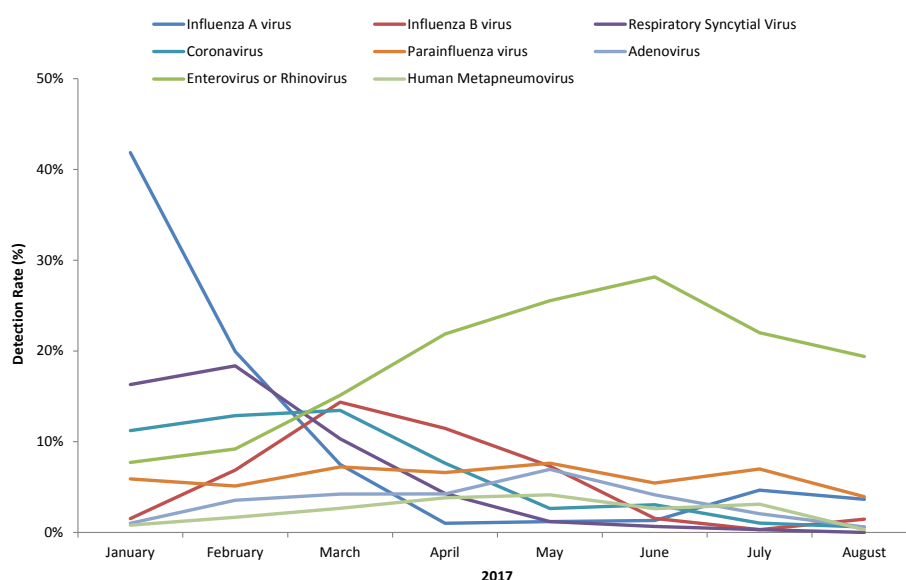
This past influenza season has been dominated by influenza A(H3). At its peak in January, the rate of influenza A detection was 42% before falling to 20% in February and further throughout the spring. Rates of influenza B (0-14%), predictably, increased as influenza A rates fell, tapering off in the summer months. Rates of respiratory syncytial virus (0-18%) also declined throughout the spring and early summer as did coronavirus (1-13%). Detections of parainfluenza (4-8%) have been steady as have human metapneumovirus (0-4%) and adenovirus (1-7%) to some extent except for a slight increase in May. As is typical over the summer months, rates of entero/rhinovirus increased from 8% in January to 28% in June before decreasing again (Figure 6).

Enhanced Surveillance for Enterovirus D68

Enterovirus D68 (EV-D68) associated with mild to severe illness emerged in the United States in mid-August, 2014 causing a nationwide outbreak in the US (1) followed by detections in Canada, Europe and Asia, with cases found in a total of 20 countries (2). In BC our first case appeared at the end of August with a total of 246 cases that year. In 2015, despite enhanced surveillance, no cases of EV-D68 were detected. In 2016, the strain resurfaced in 84 patients in the province.

In 2017, the BCCDC PHL Virology Laboratory is once again performing enhanced surveillance until the end of the year for entero/rhinovirus positive samples from patients under 20 years old and where a specific request has been made. So far this year, of 133 samples tested, **one case of EV-D68 has been confirmed**. The positive case was in an infant that did not present with neurological symptoms. The surveillance trends are consistent with an expected 2-3 year periodicity for enteroviruses.

Figure 6. Respiratory virus detection rates by collection month, Virology Program, BCCDC PHL.



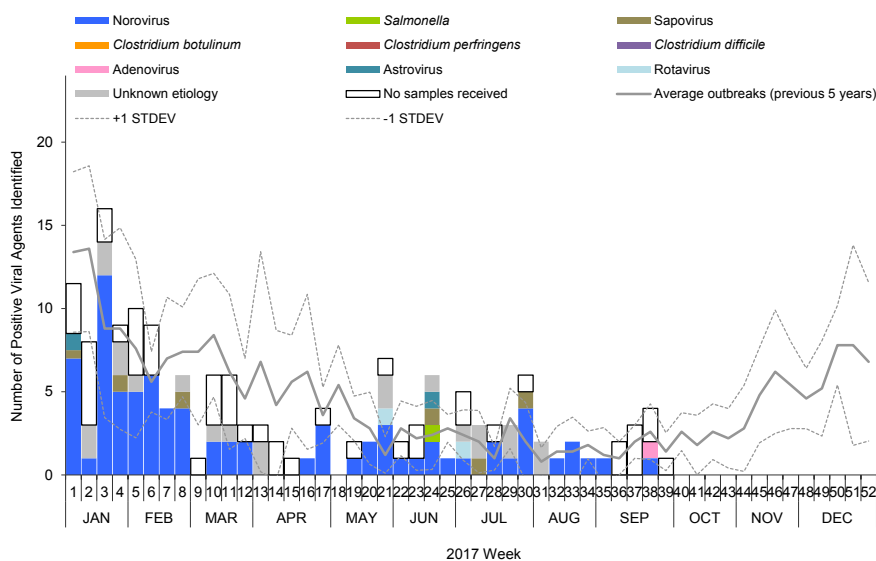
References:

1. Enterovirus D68. Centers for Disease Control and Prevention (CDC). Retrieved from: <https://www.cdc.gov/non-polio-enterovirus/about/ev-d68.html>
2. Holm-Hansen CC, Midgley E and Fischer TK. Global emergence of enterovirus D68: a systematic review. *Lancet Infect Dis* 2016; 16: e64–e75 Published Online February 23, 2016 [http://dx.doi.org/10.1016/S1473-3099\(15\)00543-5](http://dx.doi.org/10.1016/S1473-3099(15)00543-5)

Gastrointestinal outbreaks

In July and August there were 24 gastrointestinal (GI) outbreaks investigated by the BCCDC PHL (Figure 8). Outbreaks were investigated from eight longterm care (LTC) facilities (33%), five camps (21%), four daycares/schools (17%), three cruise ships (12%), two restaurants (8%), one hospital (4%) and one other facility type (4%). Samples were received from 22 (92%) of these outbreaks with norovirus detected in 13 (59%) (five from LTC facilities, four from camps, three from cruise ships and one from a restaurant outbreak). Sapovirus was also detected in two daycare outbreaks in July. So far in September there have been notifications of 10 potential GI outbreaks from seven daycares and three LTC facilities. Norovirus and adenovirus have been separately detected from samples from two different daycares to date.

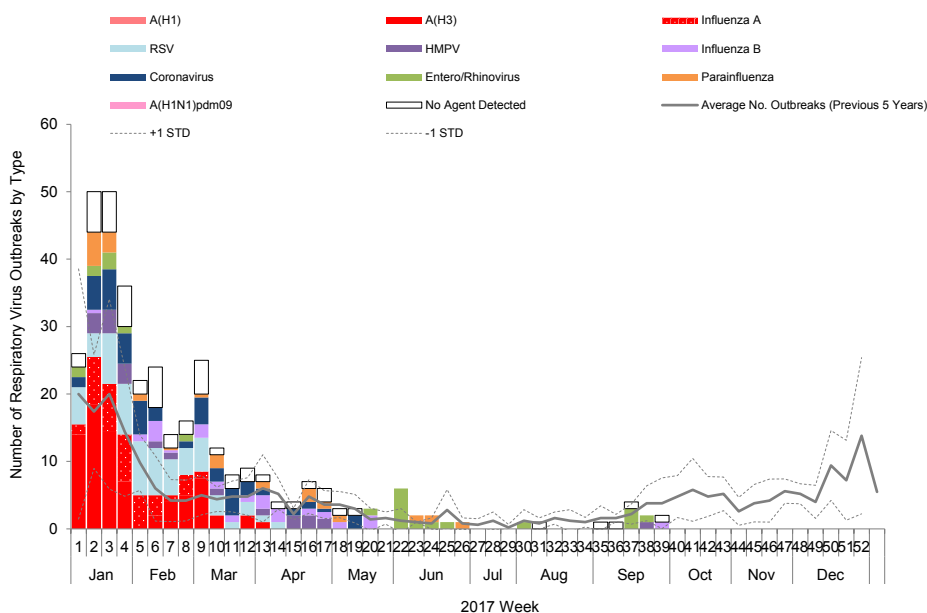
Figure 8. Gastrointestinal outbreaks investigated in 2017, Environmental Microbiology, Public Health Advanced Bacteriology & Mycology, Parasitology and Virology Programs, BCCDC PHL. The data available are from outbreaks in which the BCCDC PHL has been notified. Some acute care microbiology laboratories are also testing for norovirus in the province and these data may not include outbreaks from all health authorities.



Respiratory outbreaks

So far in September there have been 10 outbreaks investigated from LTC facilities (Figure 9). The number of outbreaks are consistent with historical weekly submissions from the past five years. Entero/rhinovirus was detected in four (40%) of the outbreaks, human metapneumovirus (HMPV) detected in another outbreak and influenza B in a separate outbreak. In one outbreak where entero/rhinovirus was detected, the patient had a mixed infection with parainfluenza virus as well as HMPV in addition to the entero/rhinovirus.

Figure 9. 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.

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.

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