



LABORATORY TRENDS



March 22, 2012

Laboratory News

Next-Generation Sequencing at the PHMRL

The Public Health Microbiology & Reference Laboratory (PHMRL) and BCCDC has acquired an Illumina MiSeq next-generation sequencer through the BCCDC Foundation. Unlike traditional Sanger-based sequencing, Next Generation Sequencing (NGS) is able to generate millions of sequences in hours. NGS provides a flexible platform for amplicon (16S), whole genome shotgun and metagenomics sequencing. Depending on the size of the genomes analyzed and the research question, multiple samples (up to hundreds) can be processed in a single sequencing run. This in-house sequencing capacity thus opens up a world of opportunities for sequencing projects, enabling work that previously would have required external submission and several months of waiting for results to come back.

Recently, the MiSeq completed its first successful sequencing run on previously sequenced TB samples from our own collection. These data will be used to validate the instrument and sequence results. Initial assessment of the sequence quality is very positive and the overall process from library construction to sequencing and data processing took the Molecular Microbiology & Genomics Laboratory 3 days to complete. In one run, near-complete draft genomes for 5 TB isolates were generated.

Upcoming sequencing projects scheduled for the MiSeq includes analyzing other microbial pathogens such as *Clostridium botulinum* and *C. difficile*, and applying it for use in the PHMRL [Genome BC Watershed Metagenomics Project](#). While most of the sequencing for the watershed

project will still be done by the Genome Sciences Centre (GSC), the MiSeq will be utilized to validate the sample preparation step prior to submission to the GSC.

Having in-house sequencing capability means that the PHMRL can ask more genomic driven questions of the bacterial, fungal and viral organisms that affect our health. It will also help ensure that the PHMRL is on the cutting edge of microbial genomic research.



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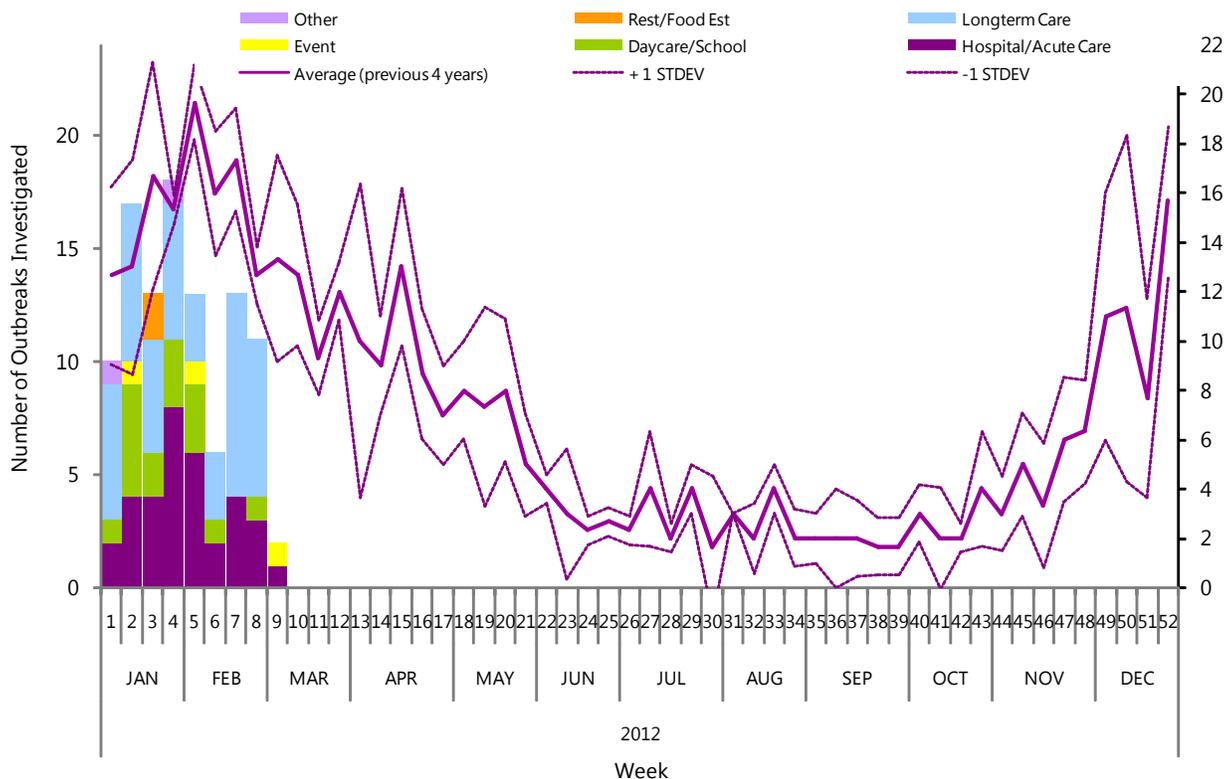


Gastrointestinal Outbreaks

In February, there were 34 gastrointestinal (GI) outbreaks investigated at the PHMRL. There were fewer outbreaks in weeks 5 and 6 compared to this time in previous years (Figure 1). Outbreaks were identified from 17 longterm care facilities, 14 hospitals, 3 daycares/schools, and 1 event. Samples for laboratory testing were submitted for 29 (85%) of these outbreaks. Of these, norovirus was confirmed in 23 (68%) outbreaks (48% in longterm care facilities, 48% in hospitals and 4% in daycares/schools). Rotavirus was confirmed in 1 daycare/school.

The data available are from outbreaks in which the PHMRL has been notified. Some acute care microbiology laboratories are also testing for norovirus in the province and these data do not include outbreaks from Vancouver Island Health Authority. Given the nature of GI outbreaks, samples are not always available for testing.

Figure 1
Gastrointestinal outbreaks investigated since January, 2012, Environmental Microbiology, Bacteriology & Mycology, Parasitology and Virology Programs, PHMRL.



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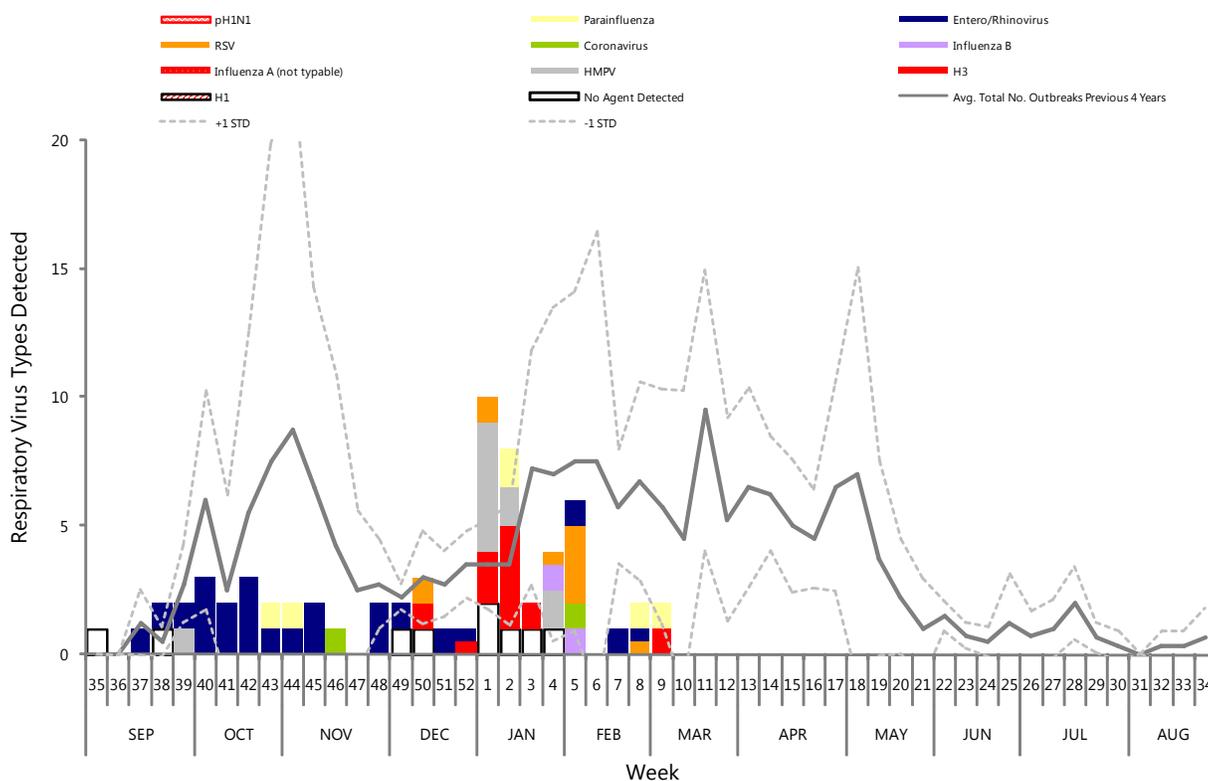
Vancouver, BC



Respiratory Outbreaks

In February, samples were submitted to the PHMRL for 11 respiratory outbreak investigations from longterm care facilities. The number of outbreaks investigated was higher in the first two weeks of the month than this time in previous years; however, the last two weeks of the month saw fewer outbreaks than the average in previous years. Using PCR and Luminex methods, RSV was detected in 5 facilities, entero/rhinovirus detected in 3 facilities, parainfluenza were detected in 2 facilities and coronavirus detected in another (Figure 2). Figure 2 reflects respiratory sample results submitted for investigation to the PHMRL and is not representative of respiratory outbreaks in the entire BC community.

Figure 2
Respiratory outbreaks investigated by respiratory season, Virology Program, PHMRL.



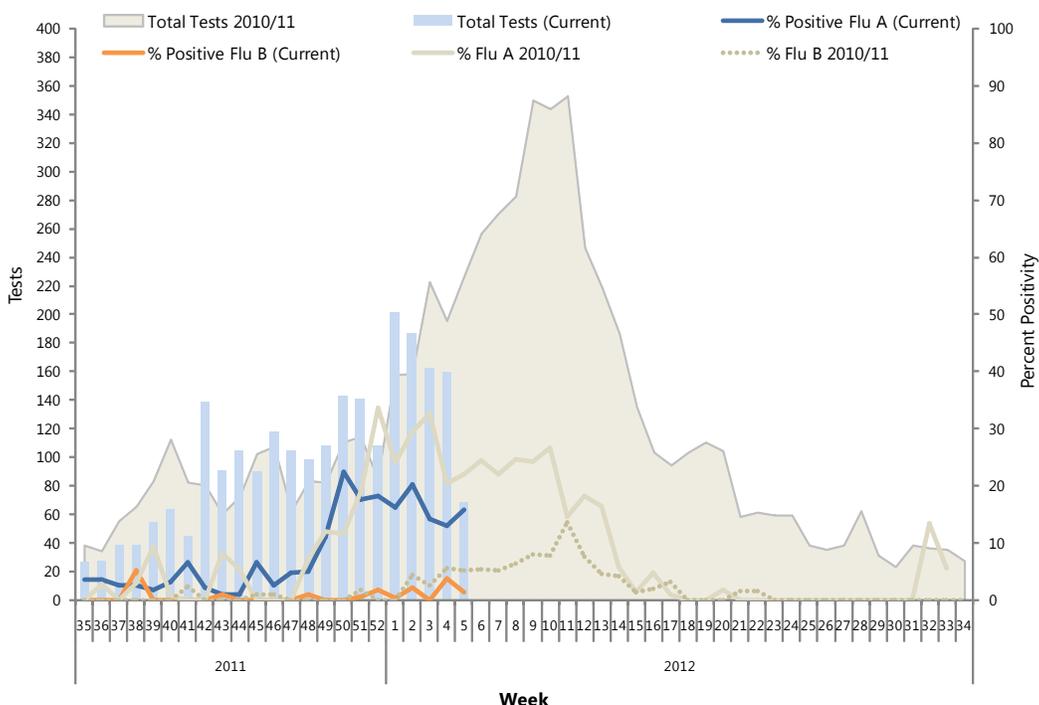


Influenza Surveillance

Volumes for respiratory testing in weeks 6-9 in the 2011/12 season continue to be below that of the same weeks from the 2010/11 season; positivity rates for both influenza A and B are also below what was seen the previous year (Figure 3).

In weeks 6-9, influenza positivity rates varied from nearly 13-23% (Table 1). Influenza A (H3N2) was the major virus type detected this period with 82 (12.1%) positive specimens; there were also 23 (3.4%) detections of (H1N1)pdm09, an increase from January. Influenza B continues to be detected at low levels with 14 (2.1%) detections this period.

Figure 3 Respiratory testing volumes and influenza percent positivity by week, 2011/12, Virology Program, PHMRL.



The PHMRL is seeing a mix of respiratory viruses detected from respiratory specimens. Rhino/enteroviruses dominated the beginning of the season with lower levels of other viruses detected (Figure 4). Levels of rhino/enterovirus have now decreased and stabilized in the last few weeks while other viruses such as RSV and human metapneumovirus has been increasing in positivity. There have also been spikes of increased positivity for parainfluenza during this season while adenovirus has been present at low levels.

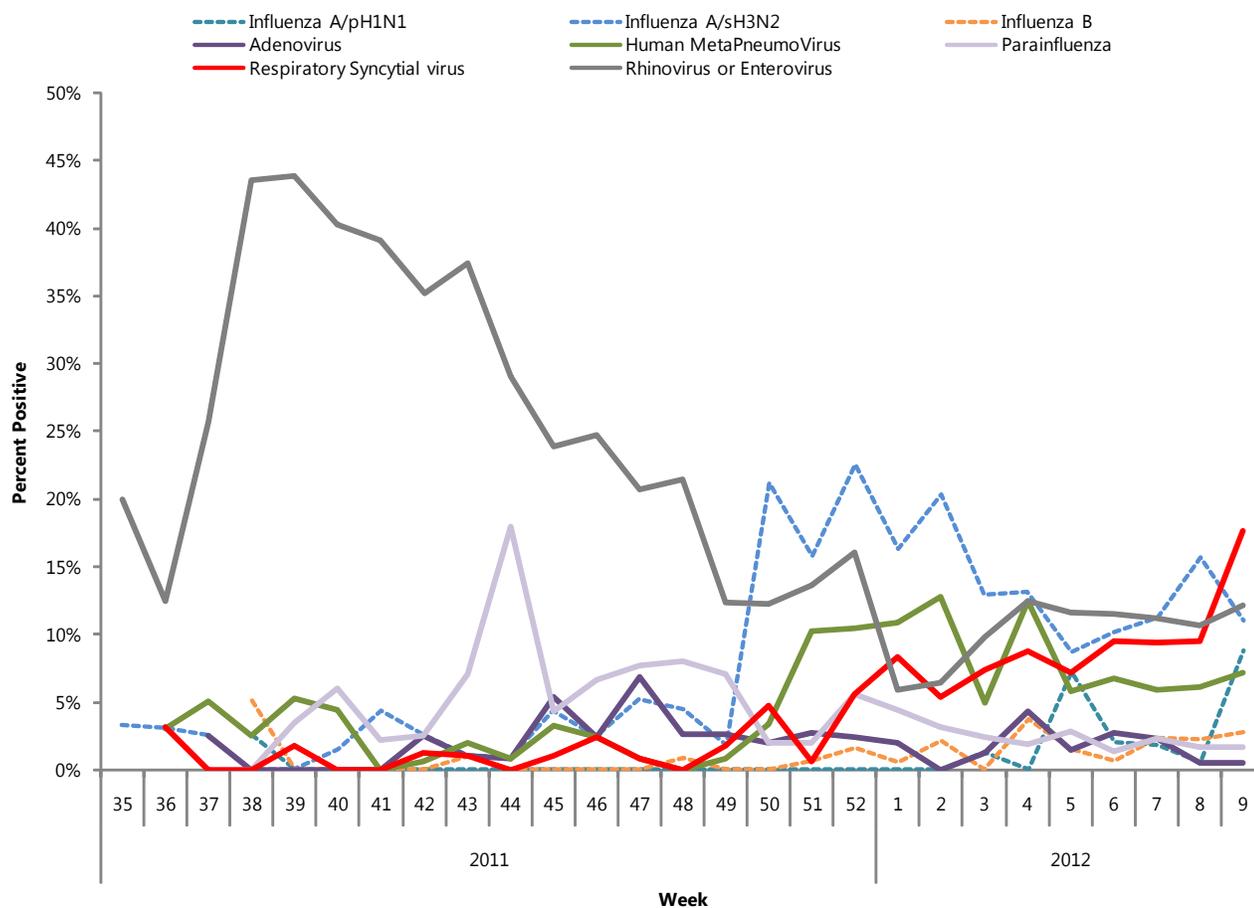
Table 1 Positive influenza A and B detections for weeks 6-9 (February 5 - March 3, 2012, Virology Program, PHMRL. (H1N1)pdm09 refers to the 2009 influenza A(H1N1) pandemic virus.

	Week 6	Week 7	Week 8	Week 9
Number of Specimens Tested	147	169	177	184
Number of Positive Specimens	19 (12.92%)	26 (15.39%)	33 (18.64%)	42 (22.83%)
Influenza A	18 (12.24%)	22 (13.02%)	29 (16.38%)	37 (20.11%)
(H1N1)pdm09	3 (2.04%)	3 (1.77%)	1 (0.56%)	16 (8.70%)
sH3N2	15 (10.20%)	19 (11.24%)	28 (15.82%)	20 (10.87%)
Not typeable				1 (0.54%)
Influenza B	1 (0.68%)	4 (2.37%)	4 (2.26%)	5 (2.72%)



Influenza Surveillance continued

Figure 4
Percent positivity of respiratory viruses by week, 2011/12, Virology Program, PHMRL.



National influenza trends are generally seeing low to moderate levels of activity. The Prairies have seen rates of 10-13% influenza A while the other provinces have less than 8% influenza A positivity. Influenza B is highest in Ontario with rates being from 6-8% positivity and seeing an increase in Quebec (3-6%) and the Atlantic Provinces (2-6%).

The World Health Organization (WHO) reports that there is low but increasing activity in North America and most of Europe. With the exception of Mexico where influenza A(H1N1)pdm09 is the predominant subtype and China where influenza type B is predominant, the most common subtype circulating in the temperate, northern hemisphere continues to be influenza A(H3N2). The vaccine strain selection committee met in February and recommended changes in the northern hemisphere vaccine formulation for the next respiratory season (WHO, 2 Mar 2012 Update).



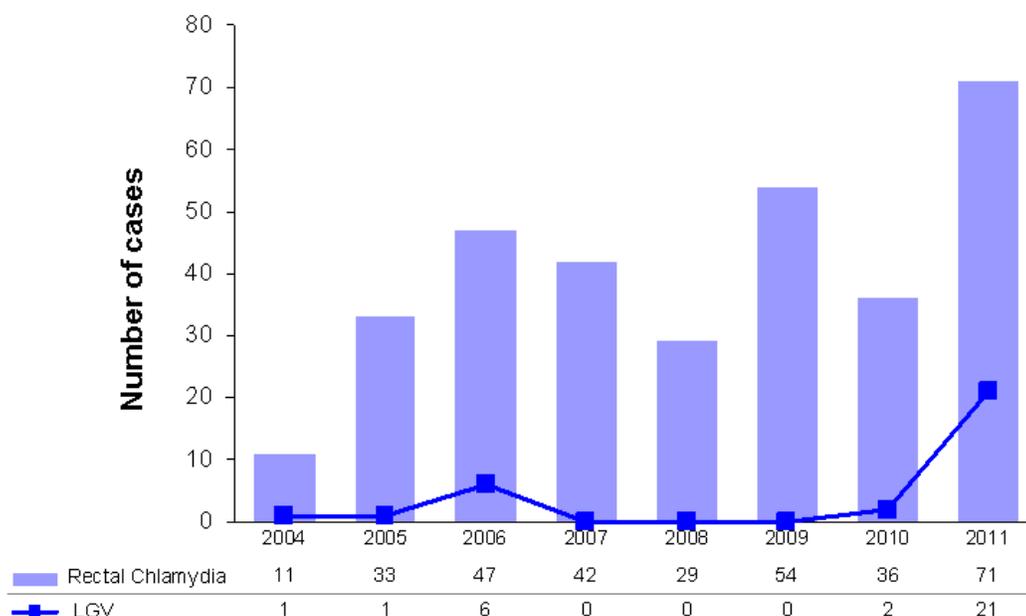
Surveillance for *Lymphogranuloma venereum*

Lymphogranuloma venereum (LGV) is an infection of the lymphatic system caused by invasive serovars of *Chlamydia trachomatis* (L1, L2, L3). Previously thought to be rare in Canada, recent outbreaks in Europe and the United States have increased focus on this infection, particularly in the men who have sex with men community where these outbreaks occurred.

Case reporting of LGV in BC started in 2004 with 1 case identified (Figure 5). The main clinical symptom in BC LGV cases has been proctitis which is an unusual presentation for LGV. Following up on rectal chlamydia cases in the province may thus be important for LGV diagnosis. Although there was an increase in the number of rectal chlamydia cases between 2005 and 2010 (29-54 cases/year) there has not been a similar increase in LGV cases. There were 6 cases reported in 2006 and none in 2007-2009. In 2011, 21 cases of LGV were reported.

Currently the most sensitive and specific approach for testing is nucleic acid testing for *C. trachomatis* with follow up testing for LGV serovar. Serology is not available due to poor sensitivity and specificity. In BC, prior to 2011, the PHMRL only forwarded *C. trachomatis*-positive rectal samples to the National Microbiology Laboratory (NML) for LGV testing when requested by ordering physicians. The PHMRL now routinely sends all *C. trachomatis*-positive rectal isolates identified to the NML for serovar analysis by molecular detection and genotyping of the major outer membrane protein (MOMP) PCR products. Since the PHMRL only performs approximately 15-20% of chlamydia testing in the province, increased vigilance is needed in the province to determine if LGV cases are indeed on the rise. This includes recognizing LGV symptomatology, performing routine rectal chlamydia tests when indicated and requesting LGV testing on any suspicious cases of proctitis or suspicious lesions. The PHMRL is working with our partners at Lifelabs and BC Biomedical Laboratories to enhance LGV testing in the province.

Figure 5
Reported cases of rectal Chlamydia (including non-LGV serovars) and Lymphogranuloma venereum (LGV) in British Columbia, 2004-2011, STI/Clinical Prevention Services, BCCDC.



Data provided by: T. Hottes, M. Lindegger, M. Gilbert, R. Lester, M. Imperial, C. Montgomery, G. Ogilvie, L. Hoang

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Vancouver, BC



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