

LABORATORY TRENDS



18 March, 2016

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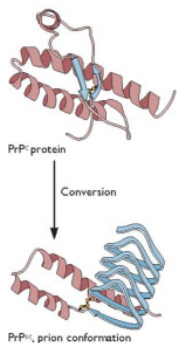
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Laboratory News

Implementation of a Quaking-Induced Conversion Assay for Creutzfeldt-Jakob Disease at the National Microbiology Laboratory

The BCCDC Public Health Laboratory (PHL) refers tests for prion diseases (transmissible spongiform encephalopathies) to the National Microbiology Laboratory (NML). The Prion Laboratory Section (PLS) of the NML has recently introduced a new test for the diagnosis of Creutzfeldt-Jakob disease (CJD). CJD is a particularly unique and challenging disease to diagnose as its etiologic agent is not bacterial or viral, the causative agents of most communicable diseases. CJD is caused by a misfolded isoform (PrPd) of the host prion protein. To date, PLS has been testing for CJD indirectly through the detection of proteins 14-3-3, Tau, and S100 (proteins found in increased concentrations in the cerebrospinal fluid (CSF) of patients with CJD) by enzyme-linked immunosorbent assay.

Figure 1
Conversion of rPrP to PrPd (1).



A recently developed end-point quaking-induced conversion (EP-QuIC) test has the ability to directly detect the presence of PrPd within CSF. With EP-QuIC testing, a sample of CSF obtained from a suspected case is added to wells with normal recombinant prion protein (rPrP) and incubated. In the presence of PrPd from an infected individual's CSF, rPrP is converted into the misfolded isoform and insoluble fibrils are formed. Figure 1 shows the conversion of rPrP to PrPd. These fibrils aggregate, bind to a specific fluorescence dye, and a resulting change in emission spectrum is detected (2). Emission readings are compared before and after the incubation period: a ≥ 4 fold increase in signal intensity in at least 2 of 3 assay replicates infers a positive test result. In the absence of PrPd, no reaction will occur, and consequently no change in emission spectrum will ensue, indicating a negative test result.

The EP-QuIC assay was added to the CJD test panel on 1 February, 2016, while S100 protein testing will be discontinued on 1 April, 2016. A retrospective analysis was recently carried out by the NML to validate the EP-QuIC test (validation of 14-3-3 protein and Tau protein testing has been previously described (3)). Performance characteristics of these tests are presented in Table 1 below. Further information regarding the implementation of the EP-QuIC assay can be found at the NML PLS website (4).

Table 1
Performance Characteristics of the NML PLS CJD Assay Panel*

Marker	Sensitivity (95% CI**)	Specificity (95% CI**)
EP-QuIC (n=91)	98% (88%-100%)	96% (85%-99%)
14-3-3 (n=1000)	88% (81%-93%)	72% (69-75%)
Tau (n=1000)	91% (84%-95%)	88% (85%-90%)

* Adapted from "A guide to the cerebrospinal fluid protein marker panel and EP-QuIC testing for sporadic Creutzfeldt-Jakob disease," client email document

** 95% Confidence Interval

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Tuberculosis Surveillance (MIRU - VNTR)

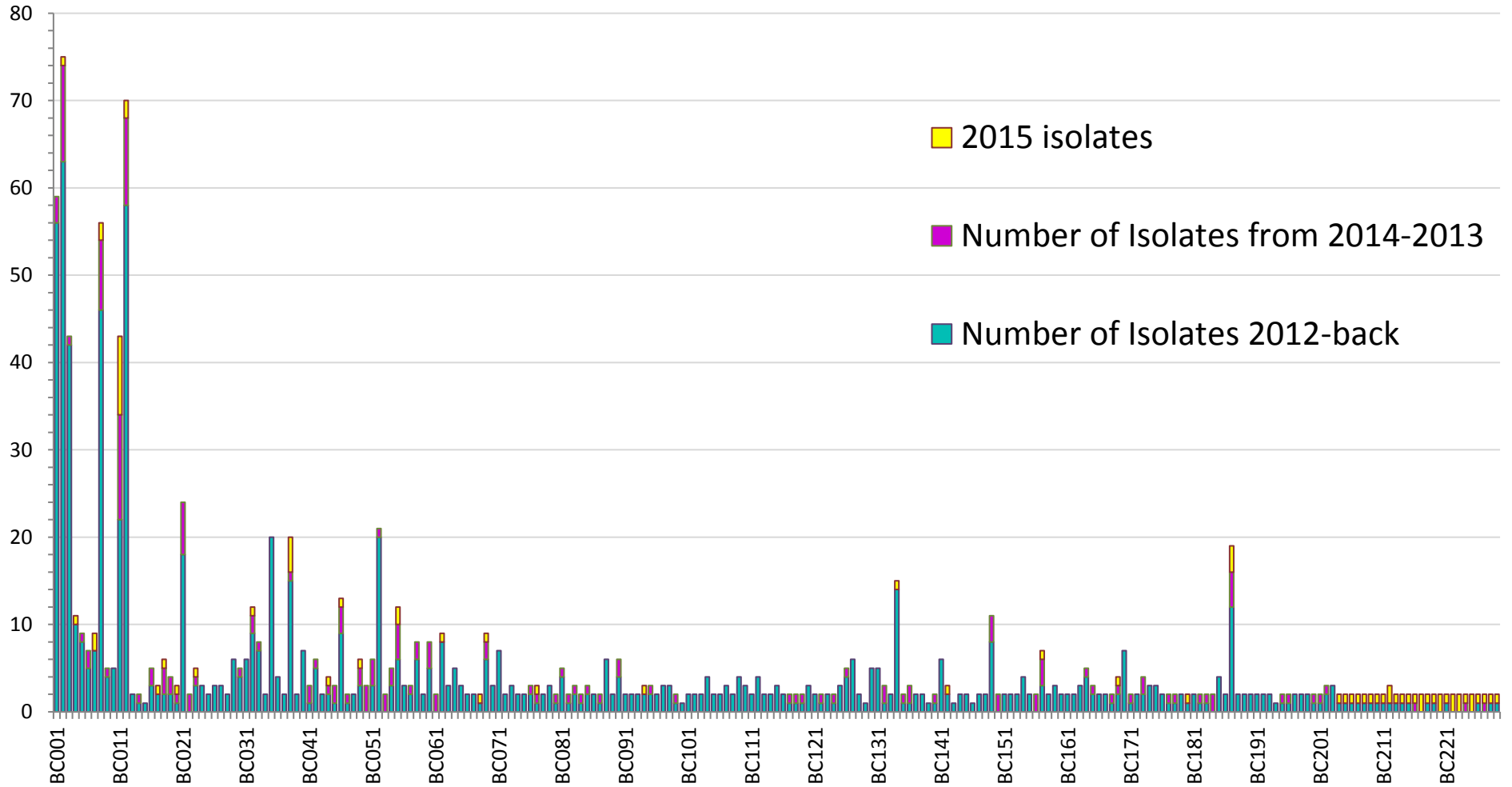
Genotyping isolates of *Mycobacterium tuberculosis* (*M. tuberculosis*) is useful in the epidemiologic study of Tuberculosis (TB) disease transmission patterns. Identifying unique genetic patterns in specific regions of genotyped *M. tuberculosis* isolates is used to distinguish different strains. These genotypic data, along with clinical and epidemiologic investigation, can help differentiate between reactivation of remotely acquired latent TB and newly acquired infection (from presumed recent transmission) in an individual with active TB disease (1). Furthermore, these genotypic data, when combined with epidemiologic data, can also identify clusters of related strains that can aid in contact investigation and outbreak investigation (2). Genotyping has also confirmed the presence of simultaneous infection with multiple TB strains, reactivation versus reinfection with a new strain of TB, false positive laboratory cultures and to refute purported associations.

The Mycobacterial Interspersed Repetitive Units - Variable Number of Tandem Repeats (MIRU - VNTR) method of genotyping *M. tuberculosis* was introduced upon request in BC in 2009 as the next generation of testing to RFLP and spoligotyping (see Laboratory Trends: "Tuberculosis Surveillance," August 2011). Briefly, in MIRU - VNTR genotyping, 24 regions of the genome that exhibit a variable number of repeated units are targeted. The number of repeats at a single region is designated a one digit number (from 0-9), and when combined, a 24-digit designation is given. Simply put, unique 24-digit patterns indicate different *M. tuberculosis* strains while like-patterns indicate same strains.

In 2015, the Tuberculosis/Mycobacteriology program at the BCCDC PHL initiated a project to further inform TB surveillance and epidemiologic investigation in BC in support of the BC Strategic Plan for TB Prevention, Treatment and Control. Using MIRU - VNTR genotyping performed at the BCCDC PHL (in association with Canada's National Centre for Mycobacteriology), this project consists of assigning unique MIRU - VNTR-derived designation patterns to *M. tuberculosis* strains dating back to 2009 that were isolated from at least two different sources (n=1147 isolates). By doing so, the Tuberculosis/Mycobacteriology program, through ongoing and systematic review of these data, will be able to pro-actively monitor trends in *M. tuberculosis* genotype patterns. Linking these genotyping data to clinical and epidemiologic information will be used to support the public health follow-up of TB, and better inform disease transmission dynamics in BC and Yukon Territory.

Since 2009, 229 previously unique strains of *M. tuberculosis* received formal designations (meaning at least one other isolate was found with the same MIRU - VNTR pattern in the database in the current year or previously), conveniently designated by BCCDC PHL as BC001-BC229. In 2015, of 186 isolates from that calendar year that were tested by MIRU - VNTR, five strains were given new designations by being matched for the first time with one other isolate with the same MIRU - VNTR pattern: BC218 (two isolates), BC221 (two isolates), BC223 (two isolates), BC224 (two isolates), and BC226 (two isolates). A further 81 isolates shared MIRU - VNTR patterns with isolates from prior years that had designations assigned to those previous isolates and were therefore assigned to that pre-existing designation (cont'd).

Figure 2
MIRU - VNTR BC Designations Assigned, 2009-2015



(cont'd) Finally, 105 isolates with an unique MIRU - VNTR pattern were not given a designation as no match was found in the existing database to date. Figure 2 (above) shows a graphical distribution of the 229 MIRU - VNTR-derived *M. tuberculosis* designations in BC, 2009-2015.

MIRU-VNTR data, updated on an ongoing basis, will be made available for review by laboratory staff, clinicians and epidemiologists to ensure the best health outcomes for British Columbians. Clustered cases with suspected epidemiologic links will be shared with public health officials to inform TB contact investigation. Clusters will also be analyzed in the context of the Provincial TB Outbreak Protocol.

With Dr. Victoria Cook, TB Control Clinic, Clinical Prevention Services; Danielle Jorgensen, Tuberculosis/Mycobacteriology

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Zika virus Surveillance

Zika virus (ZIKV) is a flavivirus transmitted by *Aedes* mosquitoes, similar to Dengue and West Nile viruses, found in South America, Latin America and the Caribbean. Originally found only in Africa and Asia, the virus was first reported in the Western Hemisphere in 2015. Mosquito-borne transmission is the dominant mechanism of ZIKV transmission; however, there have been reports, outside of Canada, of both sexual transmission and blood transfusion-related transmission. The overriding concern about ZIKV relates to the association between infection in pregnancy and microcephaly; the nature of this association is currently being investigated.

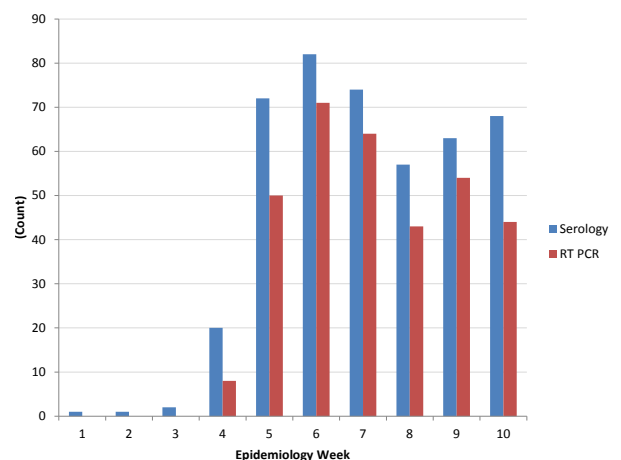
On February 1, 2016, the World Health Organization declared the recent cluster of microcephaly cases and other neurological disorders a public health emergency of international concern (1). The, BCCDC and Public Health Agency of Canada have issued recommendations around travel for pregnant women and have outlined testing algorithms depending on a number of factors including travel history, occurrence of symptoms, time since onset of symptoms, and pregnancy status (see [Laboratory Trends: "Zika virus Guidelines," January 2016](#) and [BCCDC Zika case management and testing recommendations](#) (2)).

ZIKV infection is detected by Reverse Transcription Polymerase Chain Reaction (RT PCR) in house at the BCCDC PHL or by specimen referral for serology testing (EIA-IgM) at the NML followed by confirmatory Plaque Reduction Neutralization Test (PRNT).

Since ZIKV testing of samples from British Columbia and Yukon Territory began in at the BCCDC PHL on 24 November, 2015, there have been 2 confirmed cases and 6 persons under investigation as of 9 March, 2016.

During epidemiology weeks 1-10 (3 Jan - 12 Mar), 2016, there were a total of 334 samples tested by RT PCR and 440 samples referred to NML for ZIKV detection by serology. Test volumes peaked during week 6 for serology and RT PCR testing (Figure 3).

Figure 3
Cumulative Test Volumes of Samples for Zika virus RT PCR and Serology, Epi Week 1-10 2016 (3 Jan - 12 Mar, n=774)



There were 725 samples that included information from which to derive regional variation. The largest amount of samples submitted were from Vancouver Coastal Health authority (37%) while the smallest amount of samples were submitted from Northern Health Authority (2%) (Figure 4).

Since week 1, 81% of all samples tested for ZIKV by either RT-PCR or serology were from females (82% of all serology samples and 80% of RT PCR samples) (Figure 5). Since week 1, 88% of all samples tested were from residents in the 16-49 year age group with the majority (85%) being from females of reproductive age (Figure 6).

Figure 4
Zika virus Test Volumes by Regional Health Authority (Epi Week 1-10 2016, n=725)

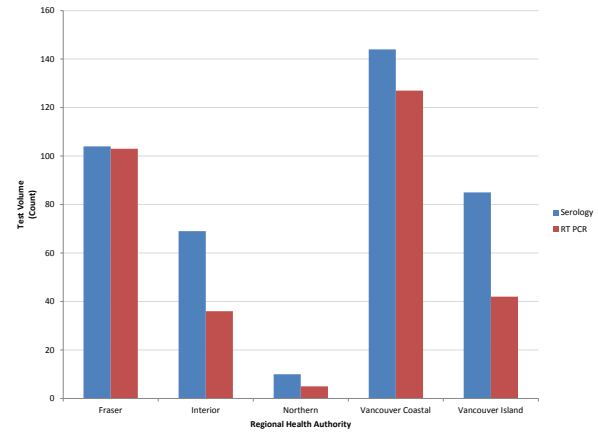


Figure 5
Zika virus Test Volumes of Samples for RT PCR and Serology by Gender (EpiWeek 1-10 2016, n= 774)

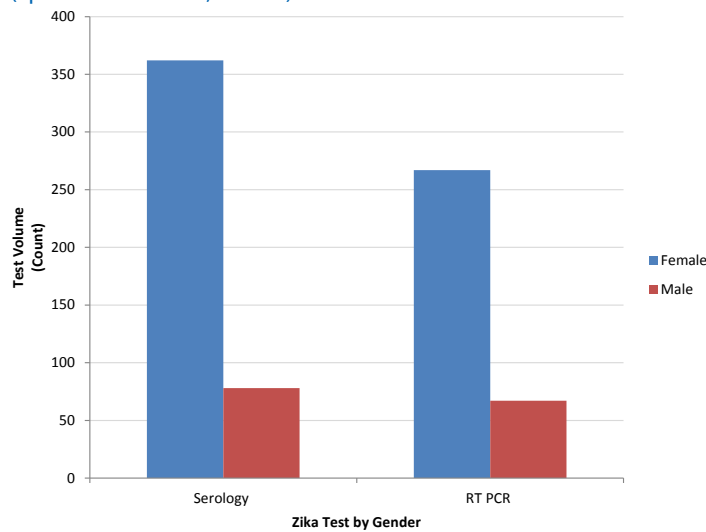
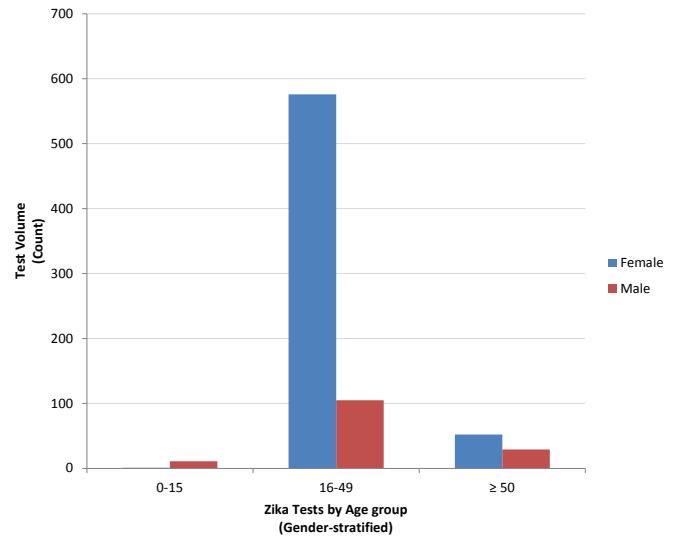


Figure 6
Zika virus Test Volumes of Samples for RT PCR and Serology by Age Group (Gender-stratified, Epi Week 1-10 2016, n=774)



Further information on ZIKV and ZIKV infection/disease can be found at the BCCDC website (<http://www.bccdc.ca/health-info/diseases-conditions/zika-virus>); testing guidelines and other information for health professionals can be found at the following website: <http://www.bccdc.ca/health-info/diseases-conditions/zika-virus/information-for-health-professionals>.

With Diana George, Communicable Disease Prevention and Control Services

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Influenza Surveillance

After initial sporadic influenza A incidence in BC, both influenza B and A rates began increasing in December 2015 and January 2016, respectively. Influenza B rates surpassed influenza A rates in week 51, 2015, and remained above influenza A rates until week 8, 2016. Influenza B rates peaked in week 4, 2016, with 23% positivity and appear to be declining while influenza A rates continue an increasing trend with 32% positivity in week 10 (Figure 7). Relative to other Canadian regions, there was an earlier rise in incidence of influenza B in BC, with influenza B rates in BC remaining above all other Canadian regions since week 48, 2015. After an initial rise in positivity rates above other Canadian regions, BC influenza A rates have been below the national average since week 5, 2016 (Figure 8). During the early flu season - weeks 40-52, 2015 - the dominant influenza A subtype was A(H3), ranging between 83.3-100% of influenza A subtypes. Since week 3 to week 10, 2016, influenza A(H1N1)pdm09 has been the predominant influenza A subtype with rates increasing from 52.7-90.3% in week 10, 2016.

Figure 7
Respiratory testing volumes and influenza detection rates, Virology Program, BCCDC PHL.

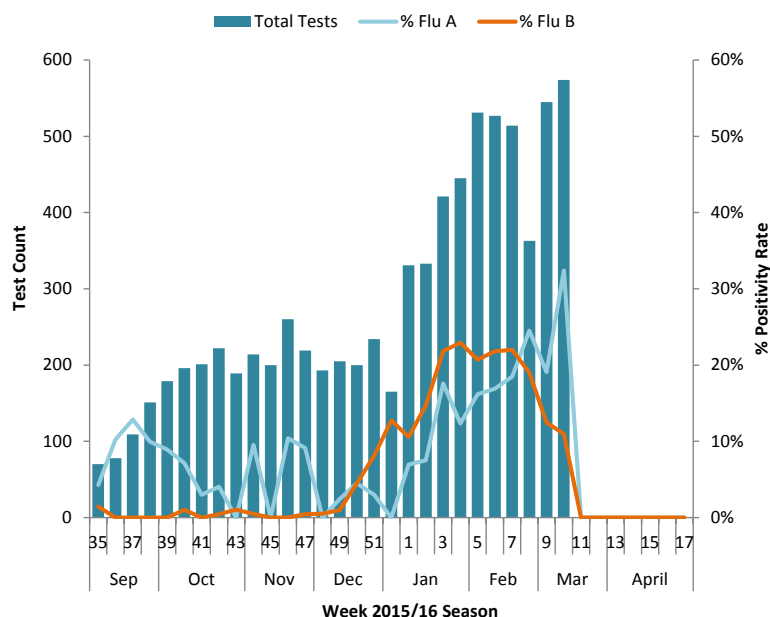
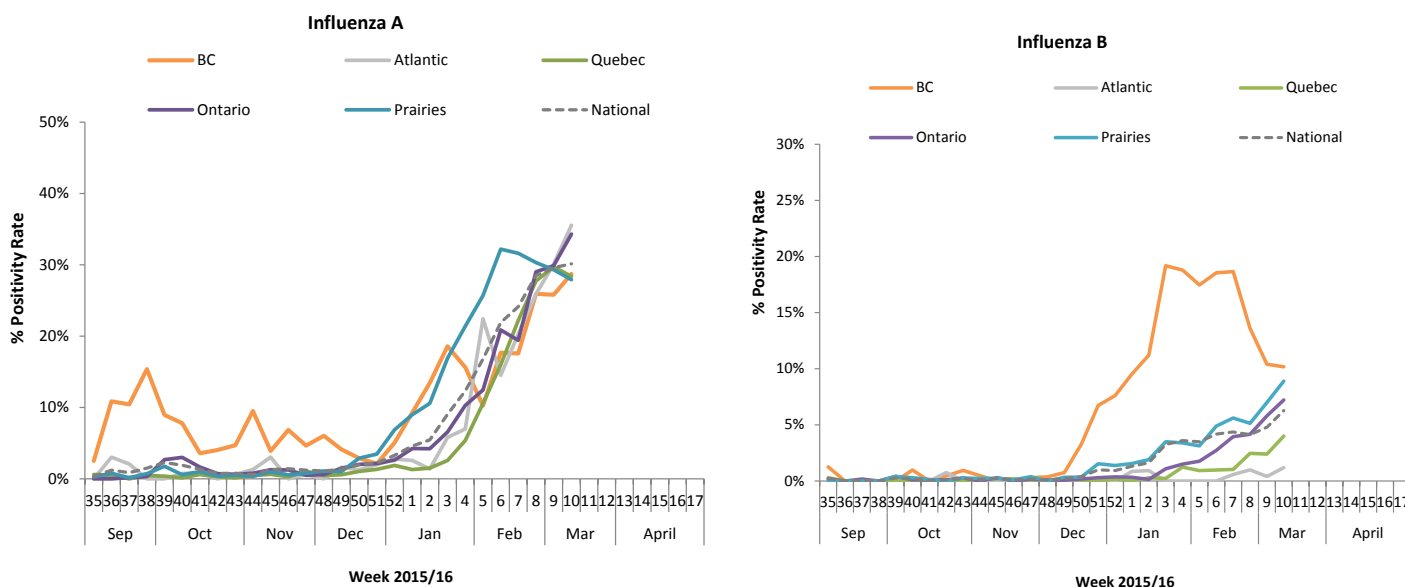


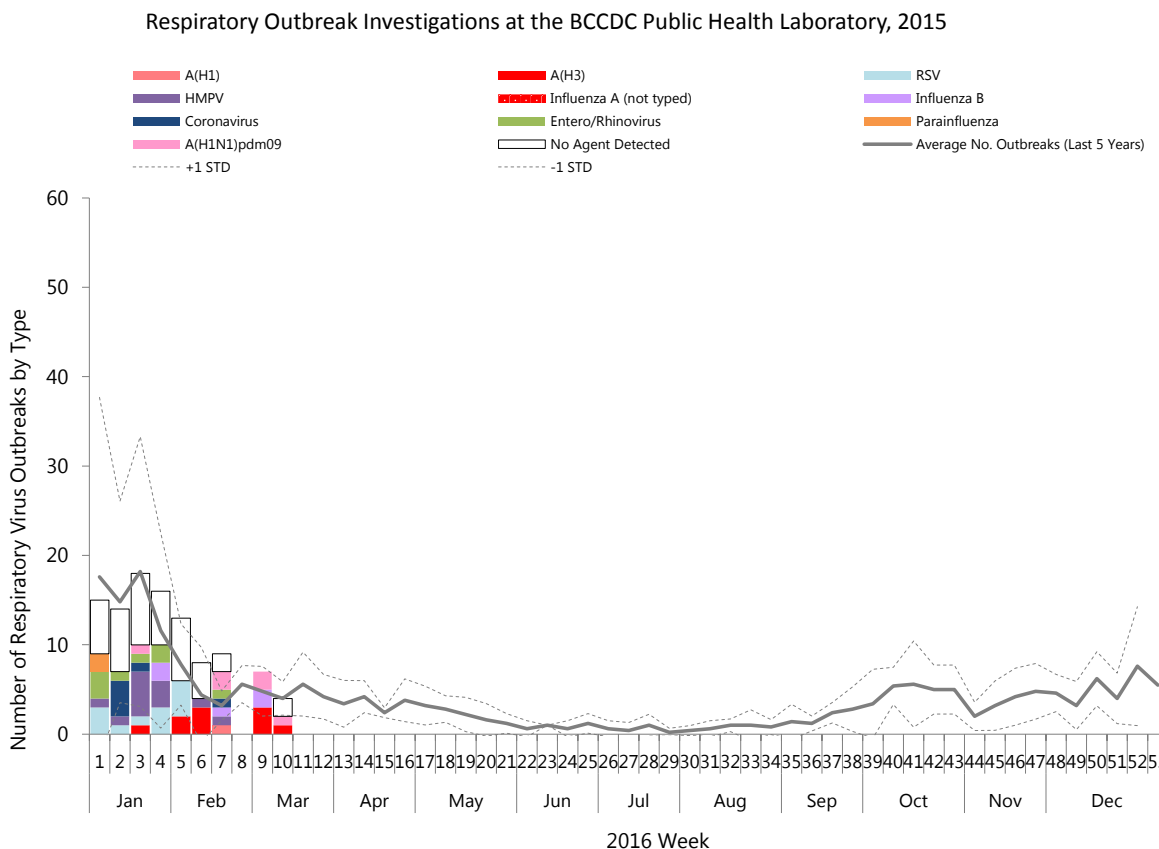
Figure 8
Influenza A and B detection rates across Canada, September 2015 to present. Data derived from FluWatch reports. Note: Reported detection rates may be different from actual detection rates (Figure 7) if subtyping is completed in subsequent weeks.



Influenza-Like Illness Outbreaks

In January there were 64 influenza-like illness outbreaks investigated by the Virology Program which is within the expected trend (Figure 9). There were 11 (17%) human metapneumovirus detections (hMPV), eight (13%) respiratory syncytial virus (RSV) detections, seven (11%) entero/rhino virus detections, five (8%) coronavirus detections, two (3%) parainfluenza detections, two (3%) influenza B detections, one (2%) influenza A(H1N1)pdm09 detection, and one (2%) influenza A(H3) detection. In February there were 30 influenza-like illness outbreaks investigated with seven (23%) influenza A(H3) detections, four (13%) RSV detections, 2 (7%) influenza A(H1N1)pdm09 detections, one (3%) influenza B detections, one (3%) hMPV detection, one (3%) entero/rhinovirus detection, and one (3%) coronavirus detection. Of the 104 ILI outbreaks reported to the BCCDC PHL during epidemiology weeks 1-10, 102 (98%) were reported from long-term care (LTC) facilities, one (1%) from a hospital, and one (1%) from a community setting.

Figure 9
Influenza-like illness outbreaks investigated* in 2016, Virology Program, BCCDC PHL.

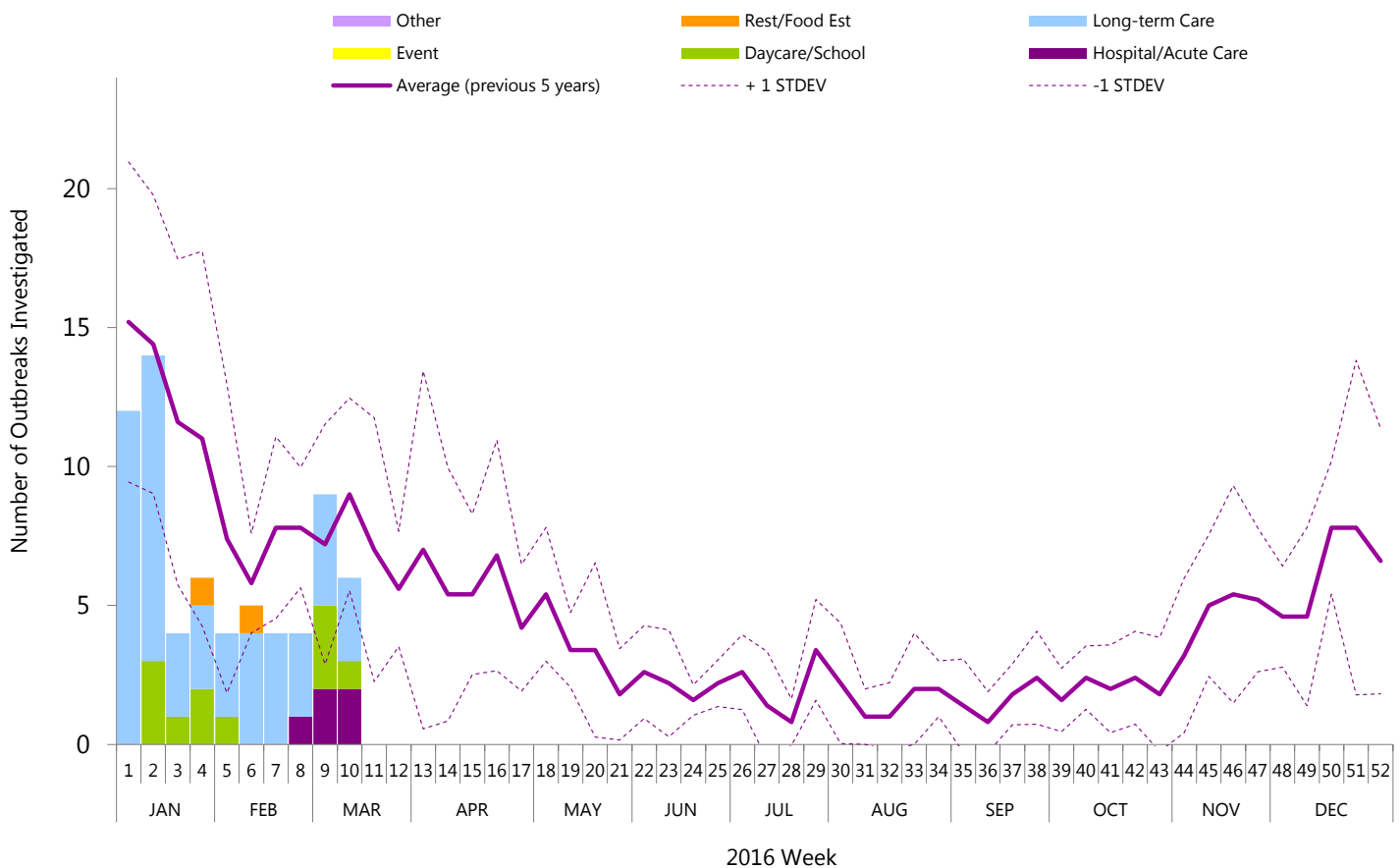


* The data available are from outbreaks in which the BCCDC PHL has been notified. Some acute care microbiology laboratories are also testing for influenza in the province.

Gastrointestinal Outbreaks

During epidemiologic weeks 1-10, there were 68 gastrointestinal outbreaks investigated by the BCCDC PHL (Figure 10), within the expected historical trend. Outbreaks were investigated from 50 (74%) LTC facilities, 11 (16%) daycares/schools, and five (7%) hospitals. Samples were received from 52 (76%) of these outbreaks with norovirus detected in 38 (56%) outbreaks (33 LTC facilities, two hospitals, two daycares/schools, and one restaurant) and sapovirus detected at one daycare/school and one LTC.

Figure 10
Gastrointestinal outbreaks investigated* in 2015, 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. Given the nature of GI outbreaks, samples are not always available for testing.

A Report of the BCCDC Public Health Laboratory,

Vancouver, BC

The BCCDC Public Health Laboratory at the BCCDC site provides consultative, interpretative testing and analyses for clinical and environmental infectious diseases in partnership with other microbiology labs and public health workers across the province and nationally. The BCCDC Public Health Laboratory is the provincial communicable disease detection, fingerprinting and molecular epidemiology centre providing advanced and specialized services along with international defined laboratory core functions province-wide.

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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