Alberta Health Services

PROVLAB ALBERTA

Laboratory Bulletin

Date: **February 28, 2013**

To: **Alberta Health, Alberta MicroNet, Communicable Disease Nurses, Infectious Diseases Physicians, Infection Prevention and Control, Medical Officers of Health, TARRANT Sentinel Physicians, and Laboratory Directors and Managers**

From: **Provincial Laboratory for Public Health (ProvLab)**
Dr. Kevin Fonseca, Program Lead; Kevin.Fonseca@albertahealthservices.ca

Re: **Summary of Current Respiratory Season and Genetic Analysis of Influenza Virus Circulating in Alberta**

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The current respiratory season (2012/2013) has been characterized by an earlier onset and greater peak of influenza activity when compared with the past two seasons (Table 1). Although the current respiratory season is not yet over, the number of samples tested by the middle of January 2013 is likely to be similar to or even exceed the total for the 2010/11 season. During the 2009 pandemic, the H1N1subtype was dominant and displaced both influenza B and influenza A H3 (Flu A H3). In the successive seasons following the pandemic, Flu A H3 has regained its ascendancy based upon the ProvLab subtyping data of the lab-confirmed cases.

Table 1: Comparison of numbers of samples tested and confirmed influenza cases by type and subtype for each respiratory season in Alberta

Respiratory Season (Sept-April)	Number of samples tested	Number of influenza A positives				Number of influenza B positives
		Total	Flu A H3	Flu A pdm09	Untyped	
2010-2011	15 286	876	569	276	31	698
2011-2012	13 962	1159	925	188	46	189
2012-2013*	11 784	2077	1586	243	109	139

** Data compiled up to 31st January 2012*

Figure 1 below, illustrates the current season's activity, based upon samples tested at the ProvLab (data provided by DIAL). The peak occurred in early January, as influenza activity now appears to be in decline. As in previous respiratory seasons, much of the activity is due to influenza A, and about 6% (139/2216) attributed to influenza B. The predominant influenza A subtype in the province is Flu A H3, with the (H1N1) pdm09 subtype [Flu A pdm09] accounting for 12% (243/2077) of all influenza A confirmed cases. Of interest is that the proportion of Flu A pdm09 positive samples was the highest in the Edmonton zone (19%), followed by the North (13%) and South zones (9%); Calgary and Central zones were 4% and 6% respectively.

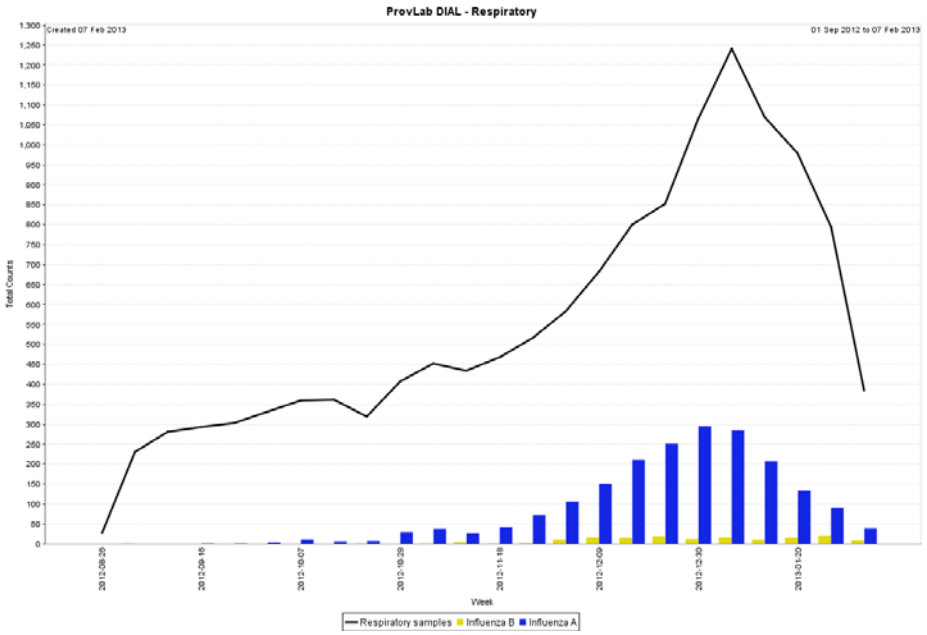

Figure 1: Comparison of numbers of samples tested, and influenza A and B detections by month (data from DIAL)

Although the reason for these differences between the zones is not apparent, a similar picture has been observed in previous seasons, and may be linked to the prevalence of the influenza type and subtype in the segment of the patient population who are being tested for respiratory illness.

Figure 2 below shows the changing distribution of influenza virus by type, subtype and month. A few cases early in September 2012 were caused by Flu A pdm09, whereas from October to January 2013, Flu A H3 has been dominant. Recently there has been a resurgence of Flu A pdm09 accounting for 21% (192/923) of cases in January 2013. Influenza B has been a minor component of confirmed cases and has not been detected from any respiratory outbreaks investigated by ProvLab. Of the 74 outbreaks with a confirmed influenza etiology, three were due to Flu A pdm09 (data not shown).

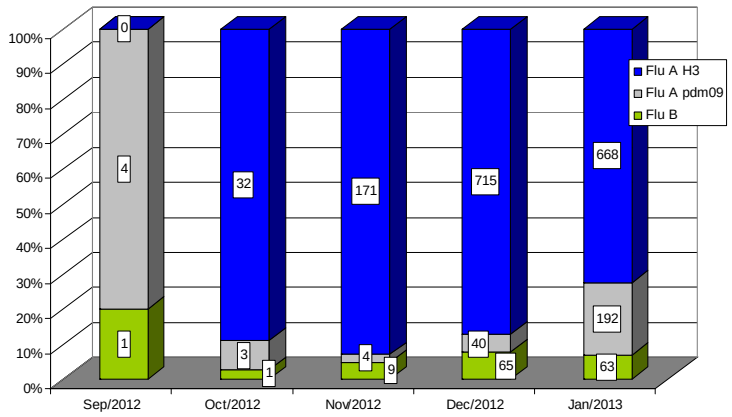

Figure 2: Distribution of influenza virus by type, subtype and month (data from DIAL)

As part of the on-going influenza surveillance at ProvLab, a selection of strains from outbreaks, community and hospitalized patients were chosen for further analysis. Sequencing of the haemagglutinin (HA) gene was performed to compare how closely strains from this season match with those from the previous season, with clades deposited in GenBank and also the current influenza vaccine components.

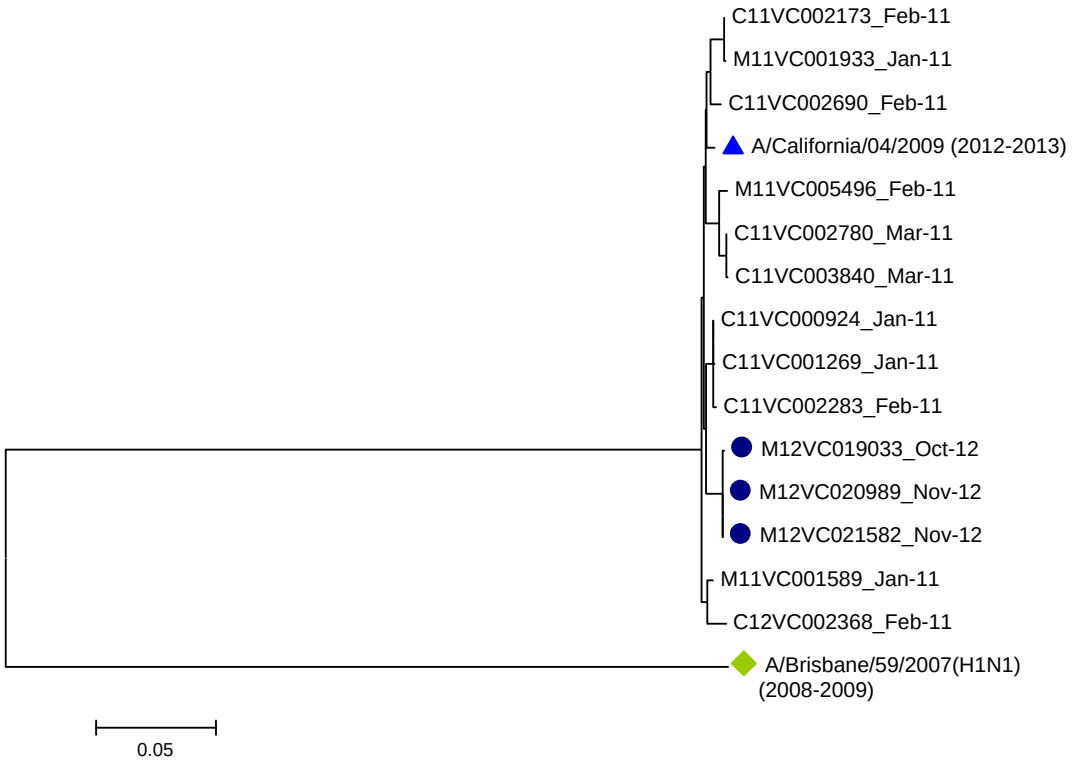

Figure 3: Phylogenetic tree of Flu A pdm09 viruses showing the relationship between the currently circulating strains (blue circles) and last season strains (no markings) from clinical samples, the 2012/13 vaccine component (A/ California/7/2009; blue triangle) and 2008/09 vaccine component (A/Brisbane/59/2007; green diamond). Clinical samples are identified by lab accession numbers, month and year of detection.

The phylogenetic tree above (Figure 3) shows that the Flu A pdm09 strains, which caused the pandemic in 2009, remains essentially unchanged. Strains from this and last season are still a good match with the vaccine component, based upon the HA gene sequence and antigenic characterization by the hemagglutination inhibition assay, performed at the National Microbiology Laboratory (NML), Winnipeg (data not shown).
However these strains are clearly antigenically distinct from the H1 seasonal strains that circulated prior to the pandemic, as exemplified by the A/Brisbane/59/2007 vaccine component.

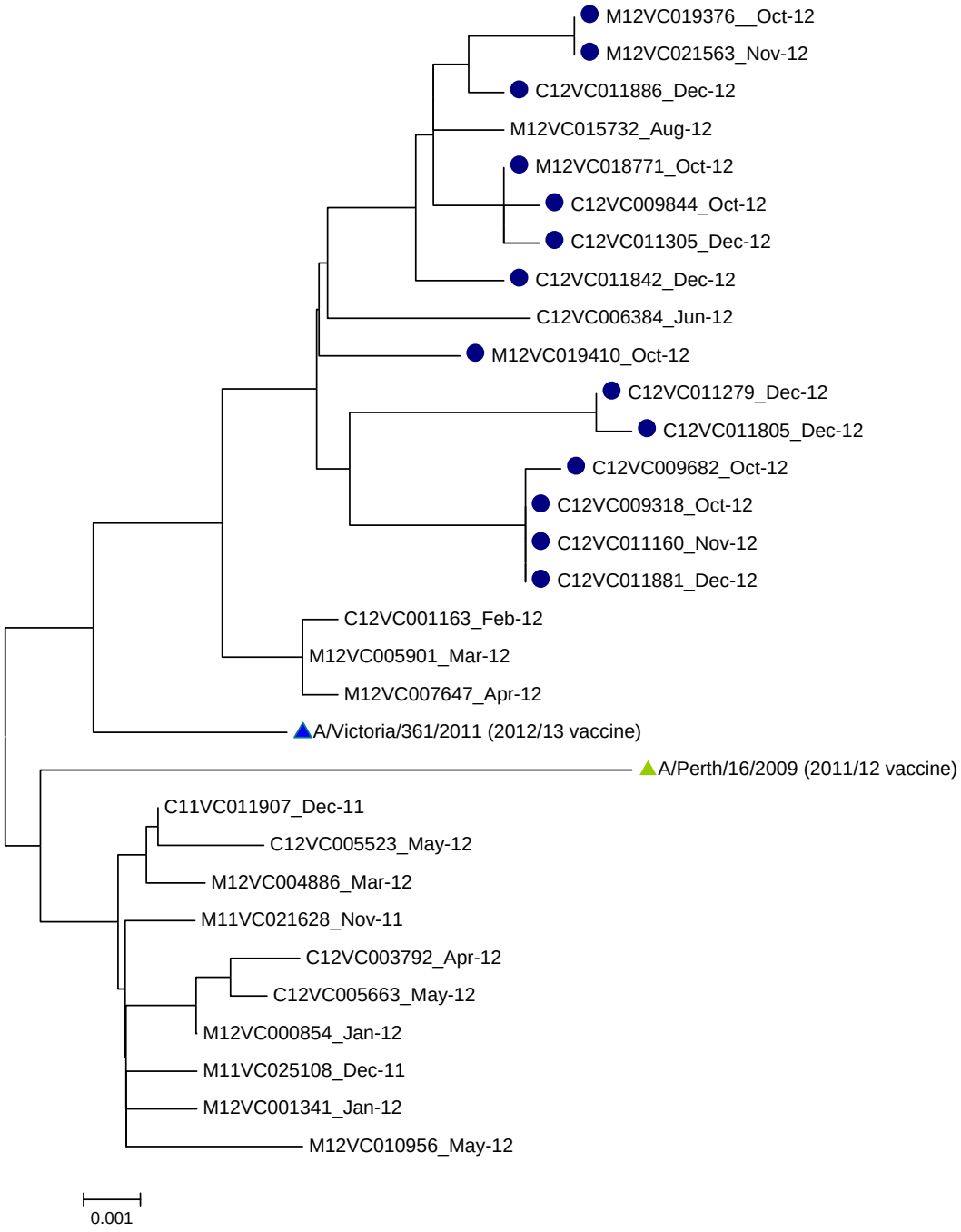

Figure 4: Phylogenetic tree showing the relationship between the currently circulating Flu A H3 viruses (blue circles) and last season strains (no circles), the 2012/13 vaccine strain (A/Victoria/361/2011, blue triangle); and last season (2011/2012) vaccine strain (A/Perth/16/2009; green triangle). The samples are identified by accession numbers followed by the month and year of detection. Clinical samples are identified by lab accession numbers, month and year of detection.

The phylogenetic tree of the Flu A H3 above (Figure 4) compares the relationship between strains from the current and previous respiratory seasons and the 2012/13 vaccine component. Strains from this season and a few from the end of last respiratory season show a good match to the current vaccine component (A/Victoria/361/2011), based upon antigenic characterization of strains by the NML. Strains from the previous respiratory season (2011/12) were also well-matched to the vaccine based upon antigenic characterization performed at the NML, and cluster with the 2011/2012 vaccine component (A/Perth/16/2009) shown in the lower part of the tree.

Sequencing of the HA gene from the current season's circulating strains has identified changes in amino acid residues at five antigenic sites (Table 2), that have been associated with the emergence of variants that can cause severe outbreaks [1,2]. Recent publications (4, 5, 6) have commented upon the reduced efficacy of the current influenza vaccine based upon the intensity of the season, numbers of outbreaks in facilities with high rates of vaccination, and visits to the ER departments. Two of the three publications also compared amino acid residue changes at antigenic sites in the HA gene and inferred that there might be some impact on the protectiveness of the vaccine. However there are differences in the numbers and locations of the amino acid changes reported by each of the two groups (4.6), which could indicate clonal distribution of influenza in the geographic regions where the studies were conducted and their respective methodologies and study populations. Additionally two of the asterisked locations in Table 2 below, where amino acid changes have been noted (Site B N161S and Site C Q49R) were not previously assigned to these respective antigenic sites. As these sites have now been recently proposed for inclusion (3), their contribution to significant changes in the antigenic properties of the HA require additional correlative studies.

Table 2: Changes in amino acids at the antigenic sites of the HA glycoprotein (3) for currently circulating Flu A H3 viruses compared to the current vaccine component (A/Victoria/208/2009)

Strain	**Changes in amino acids at 5 antigenic sites (A-E) of HA region**										
	Site A		***Site B***		***Site C***		***Site D***		***Site E***		
	122	**144**	**158**	**161***	**49***	**294**	**172**	**235**	**67**	**70**	**83**
A/Victoria/361/2011 (2012-2013 vaccine)	**A**	**T**	**R**	**N**	**Q**	**N**	**Q**	**Y**	**I**	**S**	**I**
C12VC001163_Feb-12	-	-	-	-	R	K	H	S	-	-	-
M12VC007647_Apr-12	-	-	-	-	R	K	H	S	-	-	-
C12VC009318_Oct-12	-	-	-	S	R	K	H	S	-	-	V
C12VC011160_Nov-12	-	-	-	S	R	K	H	S	-	-	V
C12VC011805_Dec-12	-	-	-	S	R	K	H	S	M	-	-
C12VC011886_Dec-12	T	-	-	S	R	K	H	S	-	-	-
M12VC019376_Oct-12	-	-	-	S	R	K	H	S	-	G	-
M12VC019410_Oct-12	-	A	G	S	R	K	H	S	-	-	-

* Amino acid residue locations proposed to be included in this antigenic site

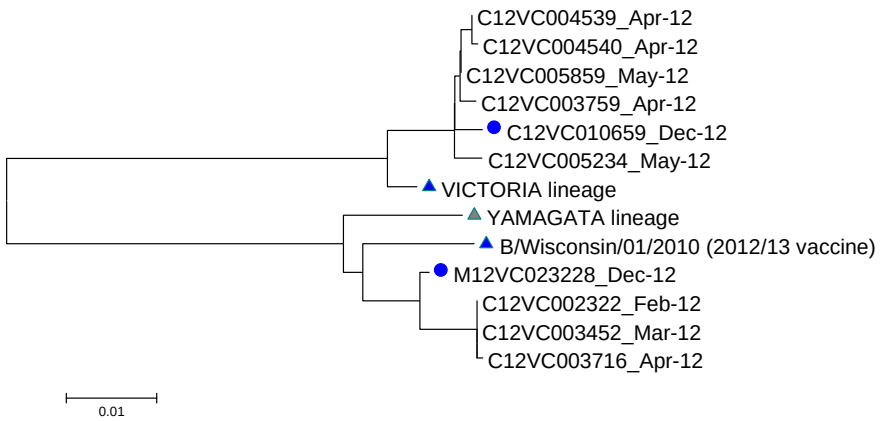

Figure 5: Phylogenetic tree showing the relationship between the circulating influenza B viruses (blue circles), last season stains (no circles), the 2012/13 vaccine component (B/Wisconsin /1/2010; blue triangle) and prototypes representing the Victoria (blue triangle) and Yamagata (grey triangle) lineages. Clinical samples are identified by accession numbers, month and year of detection.

Analysis of the influenza B strains currently circulating (Figure 5) shows that strains from both lineages are present although only those of the Victoria lineages are covered by the vaccine. However as the proportion of influenza B cases detected is approximately 6% of the total, the suboptimal coverage of the other influenza B lineage does not seem to be problematic, and there is reportedly some degree of cross-protectiveness between the two lineages.

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