



July 22, 2015

**Laboratory News**

**Tuberculosis IGRA Test Expanded for Use in Chronic Kidney Disease Dialysis Patients**

Tuberculosis (TB) infections, caused by *Mycobacterium tuberculosis* (Mtb), causes significant morbidity and mortality world-wide. Many people with active pulmonary or disseminated TB have life threatening disease. However, many patients survive and pass through the active disease stage to go on to a latent stage. This dormant infection with Mtb is referred to as Latent TB Infection (LTBI). Among LTBI patients, approximately 5-10% of people will go on to reactivating to have active TB disease again. Thus, monitoring patients with LTBI is important, particularly in vulnerable populations.

Funded by Provincial Health Services Authority (PHSA), BC is the first province to start offering Interferon Gamma Release Assay (IGRA) testing to select, particularly vulnerable, populations (TST-negative, immuno-compromised; BCG positive and TST positive, TST positive Aboriginals and foreign born). To further decrease the incidence of TB, the BC Provincial Renal Agency (BCPRA) in partnership with the BC Centre for Disease Control and BC Public Health Microbiology and Reference Laboratory (BCPHMRL) most recently obtained support to start to provide IGRA testing for Chronic Kidney Dialysis (CKD) patients. This improved screening will be done first by history taking (identify risks), a chest radiograph then by IGRA testing. This patient safety initiative was built on the pilot project results done with St Paul’s Hospital, Vancouver General Hospital and BCPHMRL. Results showed a decrease of active TB from six cases per year (2002-2012) to none in 2013.

In support of this partnership, BCPHMRL will expand IGRA testing for the CKD population within the public health

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laboratory network. Expanding from the current seven Regional Sample Collection Sites (RSCS), to five more sites, samples will be initially processed using common processes and protocols within a Quality Management System. Final complex testing is done centrally at the BCPHMRL.

Following the BC Renal Program Clinical Lead, the BCPHMRL has started to work with site specific medical microbiologists and operations managers to establish sample collection/ initial processing. Quality Management System materials (training checklists, SOPs, QC/QA documents) will be made available for use as our network expansion is rolled out.

For IGRA laboratory related questions, contact Dr. Muhammad Morshed (604-707-2622, [Muhammad.Morshed@bccdc.ca](mailto:Muhammad.Morshed@bccdc.ca)) or Ms. Quantine Wong (604-707-2612, [Quantine.Wong@bccdc.ca](mailto:Quantine.Wong@bccdc.ca)).

**New Tick Submission Guidelines**

The Parasitology Program of the BCPHMRL has updated their submission guidelines for tick testing: All ticks will be identified and may be sent alive or dead (with no preservative) for PCR. PCR for *Borrelia burgdorferi* will be performed on all tick species except *Dermacentor andersoni*. Tick culture may also be performed on all PCR positive samples, but only if live ticks were submitted (submit with slightly moistened cotton).



## Laboratory News

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### Updates to Forms for Nucleic Acid Testing (NAT) for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

**Sample Container Order Form adjusted for ordering kits for Nucleic Acid Testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*:** Now available [online](#).

Please note that there are now 3 types of swabs listed for testing for GC/CT NAT:

Collection Kit	Description	Container Type	Target Organism and Test
Unisex Swab Sample Collection Kit for Endocervical and Male Urethral Swab specimens (purple label)	Can be used for throat and rectal sites. Has a cleaning swab to be used for collection of cervical samples.	NUCLEIC ACID TESTING (NAT) SWAB	<i>Chlamydia trachomatis</i> AND <i>Neisseria gonorrhoeae</i> NAT
<b>New:</b> Vaginal Swab Sample Collection Kit for collection of vaginal specimens (orange label)	Can be used for throat. This type of swab has a thicker head and shaft and no need for a cleaning swab. Recommended for vaginal sampling.	NUCLEIC ACID TESTING (NAT) SWAB	<i>Chlamydia trachomatis</i> AND <i>Neisseria gonorrhoeae</i> NAT
Urine Sample Transport Kit (yellow label)	Used for collection and transport of urine samples.	NUCLEIC ACID TESTING (NAT) URINE	<i>Chlamydia trachomatis</i> AND <i>Neisseria gonorrhoeae</i> NAT

We calculate that the Vaginal Swab Sample Collection Kit will be replacing numbers of Unisex Swab Sample Collection Kit for Endocervical and Male Urethral Swab and not add to the total numbers. It is suggested that sites calculate their numbers accordingly as these all have expiry dates. Please bear with us as we try to adjust to requests during this introductory period.

### New Look to the Sexually Transmitted Infections section on the Bacteriology and Mycology Requisition:

Appreciating the feedback from our onsite STI Clinic the Bacteriology and Mycology Requisition (Form DCBM\_100\_1001F Version 4.0 07/2015) is also updated to minimize inappropriate requests; these are presented as blocked tests for STI requests. Please also note that *Trichomonas* testing is now available by NAT.

SEXUALLY TRANSMITTED INFECTIONS				
Source	Test Requests			
	Chlamydia & Gonorrhea NAT	Gonorrhea Culture	Trichomonas NAT	Direct Smears
Cervix	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Vagina	<input type="checkbox"/>	No cervix <input type="checkbox"/>	<input type="checkbox"/>	Bacterial vaginosis & yeast <input type="checkbox"/>
Urethra	<input type="checkbox"/>	<input type="checkbox"/>		Gonorrhea & pus cells <input type="checkbox"/>
Urine	<input type="checkbox"/>		Female only <input type="checkbox"/>	
Rectal	<input type="checkbox"/>	<input type="checkbox"/>		
Throat	<input type="checkbox"/>	<input type="checkbox"/>		
Eye	Dry swab <input type="checkbox"/>	<input type="checkbox"/>		Gonorrhea <input type="checkbox"/>
Nasopharyngeal aspirate or swab (neonates only)	Chlamydia DFA <input type="checkbox"/>			
Tracheobronchial aspirate	Chlamydia DFA <input type="checkbox"/>			

This requisition is now available [online](#). Printed versions will be available as well. Old versions are still valid and can be used.

## Outbreak of Adenovirus Keratoconjunctivitis

Adenoviruses are non-enveloped, double-stranded-DNA viruses found in animals, birds, reptiles and mammals. Since their discovery in 1953 [1], seven taxonomic species (A to G) containing 68 types known to be pathogenic in humans have been identified. Species A are mostly associated with the gastroenteritis, while species B and C are mostly associated with respiratory infections. Species D are the most prevalent cause of conjunctivitis in humans while species E are found as causes of respiratory infections, but more commonly cause conjunctivitis. Species Group F (types 40, 41) and the more recently discovered Group G [2] are causes of gastroenteritis.

Adenovirus ocular infections are classified into four distinct syndromes: pharyngoconjunctival fever (PCF); epidemic keratoconjunctivitis (EKC); acute nonspecific follicular conjunctivitis and chronic keratoconjunctivitis (CK). PCF is most commonly found in children, EKC commonly seen during the fall and winter is the most serious form of ocular adenoviral infection, and CK is the rarest adenoviral infection with symptoms lasting up to 18 months. Transmission of EKC has been associated with close personal contact as well as during non-sterile eye examinations with contaminated ophthalmic instruments. Close-quarter environments, such as hospitals, doctors' offices, industrial areas and swimming pools are prime environments for the spread of the disease.

In early January 2015 the Virology Laboratory at St Paul's Hospital (SPH) became aware of an increase in adenovirus detected from eye swabs and in collaboration with the BCPHMRL have been involved in detecting and typing of adenovirus from an apparent EKC outbreak. This outbreak appears to be confined to the greater Vancouver regional district with no reported cases detected outside the region. To date (July, 1st 2015) the SPH virology laboratory has referred 75 adenovirus positive eye/conjunctiva specimens from 74 patients to the BCPHMRL for typing. Sequencing of hypervariable region 7 of the adenovirus hexon gene [3] using an in-house improved primer pair allowed the BCPHMRL to determine adenovirus types or relationships to taxonomically accepted virus for 71 of the 74 specimens. The failure to identify the virus from 3 specimens occurred due to poor or no sequence being obtained due to insufficient DNA from the patient specimen.

Adenovirus types were determined by comparing adenovirus sequence derived from the patient to sequences deposited in GenBank. A patient's unknown adenovirus sequence was considered to match a deposited sequence when the patient's sequence had greater than 99% match with a reference sequence in GenBank and a distinct separation from the next adenovirus type sequence(s).

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## Outbreak of Adenovirus Keratoconjunctivitis

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Sequencing of patient's specimens identified 9 separate adenovirus types circulating and causing infections: 3, 4, 8, 17, 25, 37, 53, 56, and 64 (formally 19). In the present cluster evaluated, 41/71 (58%) could be assigned as species D, 19/71 (27%) as species E and 11/71 (15%) as species B (Figure 1). Serotypes 3, 4, 8, 37, 53, 56, 64, have all been associated with EKC while types 8, 37 and 64 can cause severe infection. Species B, primarily type 3, has been isolated from patients with EKC infections but is more commonly associated with PCF [4,5,6].

Temporal analysis of the data shown in Figure 1 indicates that adenovirus type 8 was the predominate adenovirus during the period from early January to the end of March 2015 accounting for 41% of adenovirus detected during this period. Seventy-six percent of all type 8 adenovirus was detected at this time. From early April to the end of June 2015, adenovirus type 8 was replaced by type 4 accounting for 41% of adenovirus detected during this period and 84% of all type 4 adenovirus detected to date. The replacement of type 8 by type 4 was also followed by a spike in types 37 and 3 detected accounting for 23% and 18% of adenovirus detected during this period.

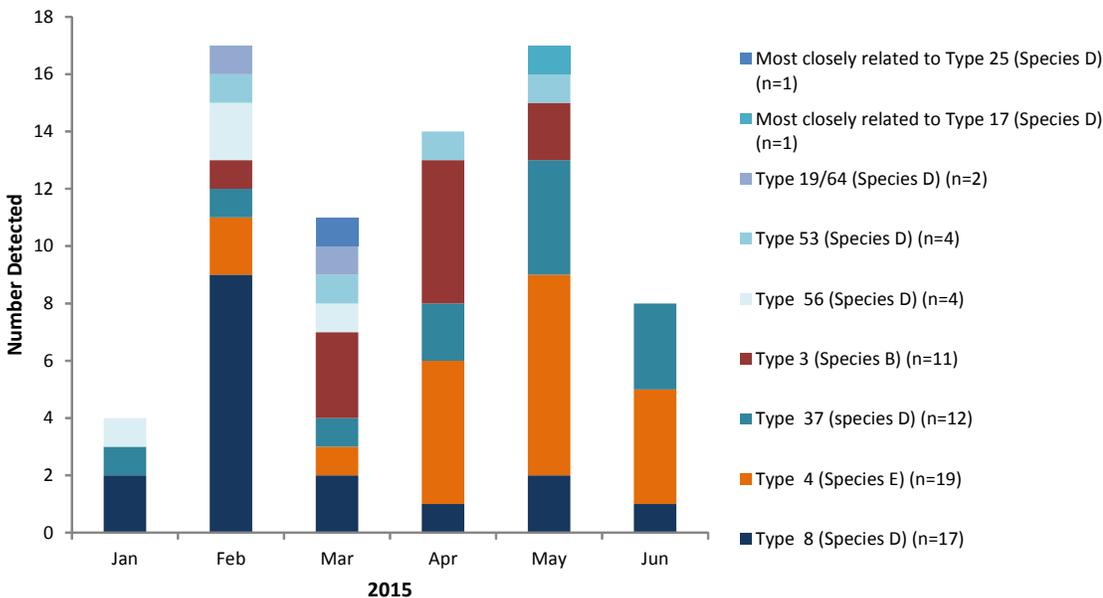


Figure 1 In-house sequencing results of referred adenovirus specimens between January and July 2015, using the adenovirus hexon gene hypervariable region 7, Molecular Microbiology & Genomics Program, BCPHMRL. Three specimens could not be typed with the limited DNA available. (n=71).

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## Outbreak of Adenovirus Keratoconjunctivitis

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Phylogenetic analysis of the predominate types 4 and 8 indicated that there was sufficient sequence similarity to hypothesize that a single strain of both types was circulating within the population and causing disease.

Adenovirus infections of the eye represent a serious public health risk due to their rapid spread and their ability to cause severe symptoms. The disease is most easily missed or confused with other conditions at the earliest stages when the physician may contaminate themselves or spread the disease to others. Therefore rapid identification of adenovirus infections as provided by PCR detection will allow a physician to treat and limit patient spread of disease. Although EKC is most common during late fall and winter we are still detecting and typing adenovirus from patients in the Lower Mainland, suggesting that conditions are allowing transmission to persist outside these seasons.

### References:

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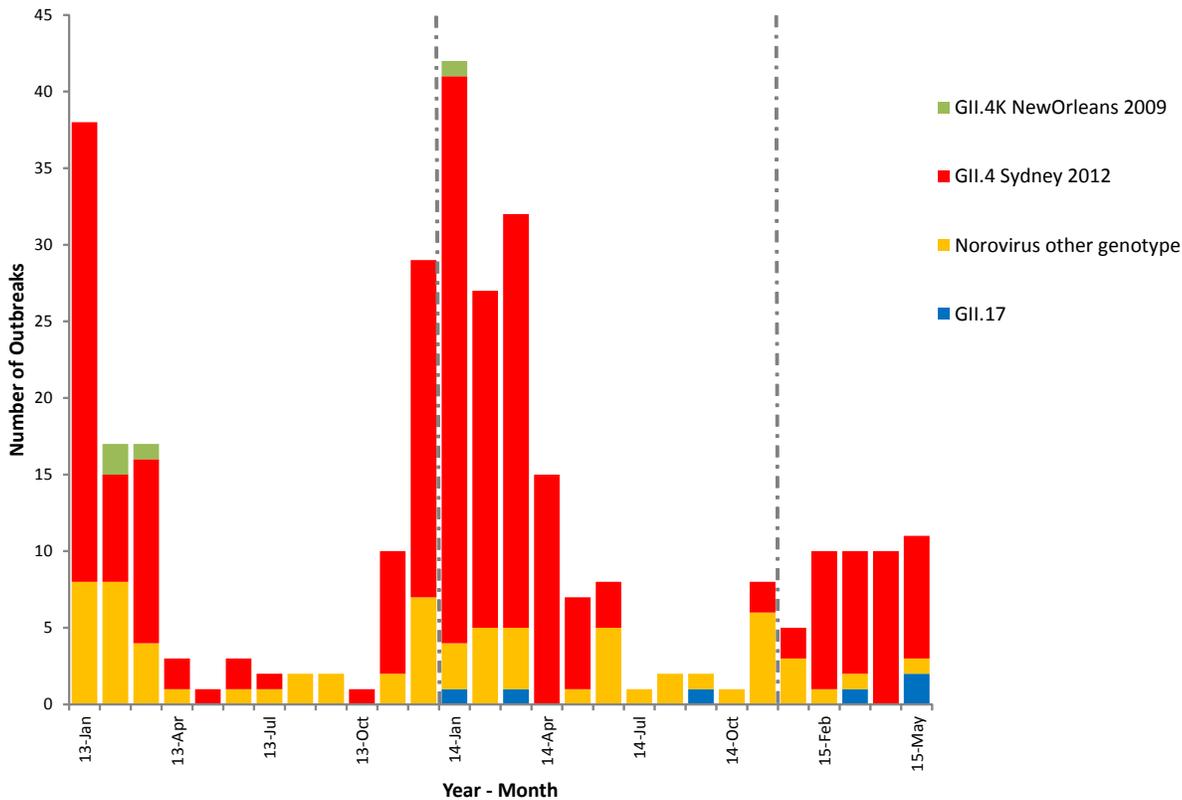
## New Norovirus GII.17 Variant in BC

Since its emergence in 2012 and subsequent spread worldwide, GII.4 Sydney 2012 has been the dominant norovirus genotype. However, recent accounts of outbreaks of a new norovirus GII.17 variant in China and Japan suggest that this strain can have high epidemic capability. GII.17 became the dominant strain in those regions during late winter/early spring (2014/2015), replacing GII.4 Sydney 2012 [1,2,3]. Norovirus GII.17 was first reported in 1978 in French Guiana, in water samples in Kenya, and clinically sporadic cases in South Korea, North America, Europe, New Zealand and Russia [4].

In British Columbia, GII.4 Sydney 2012 continues to be the dominant norovirus genotype in BC (Figure 2). However, the new norovirus GII.17 variant has been observed in our population, originally detected in patient samples from an outbreak in a BC long term care facility in January 2014 and again during an outbreak at the same facility in March 2014. This genotype was not seen again until September 2014 and since then, has only been seen sporadically in outbreak (Figure 2) and non-outbreak settings (Figure 3).

Genotyping these outbreak samples has demonstrated that norovirus GII.17 sequences from BC clusters with the recent Japanese and Chinese strains (Figure 3). Based on the region C of norovirus GII strains, sequences from BC patient samples in 2014 cluster in one clade with the majority of sequences from Japan while BC patient samples in 2015 cluster with the Chinese (Guangdong) sequences. These two clades appear to be genetically distinct from the previously

Figure 2  
Norovirus genotyping from gastrointestinal outbreaks investigated in BC (January 2013 - May 2015), Environmental Microbiology, BCPHMRL.



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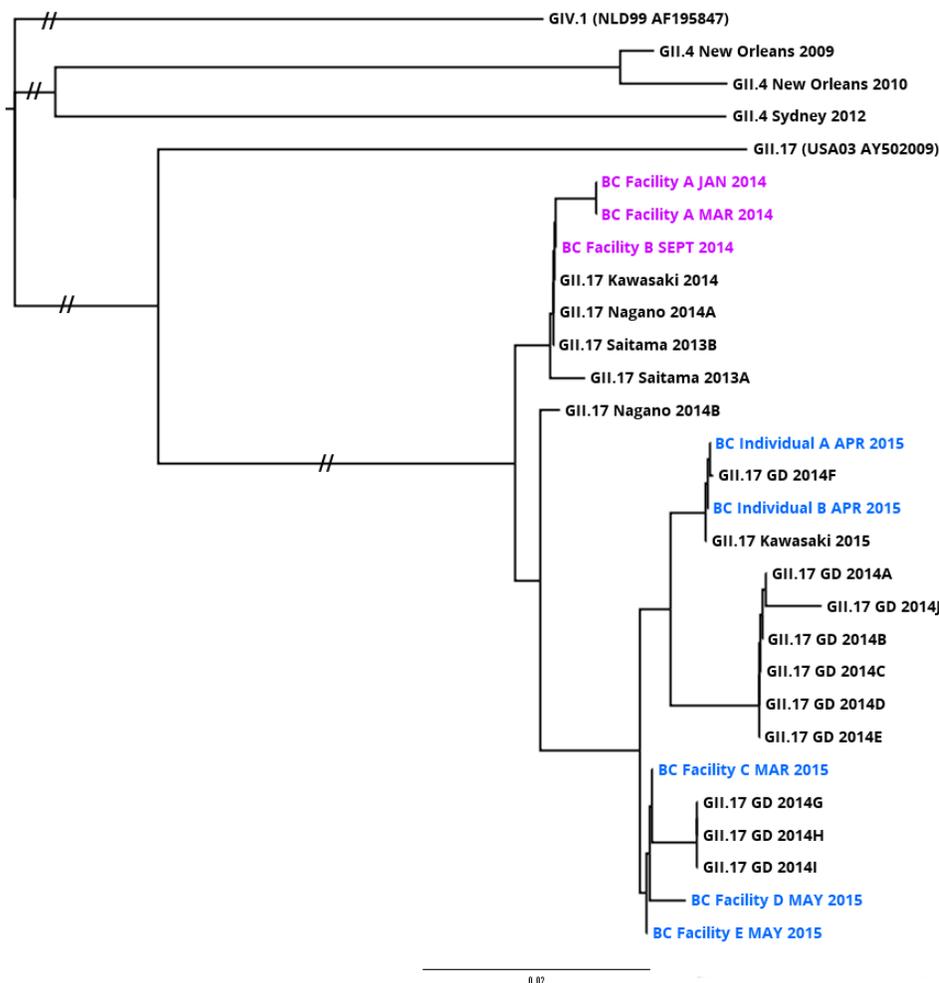
## New Norovirus GII.17 Variant in BC

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circulating GII.17 strain as suggested in recent literature [1,2,3]. It remains to be seen if other regions in the world are also observing this new norovirus strain, what clinical or public health implications are (if any) and whether it will have the capacity of replacing norovirus GII.4 Sydney 2012 globally in coming seasons.

Figure 3

Phylogenetic tree of norovirus GII.17 circulating in BC between 2014 and 2015 in comparison to GII.Sydney 2012, GII.New Orleans, and other publicly available GII.17 sequences. A neighbour joining tree was generated using Tamura-Nei distances on the C-region of norovirus GII sequences from the United States, Japan [3], China [2] and British Columbia. Each label indicates the country or city of the detected norovirus. GD: Guangdong Province, China. BC sequences are colored by year (violet for 2014 and blue for 2015). GIV.1 (NLD99 AF195847) was used as an outgroup. Double hashes (//) indicate a shortened branch length.



### References:

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## Tuberculosis Susceptibility Testing Trends

The TB/Mycobacteriology Program of the BCPHMRL performs *Mycobacterium tuberculosis* complex (MTBC) drug susceptibility testing using the BACTEC® 960 fluorometric proportion method. Isolates are tested against critical concentration levels of first-line anti-tuberculosis drugs including isoniazid (INH, 0.1 µg/mL), rifampin (RMP, 1.0 µg/mL) and ethambutol (EMB, 5.0 µg/mL). Pyrazinamide (PZA) is only performed when isolates show resistance to isoniazid and/or rifampin or when requested. Streptomycin, a second-line anti-tuberculosis drug, is also part of this susceptibility panel.

The resistance patterns of MTBC patient isolates from 2005 to 2014 are shown in Figure 4. From 2006 to 2011, mono-resistance to one of the first-line drugs has been on the rise from 5.8% in 2006 to 11.3% of isolates in 2011. Although there was a decrease in mono-resistance in 2012 and 2013, mono-resistance was 9.9% in 2014. Poly-resistance has varied from 0% to 0.9% of isolates during this period and was observed at 0.7% in 2014. Multidrug resistance has decreased from a high of 2.0% in 2005 to 0.5% in 2011, and has risen again in 2014 to 2.2%.

The TB/Mycobacteriology Program, along with other participating provincial laboratories, report results of tuberculosis drug susceptibility testing to the Canadian Tuberculosis Laboratory Surveillance System (CTBLSS) for national monitoring of tuberculosis drug resistance patterns. The various resistance patterns have shown relative stability from 2003-2013 with mono-resistance fluctuating between 6.7% and 9% and multi-drug resistance varying between 0.6% and 1.6% during this period (Figure 5). BC has had years (2008-2013) where levels of mono-resistance have been slightly higher than what CTBLSS has reported nationally.

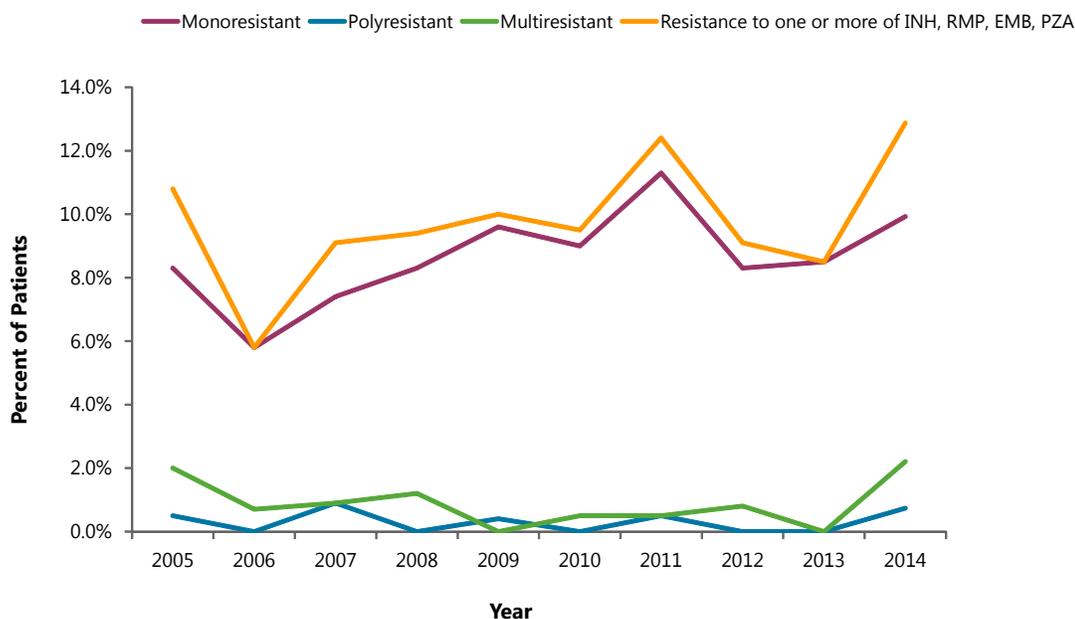
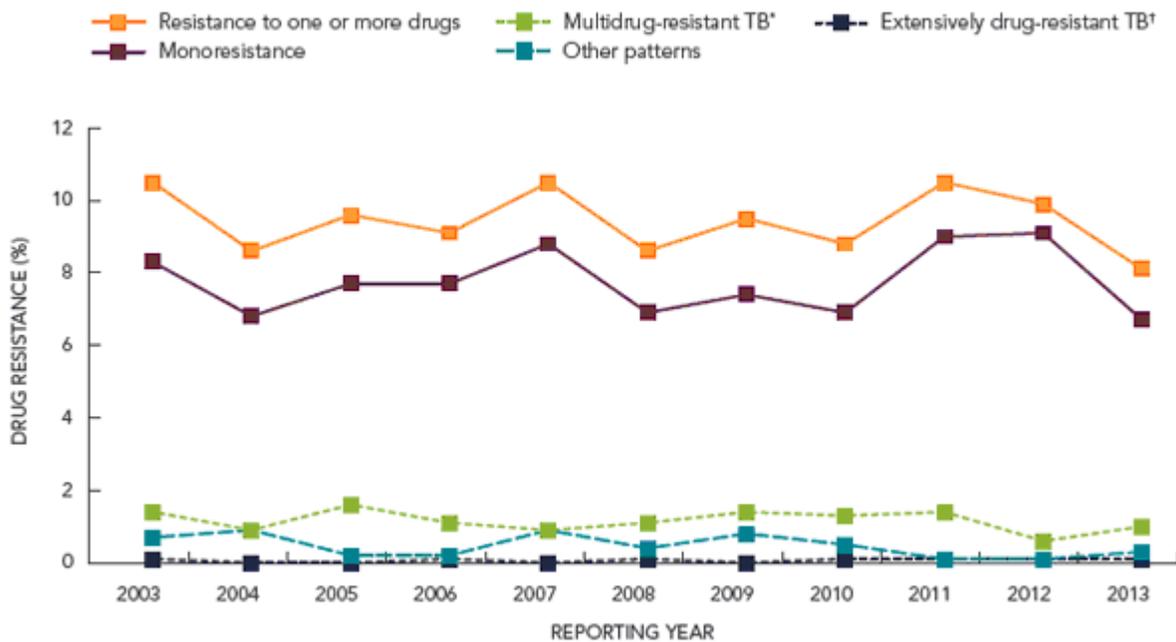


Figure 4  
Percent of MTBC patients who have MTBC isolates that are mono-resistant, poly-resistant and multi-resistant to first-line TB drugs in British Columbia, 2005-2014. Resistance profiles are defined as: mono-resistance, resistance to one of the first-line drugs (INH, RMP, EMB or PZA); poly-resistance, resistance to two or more first-line drugs not including the combination of isoniazid and rifampin; and, multidrug-resistance (MDR-TB), resistance to at least the two best first-line anti-tuberculosis drugs, isoniazid and rifampin, but which does not meet the definition of extensively drug-resistant TB.

## Tuberculosis Susceptibility Testing Trends

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Figure 5  
Tuberculosis drug resistance patterns as a percentage of isolates tested, 2003-2013. Source: Tuberculosis: Drug resistance in Canada – 2013, CTBLSS. <http://www.phac-aspc.gc.ca/tbpc-latb/pubs/tb-dr2013/index-eng.php>



## Neisseria gonorrhoeae Susceptibility Trends

The Public Health Advanced Bacteriology & Mycology (PHABM) Program routinely performs surveillance of *Neisseria gonorrhoeae* to monitor for trends of antimicrobial resistance. Culture-positive isolates from the province are evaluated by E test® for resistance to first-line cephalosporins as well as alternative antimicrobials including azithromycin, ceftriaxone, cefixime, ciprofloxacin, penicillin, spectinomycin, and tetracycline.

Since 2010, the PHABM Program continues to see trends of overall decreasing minimum inhibitory concentrations (MICs) for cefixime, ceftriaxone and azithromycin (Figure 6). These trends have been consistently lower than national trends as reported by Martin et al, 2015. In 2014, 0% of isolates demonstrated decreased susceptibility to cefixime (MIC  $\geq 0.25$   $\mu\text{g}/\text{mL}$ ) and 0.4% of isolates demonstrated decreased susceptibility to ceftriaxone (MIC 0.125  $\mu\text{g}/\text{mL}$ ). Azithromycin resistance was seen in 0.8% of isolates (MIC  $\geq 2.0$   $\mu\text{g}/\text{mL}$ ).

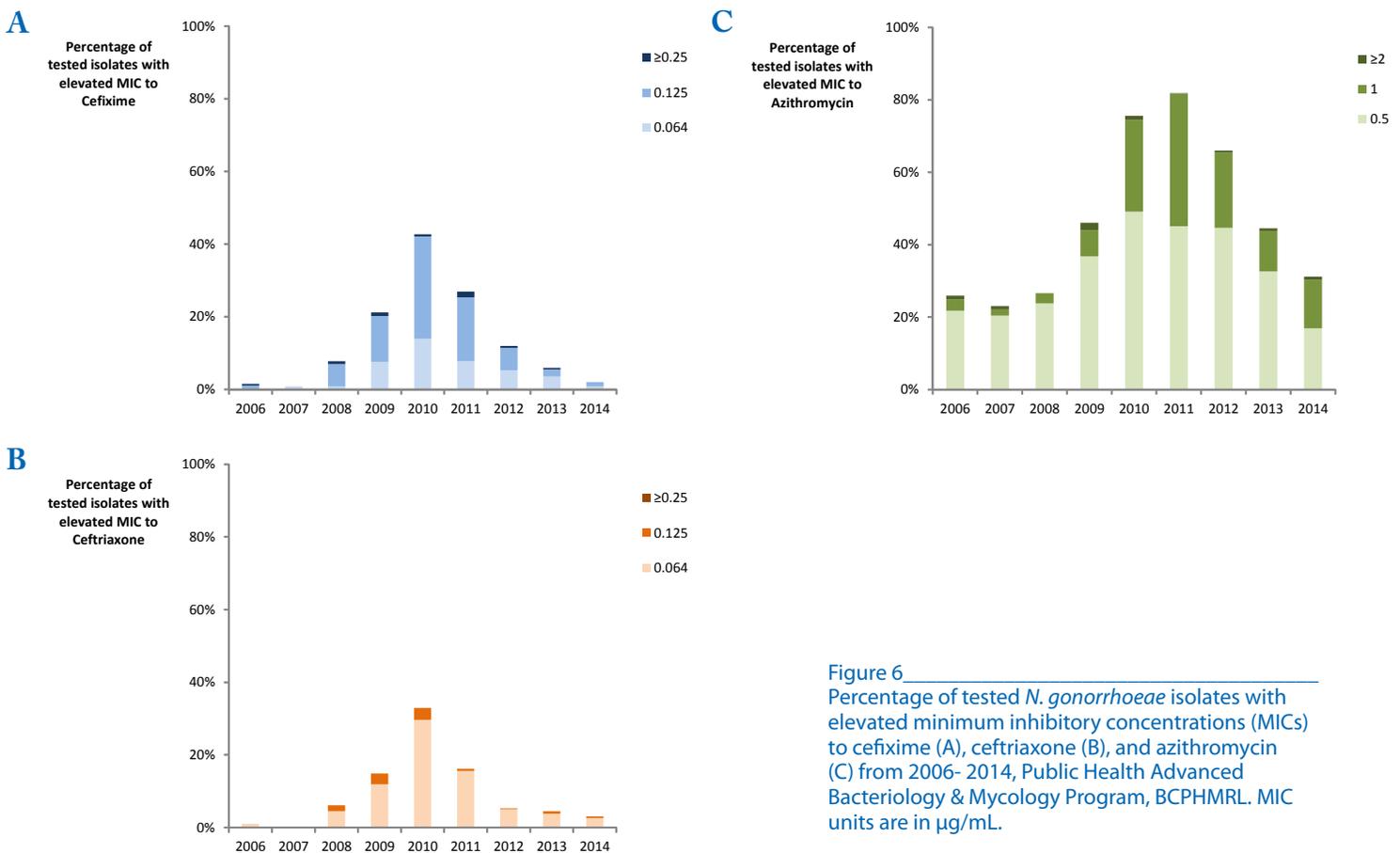


Figure 6 Percentage of tested *N. gonorrhoeae* isolates with elevated minimum inhibitory concentrations (MICs) to cefixime (A), ceftriaxone (B), and azithromycin (C) from 2006- 2014, Public Health Advanced Bacteriology & Mycology Program, BCPHML. MIC units are in  $\mu\text{g}/\text{mL}$ .

### Reference

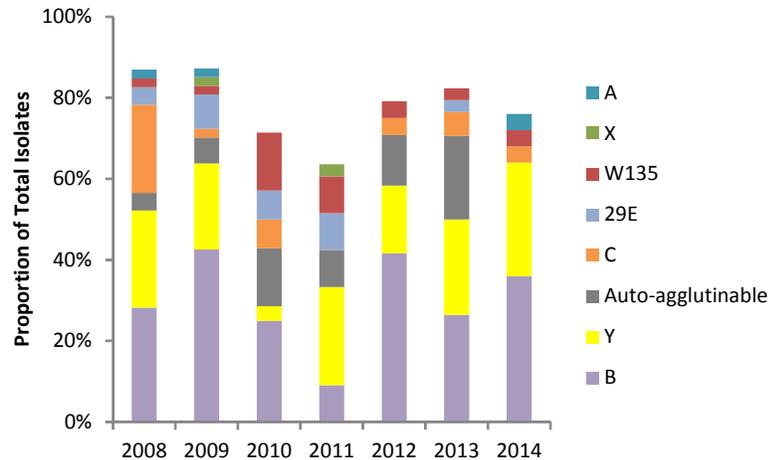
Martin I, Sawatzky P, Liu G, Mulvey MR. Antimicrobial resistance to *Neisseria gonorrhoeae* in Canada: 2009-2013. *CCDR*. 2015; 41(2): 35-41.

## Neisseria meningitidis Susceptibility Trends

*Neisseria meningitidis* is an aerobic Gram-negative encapsulated bacterium whose invasive form can cause meningococcal disease, resulting in meningitis and/or septicaemia. Thirteen serotypes distinguished by differences in immunologic reactivity of the polysaccharide capsule of *N. meningitidis* have been described. Five of these serogroups (A, B, C, Y and W135) account for the majority of meningococcal disease in Canada.

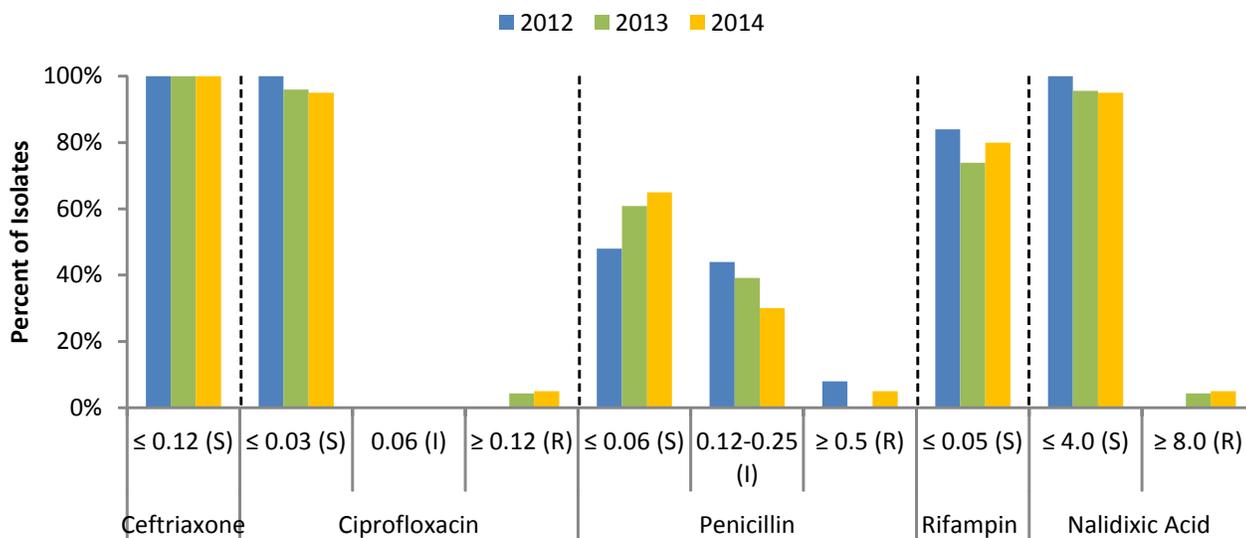
The BCPHMRL PHABM Program performs *N. meningitidis* serotyping using sero-specific antisera after confirming isolates submitted by microbiology laboratories in the province. In BC, serogroups B and Y cause the majority of infections (Figure 7). In 2014, 36% of *N. meningitidis* serotyped belonged to serotype B and Y cause the majority of infections (Figure 7). In 2014, 36% of *N. meningitidis* serotyped belonged to serotype B while 28% were serotype Y.

Figure 7  
*N. meningitidis* serotyping results, 2008-2014, Public Health Advanced Bacteriology & Mycology Program, BCPHMRL.



The BCPHMRL PHABM Program also evaluates *N. meningitidis* isolates for antimicrobial susceptibility by E test® to ceftriaxone, ciprofloxacin, penicillin, rifampin and nalidixic acid. Resistant isolates have been observed for ciprofloxacin in 2013 (4%) and 2014 (5%), for penicillin in 2012 (8%) and 2014 (5%), and for nalidixic acid in 2013 (4%) and 2014 (5%). Penicillin-intermediate isolates have also been seen in 2012 (44%), 2013 (39%) and 2014 (30%) (Figure 8).

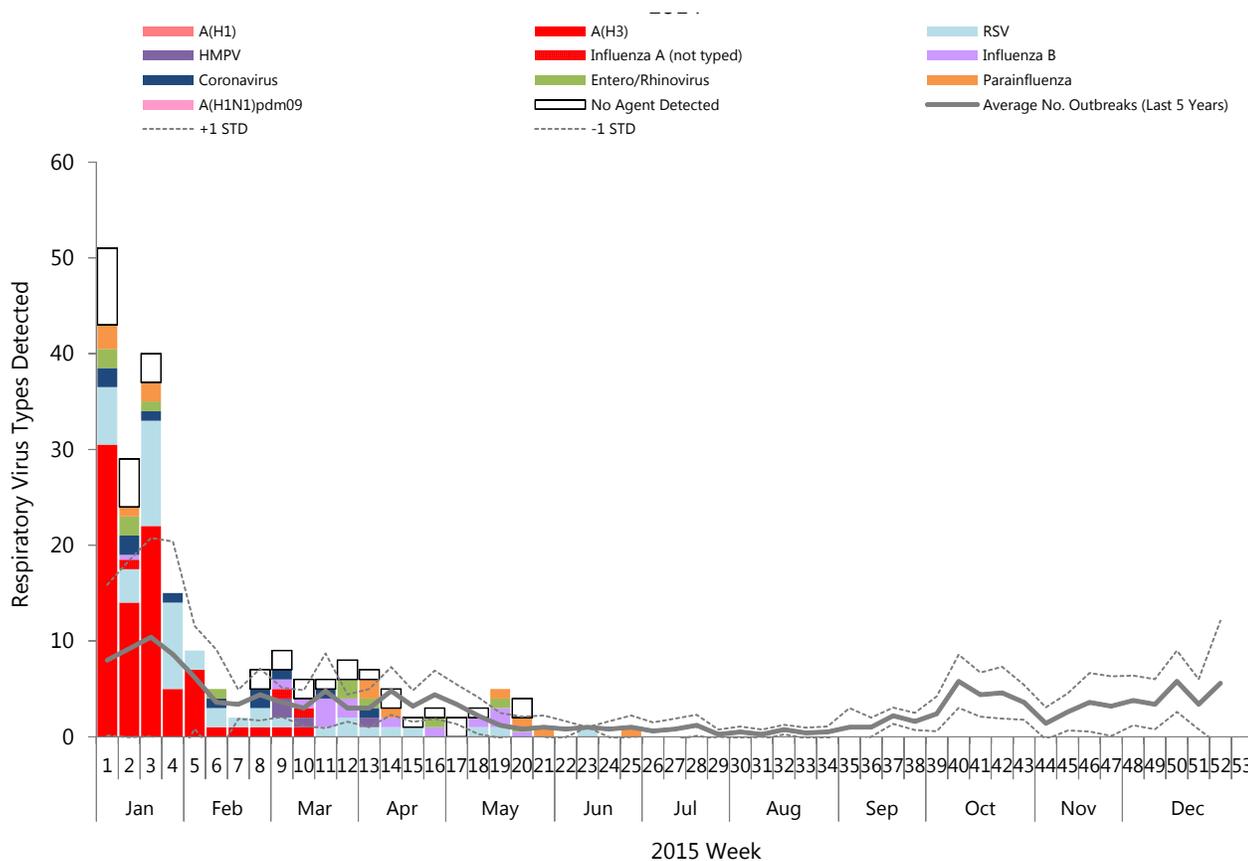
Figure 8  
*N. meningitidis* susceptibility results, 2012-2014, Public Health Advanced Bacteriology & Mycology Program, BCPHMRL. Antimicrobial MIC breakpoints are identified as S=susceptible, I=intermediate, and R=resistant.



## Influenza-Like Illness Outbreaks

In May and June, the Virology Program investigated 15 influenza-like illness outbreaks (Figure 9). This was on the higher end of historical trends in some weeks in May, but decreased to expected, lower levels in June. During this time, outbreaks occurred at 12 (80%) longterm care facilities with the following detected: 25% (3) respiratory syncytial virus (RSV), 25% (3) parainfluenza virus, 17% (2) influenza B and 17% (2) entero/rhinovirus. There were three (20%) hospital-related outbreaks where influenza B and parainfluenza viruses were detected at two separate facilities.

Figure 9  
Influenza-like illness outbreaks investigated\* in 2015, Virology Program, BCPHMRL.

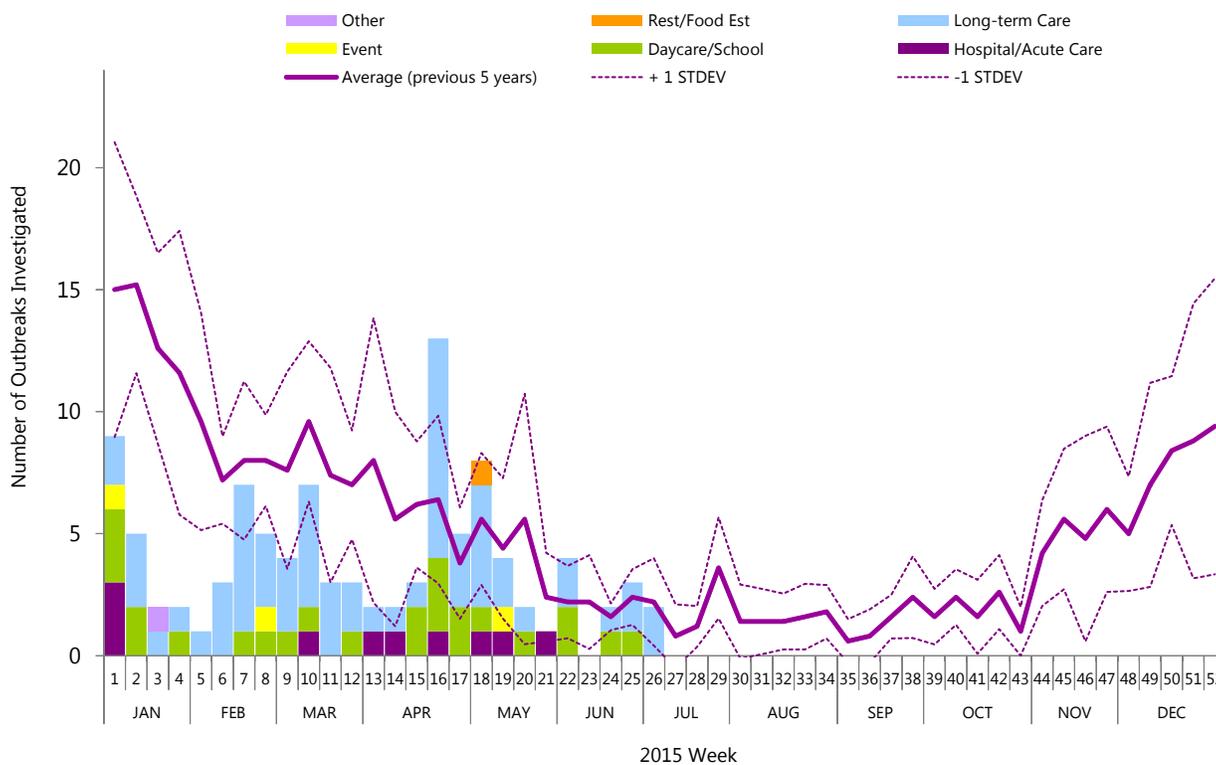


\* The data available are from outbreaks in which the BCPHMRL has been notified. Some acute care microbiology laboratories are also testing for influenza in the province.

## Gastrointestinal Outbreaks

In May and June, there were 26 gastrointestinal outbreaks investigated by the BCPHMRL (Figure 10). The number of outbreak investigations were at the high end compared to historical averages at the beginning of May and then decreased to average historical levels by the end of June (Figure 10). Outbreaks were investigated from 15 (58%) LTC facilities, 6 (23%) daycares/schools, and three (11%) hospitals one event (4%) and one restaurant (4%). Samples were received from 22 (85%) of these outbreaks with norovirus detected in 16 (73%). Sapovirus was also detected in two (9%) outbreaks while rotavirus was detected in another (5%). Three (14%) outbreaks revealed unknown etiologies.

Figure 10  
Gastrointestinal outbreaks investigated\* in 2014, Environmental Microbiology, Public Health Advanced Bacteriology & Mycology, Parasitology and Virology Programs, BCPHMRL.



\* The data available are from outbreaks in which the BCPHMRL 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 BC Public Health Microbiology & Reference Laboratory, Vancouver, BC

The BC Public Health Microbiology Reference Laboratory (BCPHMRL) 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 PHMRL 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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