



# LABORATORY TRENDS



July 13, 2012

## Laboratory News

The BC Public Health Microbiology & Reference Laboratory (PHMRL) is committed to continuous quality improvement for better patient care and population health. Consequently, we strive for testing enhancements by improving efficiencies in turnaround times and through new test development and implementation. As with most new tests these days, molecular methods replacing older, slower tests are the means to faster and more sensitive results.

Below are introductions to two new polymerase chain reaction (PCR) tests for Shiga toxinogenic *Escherichia coli* (STEC) and Lyme disease spirochete detection. In addition, the PHMRL has improved on the algorithm for hepatitis C RNA testing by conversion to hepatitis C PCR quantitative (only) testing.

### PHMRL Changes: PCR for the Detection of STEC

The timely diagnosis of STEC is important both for clinical management and effective public health intervention. Infection by STEC often causes bloody diarrhea and may cause hemolytic uremic syndrome (HUS). In the past, most infections with STEC were thought to be caused by *E. coli* serotype O157:H7. However, recent data has shown that roughly half of all STEC infections in BC are caused by serotypes other than *E. coli* O157:H7. The recent 2011 outbreak of *E. coli* O104 originating in Germany highlighted the ability of non-O157:H7 strains to cause outbreaks and HUS. Although most front-line labs in BC are able to detect *E. coli* O157:H7 in stool, the majority are not set up to detect these non-O157:H7 serotypes from stools. Identification of STEC requires demonstrating the ability of the *E. coli* isolate to produce Shiga toxin (usually by vero-cell assay) or by detection of the toxin genes (*stx1*/*stx2*) in fecal samples or culture isolates. For many years, PHMRL has been performing the vero-cell

assay for the detection of STEC toxins in a fecal sample. Although the gold standard method, it is labour intensive, time-consuming and demanding. Results are not available for at least 2-4 days.

The advent of PCR technology for the detection of Shiga toxin genes provides the ability to discriminate between *stx1* and *stx2* gene variants and reduces turnaround time to 1-2 days. Validation of the method has demonstrated improved sensitivity and specificity compared to conventional vero-cell assay. PCR is done either directly from enriched broth culture of stool or from isolates referred in from referring laboratories. If a stool sample is positive by PCR, every attempt is made to isolate the actual *E. coli* strain which harbours the *stx* gene so that the organism may be further characterized and serotyped.

As of June 25, the PHMRL will no longer be performing the vero-cell assay. All submitted stool samples for enteric pathogen testing will be screened using the STEC PCR. Additional isolation of STEC and serotyping will continue to be performed at PHMRL. Any non-O157:H7 *stx* gene-positive isolates will still be forwarded to the National Microbiology Laboratory for confirmation by both PCR and vero-cell assay.

PHMRL's move to using PCR will reduce turnaround times significantly and will improve on the sensitivity and specificity of STEC detection.

### Molecular Method (Polymerase Chain Reaction) for the Detection of Tick Borne Spirochetes

Lyme disease is an emerging vector-borne disease caused by three geno-species of *Borrelia burgdorferi* sensu lato.



In this Issue:	
Laboratory News .....	1
Carbapenemase Resistance .....	3
Gastrointestinal Outbreaks .....	4
Respiratory Outbreaks .....	5
Influenza Surveillance .....	6



*B. burgdorferi sensu stricto* is the most common spirochete responsible for Lyme disease in North America. Certain *Ixodes* ticks carry these spirochetes, and can transmit *B. burgdorferi* when they bite mice or other small mammals. Humans or pets, such as dogs or cats, can also acquire the disease if they are bitten by *B. burgdorferi* infested ticks. Early symptoms may include influenza like illness with erythematous rashes. However, there can be a variety of symptoms ranging from no symptoms to complications with the joints, heart and nervous system. BC data suggests that the Lyme disease rate is very low in the province.

The Zoonotic Diseases and & Emerging Pathogens (ZEP) Program at the PHMRL provides services that support public health programs across BC, including the culture of *B. burgdorferi* from ticks for Lyme disease surveillance since the early 90's. Culture is a very slow and tedious process which relies on highly specialized media. In-house preparation of the culture media is complex and performing quality control is a challenge.

To be aligned with cutting edge technology and to also provide better diagnostic sensitivity, the ZEP Program at BCCDC PHMRL validated a Real Time-PCR method and implemented it at the beginning of June 2012 (Man et al., 2012). Clients can continue to submit tick samples as they do presently for Lyme disease testing. In addition to increased test sensitivity, PCR will now provide a much quicker turnaround time for our clients.

*Reference:* Man S, Lee M-K, Fernando K, Wong Q & Morshed M. Validation of a Real Time PCR Assay for Detecting *Borrelia burgdorferi* in Ticks. Presented at AMMI Canada and CACMID Annual Meeting, May 4, 2012.

## Conversion to Hepatitis C PCR Quantitative (Only) Testing

The Virology Laboratory at the BC PHMRL performs approximately 20,000 hepatitis C (HCV) RNA tests/year. The qualitative HCV RNA test is used to detect active/current HCV infection in patients (17,500 tests/yr) and the quantitative assay is used for predicting and monitoring antiviral treatment response (2,500 tests/yr).

In the past two different HCV RNA tests were required because the qualitative assay was initially more sensitive and less costly than the quantitative HCV RNA. Now that the quantitative and qualitative tests are equally sensitive and cost effective, The Virology Laboratory has adopted a "quantitative only" HCV RNA testing algorithm. This allows the laboratory to provide one test for diagnosis or monitoring which simplifies test accessioning and improves turnaround times.

The sensitivity of the quantitative assay is 10 to 15 IU/ml. There will be four possible test results generated from the quantitative assay:

1. Target not detected which will be reported as: *No HCV RNA detected*
2. 10 to 15 IU/mL which will be reported as: *HCV RNA < 15, HCV RNA detected but below the test linear range*
3. 15 to 43 IU/mL which will be reported as: *25 IU/ mL (example) HCV RNA detected but below the test linear range*
4. 15 to 69,000,000 IU/mL which will be reported as: *1,500,000 IU/mL (example), HCV RNA detected*

We have also added the following comment to notify our clients of the change in testing algorithm and to assist with the result interpretation:

*The qualitative HCV RNA assay used to confirm HCV infection has been replaced by an equally sensitive quantitative assay (detection limit 10 to 15 IU/mL). The magnitude of the HCV RNA viral load is used to predict and monitor treatment response but does not correlate with disease progression.*



## Carbapenemase Resistant Enterobacteriaceae (CRE)

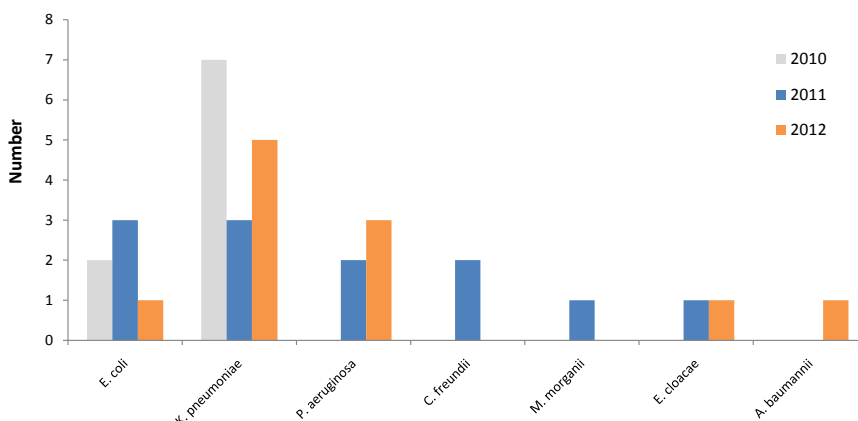
The latest counts for cases of carbapenemase resistance in BC can be found in Table 1 (updated from our June 2012 issue). Eighteen cases with the New Delhi Metallo- $\beta$ -lactamase gene (NDM) endemic to South Asia have been detected since this work began in 2010. Two cases had the *Klebsiella pneumoniae* carbapenem (KPC)  $\beta$ -lactamase gene (one case with KPC as well as a Verona integron-encoded metallo- $\beta$ -lactamase (VIM) gene) and five cases with only the VIM gene. Two cases with the IMP-type  $\beta$ -lactamase has also been detected. So far, NDM-1-producing isolates are the most predominant CRE comprising 66.7% of the cases in 2012, followed by VIM-producing (18.5%) and IMP-producing (7.4%) isolates.

Table 1 Carbapenem Resistant Enterobacteriaceae Detected, Bacteriology & Mycology Program, PHMRL.

Type	No. of Cases	Comments
NDM	18	
KPC	2	1 case also harboured the VIM gene
VIM	5	In addition to above KPC/VIM case
IMP	2	

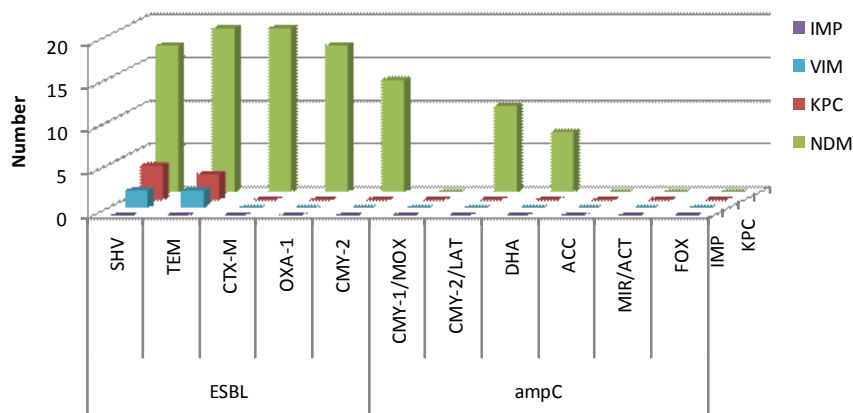
To date, carbapenem resistance has been isolated in a variety of organisms including *E. coli*, *K. pneumoniae*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Morganella morganii* and *Enterobacter cloacae* and *Acinetobacter baumannii* (Figure 1). *K. pneumoniae* has been the most frequently isolated.

Figure 1 Organisms producing carbapenemase resistance genes, isolated since 2010, Bacteriology & Mycology Program, PHMRL.



NDMs have also been associated with a range of ESBL and ampC enzymes, the majority of which produce more than one

Figure 2  $\beta$ -lactamase enzymes and their associated Extended-spectrum  $\beta$ -lactamase (ESBL) and ampC producing enzymes, isolated since 2010, Bacteriology & Mycology Program, PHMRL.



ESBL enzyme (Figure 2). In contrast, KPC and VIM have had fewer correlations with ESBL enzymes and IMP have not produced any ESBL/ampC enzymes to date.

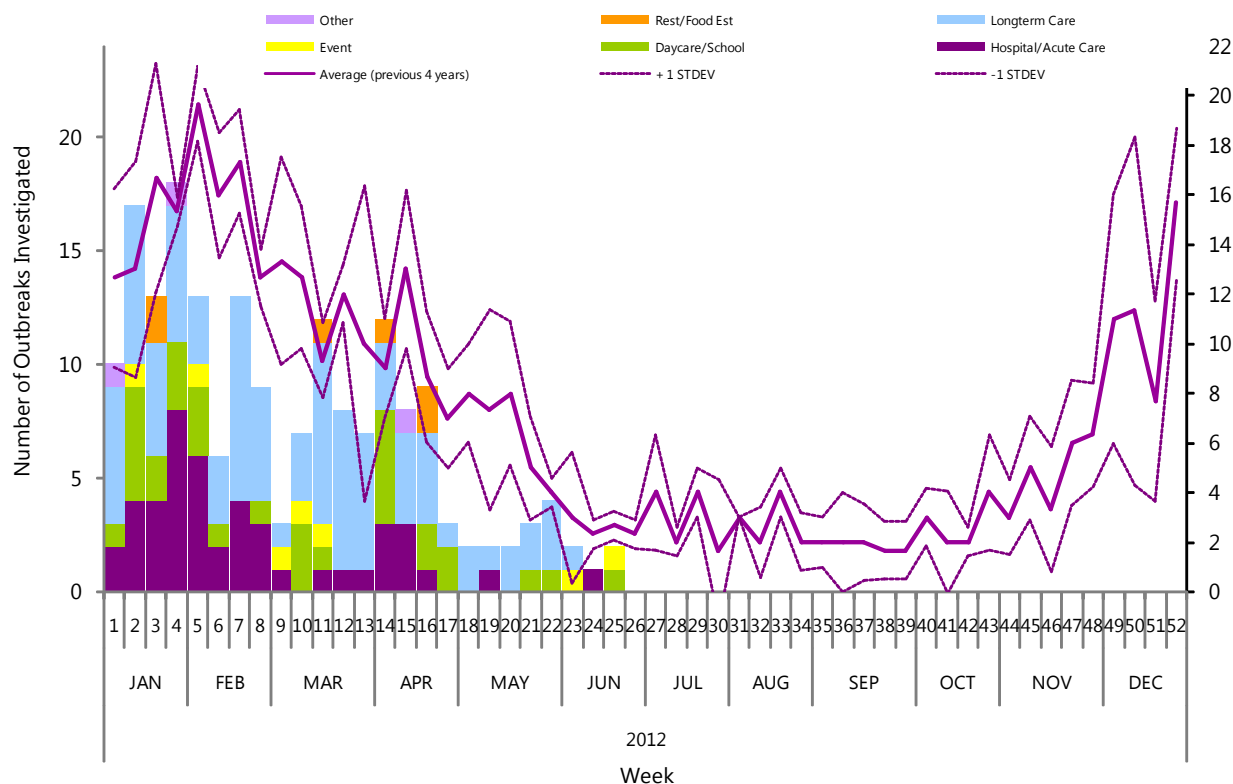


## Gastrointestinal Outbreaks

In June, there were 6 gastrointestinal (GI) outbreaks investigated at the PHMRL. This is consistent with the volumes typically seen at this time of the year when GI outbreaks are fewer (Figure 3). Outbreaks were identified from 2 longterm care facilities, 2 events, 1 daycare/school and 1 hospital. Samples were only submitted for 1 (17%) of these outbreaks and *Clostridium perfringens* was detected.

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 3  
Gastrointestinal outbreaks investigated since January, 2012, Environmental Microbiology, Bacteriology & Mycology, Parasitology and Virology Programs, PHMRL.



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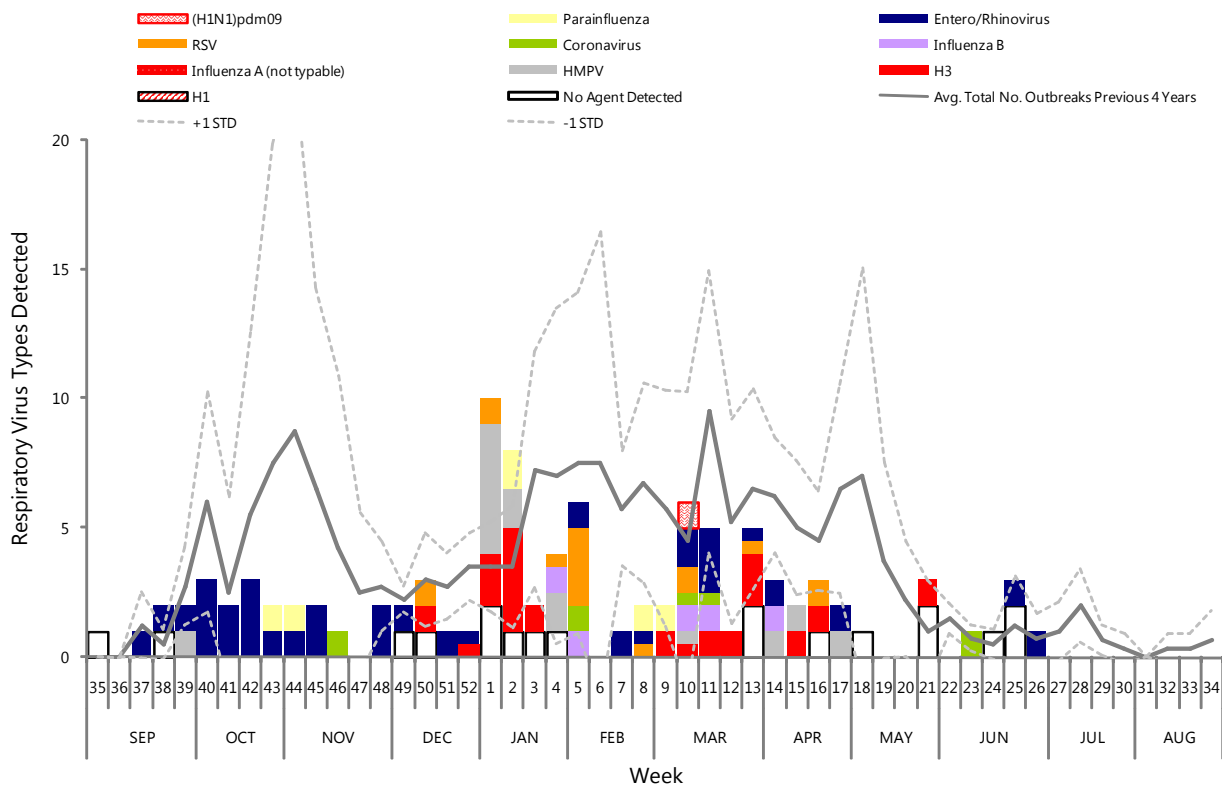
Vancouver, BC



## Respiratory Outbreaks

In June, samples were submitted to the PHMRL for 6 respiratory outbreak investigations from longterm care facilities. The number of outbreaks investigated was on the higher end of what has been previously observed at this time for week 25 (Figure 4). Using PCR and Luminex methods, entero/rhino virus was detected in 2 facilities and corona virus detected in another.

Figure 4  
Respiratory outbreaks investigated in the 2011/2012 respiratory season, Virology Program, PHMRL.

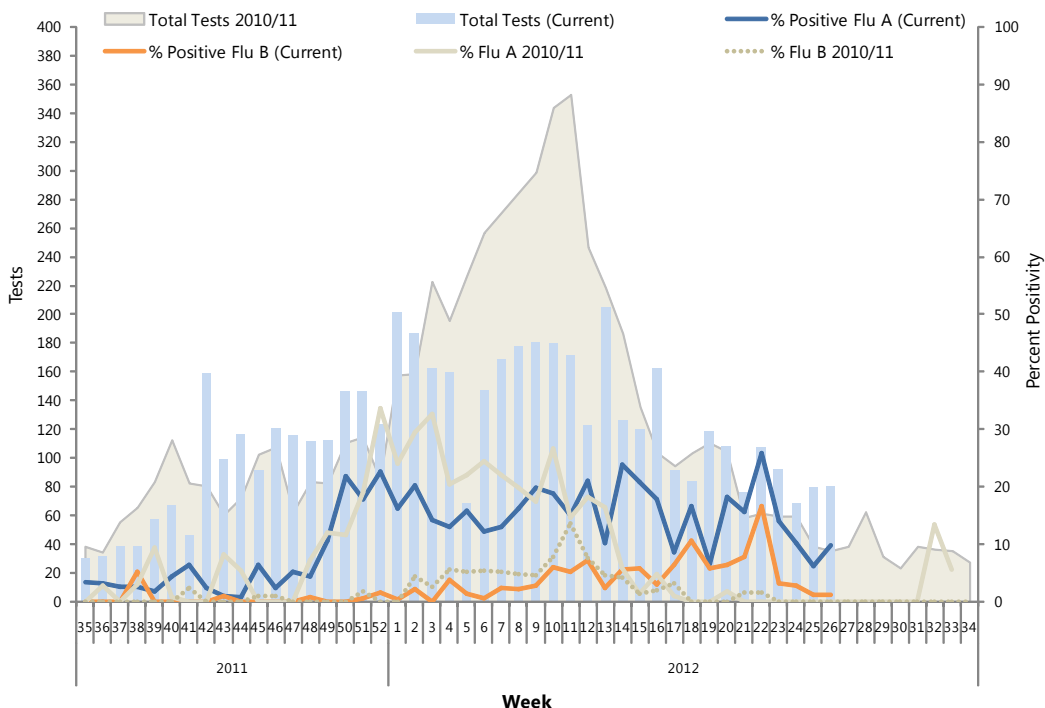




## Influenza Surveillance

Likely reflecting the unseasonably cold June we have experienced, respiratory testing volumes have been above that of the same weeks from the 2010/11 season (Figure 5). Influenza positivity rates have varied from 8-17% in weeks 23-26 (Table 2). Influenza A (H3N2) was the major virus type detected this period with 30 (9.29%) positive specimens, followed by 7 (2.17%) detections of influenza B and 3 (0.93%) detections of (H1N1)pdm09 during this time. Influenza A rates were higher this period (6-14%) compared to this time last season when the influenza season ended in May; influenza B rates have been between 1-3% this week (Figure 5).

Figure 5 Respiratory testing volumes and influenza percent positivity, Virology Program, PHMRL.



Rhino/enterovirus detection has increased in June with positivity from 16-28%. There has been a decrease of parainfluenza (2-7%) and a decrease in RSV (0-3%) this month. Human metapneumovirus and adenovirus are at rates below 4% positivity (Figure 6).

Table 2 Positive influenza A and B detections for weeks 23-26 (June 3- June 30, 2012), Virology Program, PHMRL. (H1N1)pdm09 refers to the 2009 influenza A(H1N1) pandemic virus.

	Week 23	Week 24	Week 25	Week 26
<b>Number of Specimens Tested</b>	<b>93</b>	<b>69</b>	<b>80</b>	<b>81</b>
<b>Number of Positive Specimens</b>	<b>16 (17.20%)</b>	<b>9 (13.04%)</b>	<b>6 (7.50%)</b>	<b>9 (11.11%)</b>
Influenza A	13 (13.98%)	7 (10.14%)	5 (6.25%)	8 (9.88%)
(H1N1)pdm09	2 (2.15%)	1 (1.45%)		
sH3N2	11 (11.83%)	6 (8.70%)	5 (6.25%)	8 (9.88%)
Not typeable				
Influenza B	3 (3.23%)	2 (2.90%)	1 (1.25%)	1 (1.23%)

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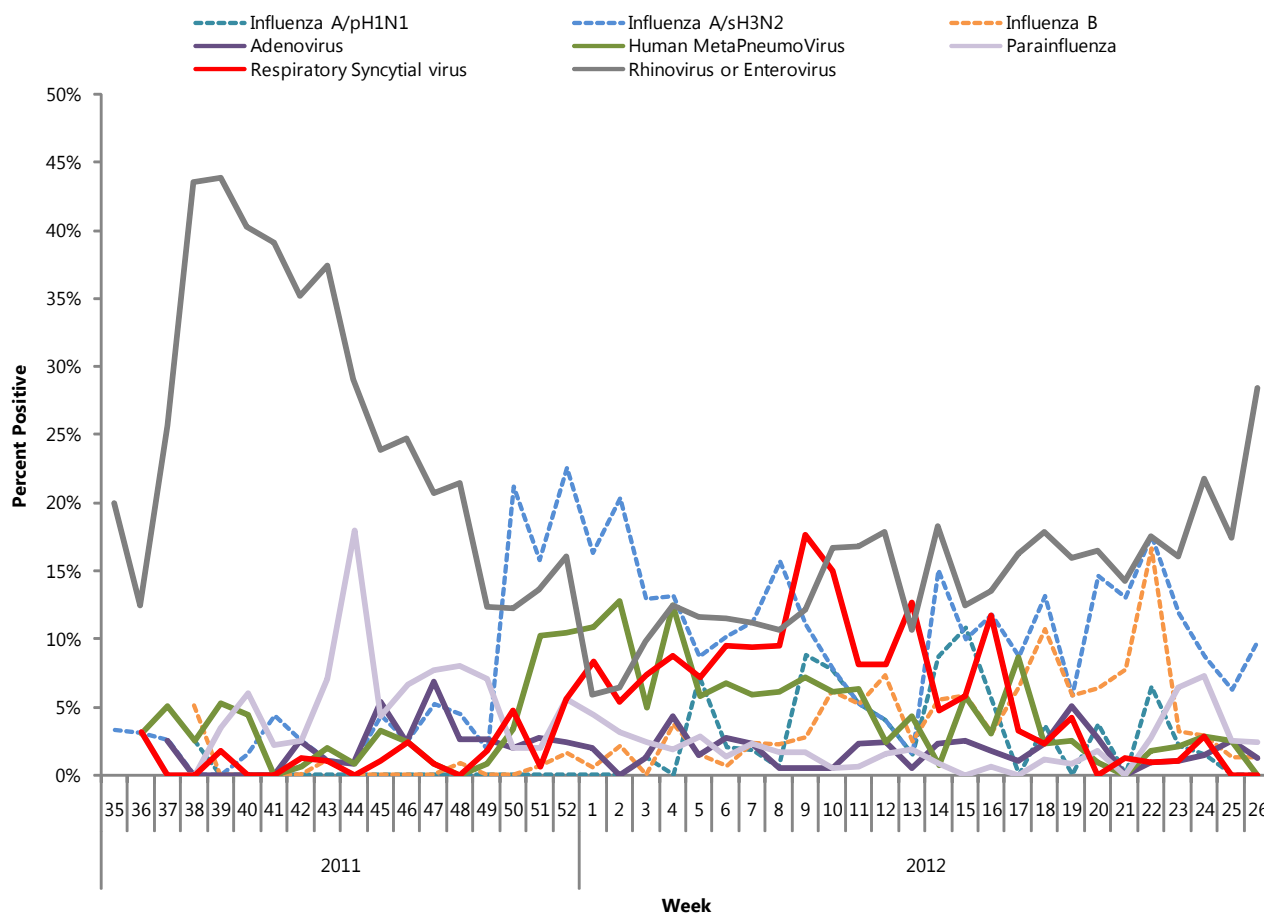
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## Influenza Surveillance continued

Figure 6  
Percent positivity of respiratory viruses by week, 2011/12, Virology Program, PHMRL.



Nationally, there were low levels of influenza activity in June. Aside from BC, rates of influenza A were less than 2% in all provinces. The Atlantic Provinces have not detected influenza A since week 23. Rates of influenza B were 0.4-1.4% in Ontario, 0-0.9% in Quebec, 1.3-1.4% in the Atlantic Provinces, and 0.9-3.9% in the Prairies. Overall for the 2011/12 season, influenza B was the most commonly detected virus (53.5%) and dominated the provinces outside of Western Canada. Where subtype data were available, influenza A(H3N2) (41.3%) was the most commonly detected influenza A virus while (H1N1)pdm09 accounted for only 18.8%.

The World Health Organization (WHO) reports that the 2011-2012 season is largely over for most regions in the temperate northern hemisphere. The 2011-2012 season started unusually late in most of North America, with activity increasing in early December and peaking in March. Mexico almost exclusively detected A(H1N1)pdm09 this season while influenza A(H3N2) was the most commonly detected virus for most of the season in the USA. Like Canada, there were also regional differences with A(H1N1)pdm09 viruses most common in the South West (WHO, 15 June 2012 and 6 July 2012 Updates).



## A Report of the Public Health Microbiology & Reference Laboratory, Vancouver, BC

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