

# LABORATORY TRENDS



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A Report from the BCCDC Public Health Laboratory



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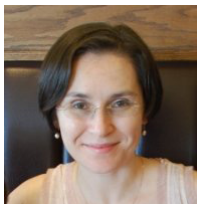
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# Welcome Dr. Sekirov Congratulations Dr. Morshed

## Welcome to Dr. Inna Sekirov!



Dr Inna Sekirov will be joining the BCCDC Public Health Laboratory (PHL) in August. She completed her Medical Microbiology Residency Training,

here at the University of British Columbia (UBC) and passing her exams to officially become a Fellow of the Royal College of Physicians of Canada. Congratulations, Inna! Prior to her residency, Dr. Sekirov also held a MD/PhD. Her PhD thesis was on the role intestinal microbiota plays in the progression of *Salmonella* gastroenteritis. During her residency she has spent much time working at the BCCDC PHL, Vancouver General Hospital, BC Children's Hospital and other microbiology laboratories in the Lower Mainland. She has published on multi-drug resistant organisms, intestinal microbiota and virulence factors of enteric pathogens.

We are thrilled to have to have Dr. Sekirov join our team! She will be the program head for Mycobacteriology/TB. She will also assist with other clinical services at BCCDC PHL. We look forward to her contributions with her interest in molecular diagnostics, genomics and research experience.

## RBC Top 25 Immigrant Award

The votes are in! After being shortlisted as one of 75 finalists, Dr. Muhammad Morshed has been chosen as one the 2017 RBC Top 25 Immigrants! This peoples' choice award recognizes immigrants who have made a positive difference in Canada and celebrates the efforts and actions of immigrants achieving success in their new homeland.

Dr. Morshed has been with the BCCDC PHL for over 20 years and has provided leadership as program head for both the Zoonotic Diseases & Emerging Pathogens and Parasitology Programs. He is also a clinical professor at UBC and is recognized nationally and internationally for his expertise in spirochetal infections, particularly Lyme disease and syphilis.

We are very proud of Dr. Morshed's achievements and are thrilled that he is being recognized across the country! Dr. Morshed will be donating his winnings to UNICEF Canada to support education for children.

**“ Being recognized as one of the RBC Top 25 Immigrant Award recipients this year was a great honour and big surprise for me. This recognition came as a result of your overwhelming support and I thank you from the bottom of my heart. ”**

Dr. M. Morshed



# New gastrointestinal panel Meningococcal nomenclature update

## Testing for gastrointestinal viruses in stool and vomitus

BCCDC PHL has implemented a new nucleic acid test (NAT) for gastrointestinal (GI) viruses in stool and vomitus. Previously, norovirus (genogroup I and genogroup II) and sapovirus were tested by NAT, while rotavirus and adenovirus were tested by enzyme immunoassay (EIA). There was no testing for astrovirus. To improve detection of GI viruses, sapovirus NAT and rotavirus/adenovirus EIA will be replaced by a molecular GI panel capable of detecting sapovirus, adenovirus, rotavirus and astrovirus. The new panel is a laboratory developed test that has been extensively validated.

In most cases, norovirus NAT will be performed first and reflexed to the GI panel if norovirus is not detected. For cases associated with daycare outbreaks, children  $\leq 5$  years old and fecal transplant patients, norovirus NAT and the GI panel will be run concurrently. This assay can be performed on both diarrhea and vomitus samples.

The following patient categories are approved for gastrointestinal viruses testing:

- Gastrointestinal outbreak samples
- Solid organ transplant patients
- Fecal transplant patients
- Hospitalized and ER patients
- Children  $\leq 5$  years old

If there are any questions regarding testing, please contact BCCDC PHL Environmental Microbiology Laboratory at 604-707-2611 or page the Medical Microbiologist on call (604-661-7033).

Testing can be requested using one of the following requisitions:

- a) [BCCDC PHL GI Outbreak notification form](#) and [BCCDC PHL GI Outbreak requisition](#)
- b) [BCCDC PHL Virology requisition](#)

For inpatient results, please refer to CareConnect. Outpatient reporting will continue via Excelleris.

## Update to the meningococcal capsule nomenclature

*Neisseria meningitidis* is the causative agent of meningococcal disease and meningococcal meningitis. The polysaccharide capsule of the bacterium is a key virulence factor, with subtypes distinguished by differences in their antigenic structure. Within these serogroups, six (A, B, C, W, X, Y) are known to cause invasive meningococcal disease. In BC, serogroup B is the most prevalent, followed by serogroup Y.

The BCCDC PHL Advanced Bacteriology/ Mycology laboratory confirms isolates of *N. meningitidis* submitted from frontline microbiology laboratories and performs serogrouping using sero-specific antisera. The isolate is then forwarded on to the National Microbiology Laboratory (NML) for further characterization. The NML has recently updated its reporting to be in line with changes to the nomenclature as proposed by Harrison et al (2013). Serogroup W135 will now be known as serogroup W while serogroup 29E will now be serogroup E. The W135 and 29E serogroup designations were historical labels that supplied no additional information.

References:

1. Harrison OB, Claus H, Ying J et al. 2013. Description and Nomenclature of *Neisseria meningitidis* Capsule Locus. *Emerg. Infect. Dis.* 19(4): 566-573.

# Diagnostic Yield of Reverse Transcription PCR and IgM Serology for Confirmation of Mumps During an Adult Outbreak in British Columbia

Jassem A, Nunn A, Masud S, Hiebert J, Kraiden M, and Naus M. 2017. Association of Medical Microbiology and Infectious Disease Canada/Canadian Association for Clinical Microbiology and Infectious Diseases Annual Conference, Toronto, ON.

In the spring of 2016, a cluster of mumps cases was identified following a mass gathering event in Vancouver. The outbreak spread to other regions within BC and persisted for months. From April 1 to October 31, 168 outbreak-related cases were identified, with 139 confirmed (either laboratory or epidemiologically). Confirmed and probable cases had a median age of 27 years and most (68%) of the 135 lab-confirmed cases had 1-2 documented or undocumented doses of MMR vaccine. The outbreak strain was identified by the NML to be genotype G related to the MuVi/Sheffield.GBR/1.05 strain that is endemic in North America.

The diagnostic utility of laboratory methods was evaluated. A total of 256 samples were collected for any combination of RT-PCR (urine, buccal or both) and IgG/IgM serology. Eighty-eight percent of these were collected within 5 days of symptom onset. Of the 135 lab-confirmed cases, 77% had RT-PCR from buccal swabs, 50% had RT-PCR from urine, 50% had IgM/IgG serology, and 64% were tested by two or more methods. The majority of the 139 confirmed cases (n=99, 71%) were confirmed by buccal/oral swab RT-PCR, with only 10 (7%) confirmed by urine RT-PCR, 14 (10%) confirmed by IgM serology, and 5 (4%) by IgG seroconversion. There was no significant difference in the ability to detect mumps virus by RT-PCR between confirmed cases with and without a history of immunization. Mumps IgM detection was significantly higher in confirmed cases with no history of vaccination than with a history of one or more mumps vaccinations (64% vs. 34%, p=0.02). This study provides further evidence that RT-PCR of buccal specimens has the highest diagnostic yield in a mumps outbreak in a partially vaccinated population.

## Laboratory testing for mumps virus

Clinical specimens are submitted to the BCCDC PHL for serology and RT-PCR. Mumps virus RNA from buccal/oral swabs and urine is detected with an in-house assay targeting the fusion (F) and small hydrophobic (SH) genes. RNA positive samples are then genotyped by SH gene sequencing by the National Microbiology Laboratory (NML).

Through a studentship award provided by the Canadian Foundation for Infectious Diseases, Dr. Jassem is currently supervising a student who is characterizing the outbreak strain using whole genome sequencing to further elucidate transmission dynamics. This study was also supported through funding from the BCCDC Foundation for Public Health with Drs. Richard Harrigan, Jennifer Gardy, and Mel Kraiden as additional co-investigators.

## Update: Current Mumps Outbreak

In February, 2017 the mumps infections once again have resurfaced in the Lower Mainland, and continue to cause sporadic disease in the community today. To date, there have been 122 laboratory-diagnosed cases, with the majority (82%) being adults 20-59 years old. All samples with genotyping performed have been identified as genotype G by the NML. The predominant circulating strains include one that has been related to the endemic strain and another unrelated strain.

# Development and Validation of a Real-Time, Reverse Transcription PCR Assay for the Rapid and Low-cost Genotyping of Hepatitis C Virus Genotypes 1a, 1b, 2, and 3a

Hepatitis C virus (HCV) infection affects millions of people and leads to liver fibrosis, cirrhosis, and hepatocellular carcinoma. Treatment regimen selection requires HCV genotype and genotype 1 subtype (1a or 1b) determination. The BCCDC PHL performs genotyping for HCV and does so by using a commercial line probe assay (LiPA). To meet increasing demands for genotyping, the laboratory investigated alternative, less expensive methods including a laboratory developed, real-time reverse transcription (RT)-PCR genotyping assay for high-throughput screening of the major HCV genotypes and subtypes in North America. Various primer/probe combinations targeting the HCV Core, E1, and NS5B genomic regions were tested. A paired, duplex real-time RT-PCR assay that targets HCV genotypes 1a and 3a in one reaction and genotypes 1b and 2 in another reaction was ultimately validated using samples genotyped by LiPA.

A total of 155 HCV-positive blood specimens tested at the BCCDC PHL were run in parallel with the laboratory developed real-time RT-PCR assay (paired, duplex) and the existing LiPA. Discordant results were tested by sequencing and an alternative PCR assay.

The laboratory developed real-time RT-PCR assay was found to have a 95% overall sensitivity, with individual sensitivity and specificity between 98-100% for genotypes 1a/3a and 85-98% for genotypes 1b/2

respectively. The RT-PCR assay detected mixed HCV genotypes in clinical and spiked samples with no false-positive reactions for rare genotypes 4, 5, or 6. Although this assay detects the major subtypes seen in BC, there will still be a need to detect rare and other genotypes not detected. These findings, along with cost analyses, have been described in a recent publication (1) and presented at the AMMI/CACMID conference (2).

## New HCV Genotyping Algorithm

In July 2017, the combination of this new assay and another commercial NAT assay will replace the LiPA. Where HCV genotyping is requested in the absence of a recent result, plasma samples positive for HCV RNA will be screened by PCR for genotypes 1a, 1b, 2, and 3a using this laboratory developed test and reflexed to the Abbott Realtime HCV Genotype II assay as needed.

The HCV genotype test name has changed to more accurately reflect testing and HCV genotyping reports will contain a revised comment reflecting the type of test used.

### HCV Genotyping Changes:

#### HCV genotype test name

Previously with line probe assay:

HCV Genotyping Innolipa

Now with NAT assays:

HCV Genotyping

#### HCV genotype report comment

Previously with line probe assay:

By line probe assay

Now with laboratory developed NAT assay:

Tested by Laboratory Developed Assay

Now with Abbott NAT assay:

Tested by Commercial Assay

### References:

- Olmstead AD, Lee TD, Chow R, Gunadasa K, Auk B, Krajden M, Jassem AN. 2017. Development and Validation of a real-time, reverse transcription PCR assay for rapid and low-cost genotyping of hepatitis C virus genotypes 1a, 1b, 2, and 3a. *J Virol Methods*. 20; 244:17-22. doi: 10.1016/j.jviromet.2017.02.009
- Lee T, Olmstead AD, Chow R, Gunadasa K, Auk B, Krajden M, Jassem A. 2017. Development and Validation of a Real-Time, Reverse Transcription PCR Assay for the Rapid and Low-cost Genotyping of Hepatitis C Virus Genotypes 1a, 1b, 2, and 3a. Association of Medical Microbiology and Infectious Disease Canada/ Canadian Association for Clinical Microbiology and Infectious Diseases Annual Conference, Toronto, ON.

# The Diagnostic Yield of Nucleic Acid Testing for Acute HIV Infection is Reduced by Fourth Generation EIA Screening

Chahil N, Cook D, Chu K, Mak A, Jassem A and Krajden M. 2017. Association of Medical Microbiology and Infectious Disease Canada/Canadian Association for Clinical Microbiology and Infectious Diseases Annual Conference, Toronto, ON.

Early diagnosis of HIV infection is important in reducing morbidity and mortality and in reducing the onward transmission of the virus. The BCCDC PHL switched from third-generation Enzyme Immunoassay (EIA) screening to fourth-generation EIA screening in June, 2015. Fourth-generation HIV assays can detect seroconversion earlier than third-generation HIV assays because they can detect both the HIV p24 antigen as well as anti-HIV antibodies. The goal of this study was to evaluate whether fourth-generation screening had an impact on the rate of acute and early HIV diagnosis by comparing the outcome of acute HIV infection (AHI) during the 15 month period before and after switching to the fourth-generation EIA screening from third-generation EIA screening for HIV.

Figure 1. BCCDC PHL algorithm for HIV testing.

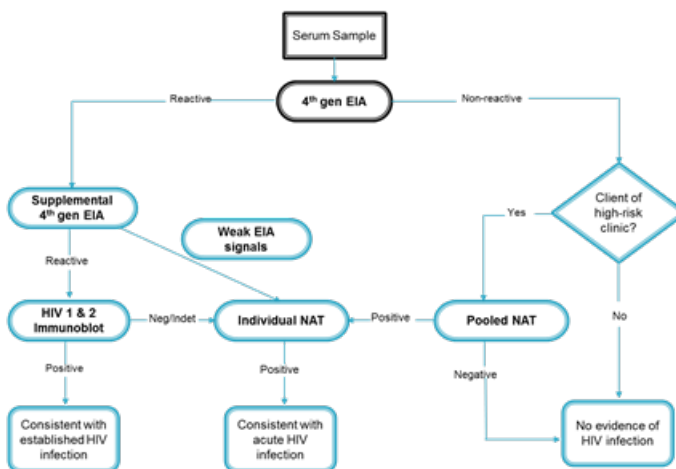
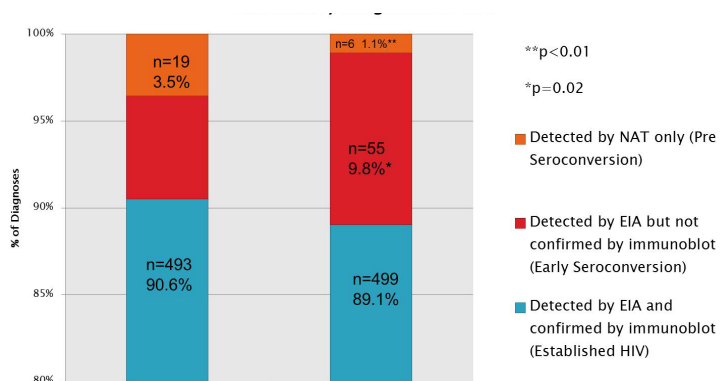


Figure 1 depicts the algorithm for HIV testing at the BCCDC PHL. Samples are screened using the fourth-generation EIA test and reflexed to another supplemental fourth-generation EIA assay when reactive. Acute infection with HIV is then confirmed by either HIV 1 & 2 Immunoblot (EIA reactive) or nucleic acid testing (NAT) (EIA prenatal non-reactive / indeterminate). For screen negative samples, (24) samples from high risk individuals are pooled for NAT and if positive, proceed to individual NAT. Infection stage can be further characterized by the following:

- Pre-seroconversion AHI: EIA negative and HIV 1 NAT positive
- Early seroconversion AHI: EIA positive, HIV Immunoblot negative or indeterminate and HIV 1 NAT positive
- Established infection: EIA positive and HIV Immunoblot positive

A total of 403,092 and 478,693 specimens were screened by EIA during the two periods, respectively. A total of 499 and 553 pooled NAT were performed (representing ~3% of samples). HIV positivity rates were found to be comparable between the two periods in the study (0.13% pre fourth-generation

Figure 2. HIV diagnosis by EIA, immunoblot and NAT for 15 week period pre fourth-generation implementation (Period 1) and 15 week period post fourth-generation implementation (Period 2).



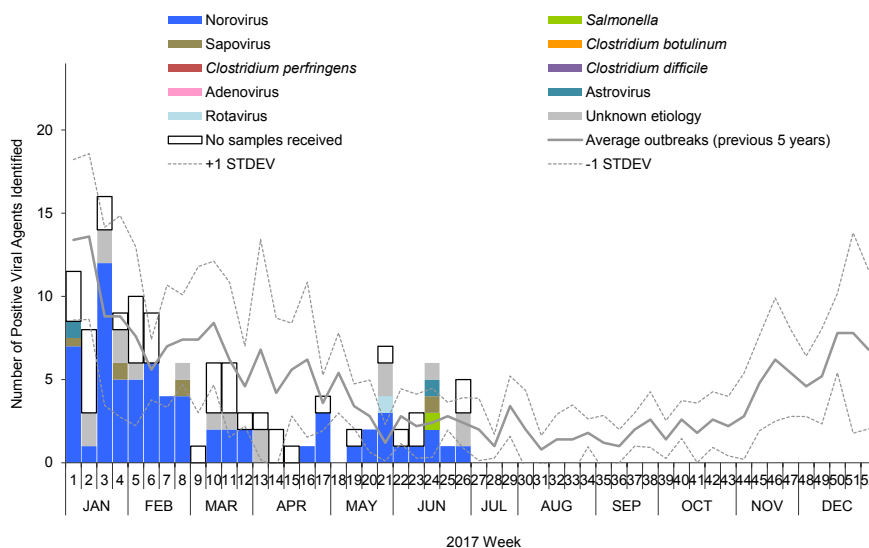
implementation vs. 0.12% post fourth-generation implementation). The percentage of samples with established HIV infection was consistent between the two periods (90.6% pre and 89.1% post) (Figure 2). Post fourth-generation EIA implementation yielded higher numbers of early seroconverters where confirmation was made by NAT rather than immunoblot (9.8% vs. 5.9%) and decreased numbers of pre seroconverters with negative

EIA and NAT confirmation (1.1% vs. 3.5%). Effectively, HIV detection by EIA increased with the implementation of the fourth-generation instruments (98.9%) compared to detection by third-generation EIA (96.5%). Concomitantly, there were fewer diagnoses by pooled NAT and confirmed by individual NAT in the period following implementation. Decreased usage of NAT for pooling and confirmation may ultimately result in lowered costs.

## Gastrointestinal outbreaks

There were 16 gastrointestinal (GI) outbreaks investigated by the BCCDC PHL in June (Figure 3). Outbreaks were investigated from eight (50%) longterm care (LTC) facilities, five (31%) daycares/camps, one (6%) restaurant/food establishment, one (6%) correctional facility (6%) and one event. Samples were received from 11 (69%) of these outbreaks with norovirus detected in five (45%) (from four LTC facilities and one daycare). Sapovirus was detected from a patient at a LTC facility, astrovirus was detected from a participant at an event and *Salmonella* was detected from the restaurant/food establishment outbreak. So far in 2017, there have been 128 GI outbreaks investigated with the majority (47%) reported from LTC followed by daycares/schools (23%).

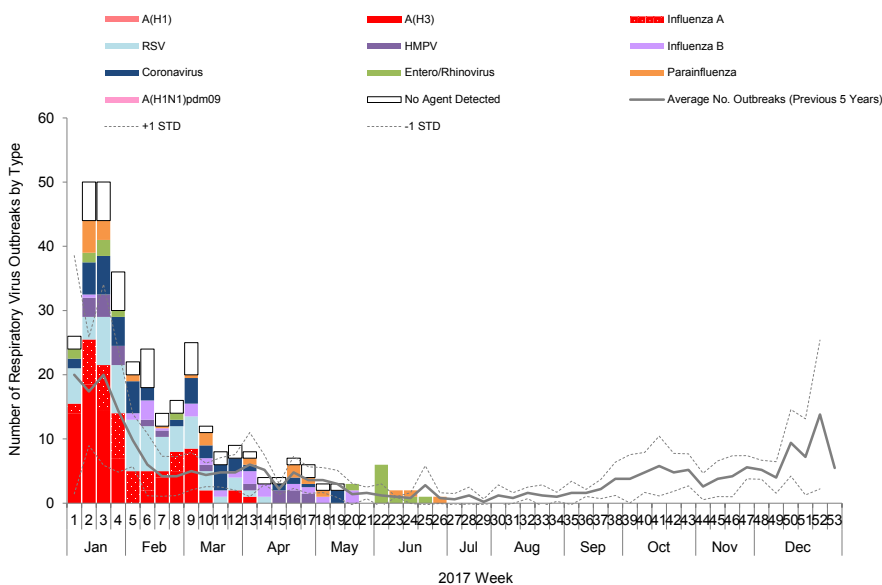
**Figure 3.** Gastrointestinal outbreaks investigated\* in 2017, Environmental Microbiology, Public Health Advanced Bacteriology & Mycology, Parasitology and Virology Programs, BCCDC PHL. The data available are from outbreaks in which the BCCDC PHL has been notified. Some acute care microbiology laboratories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



## Respiratory outbreaks

In June, there were 9 influenza-like-illness (ILI) outbreaks investigated at LTC facilities across the province (Figure 4). These outbreaks have been due to entero/rhinovirus (9; 67%) and parainfluenza (3; 33%). This is a shift in the circulating viruses in the preceding months where influenza B, coronavirus and respiratory syncytial virus dominated in facility outbreaks. So far this season, the Virology Program has investigated 346 ILI outbreaks with the bulk of the outbreaks from January-February, at the height of the respiratory season.

**Figure 4.** 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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