TECHNICAL DOCUMENT

Community Network of Reference Laboratories (CNRL) for Human Influenza in Europe

Influenza virus characterisation

Summary Europe, December 2011

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Summary

Since week 40/2011, influenza A(H1N1)pdm09, influenza A(H3N2), and influenza B/Victoria- and B/Yamagata-lineage viruses have been detected in ECDC-affiliated countries. However, the number of detections has been low and only A(H3N2) viruses and a single B/Yamagata-lineage virus have been analysed at the London WHO CC to date:

- Type A viruses predominate over type B.
- A(H3N2) viruses predominate over A(H1N1)pdm09 viruses.
- While A(H3N2) viruses collected since 1 February 2011 fall into seven genetic groups, all recent viruses analysed from ECDC-affiliated countries fall within group 3 and there is some evidence of altered antigenicity compared to the vaccine virus A/Perth/16/2009.
- Influenza B viruses of the B/Victoria/2/87 and B/Yamagata/16/88 lineages have been detected in approximately equal proportions.

Influenza activity has remained low across the ECDC-affiliated countries with sporadic detections since the start of weekly reporting in week 40/2011, indicative of a late start to the influenza season compared to the 2010–2011 season. As at week 52/2011, 807 influenza detections had been reported: 736 (91.2%) type A and 71 (8.8%) type B. Of the type A viruses, 414 were sub-typed [23 (5.6%) as A(H1)pdm09 and 391 (94.4%) as A(H3)], while the lineage of 14 type B viruses had been determined [eight (57.1%) as B/Yamagata/16/88 and six (42.9%) as B/Victoria/2/87].

Table 1 provides a summary of the 37 specimens from seven countries collected after 1 October 2011, received at the London WHO CC. At the time of this report being written, nine viruses had been recovered and subjected to antigenic and genetic analyses, eight A(H3N2) and one B-Yamagata lineage. Batches of specimens for which analysis has yet to be completed are shown as in process.

Influenza A(H3N2) virus analysis

For specimens collected since 1 October 2011, influenza A(H3N2) viruses have been successfully isolated and propagated from three ECDC-affiliated countries. The difficulties with antigenic characterisation of recent H3N2 viruses were described in the most recent influenza virus characterisation report. With HI assay using guinea pig red blood cells, in the presence of oseltamivir to reduce any effect of the virus neuraminidase on the agglutination of the red blood cells (Lin et al. 2010), all viruses show 4–16 fold reductions in titre against post-infection ferret serum raised against the

A/Perth/16/2009 vaccine virus compared to the homologous titre (Table 2). Similar reductions were observed with five of the other post-infection ferret sera where homologous titres of ≥2560 were seen, and these included sera raised against viruses in HA genetic groups 1, 5 and 6. However, reductions were not observed for sera raised against a genetic group 5 virus (A/Alabama/5/2010) that gave a homologous titre of only 160 and two genetic group 3 viruses (A/Hong Kong/3969/2011, A/Stockholm/18/2011), both of which yielded homologous titres of 640.

In the last (August and September 2011) influenza virus characterisation report seven genetic groups were identified in the H3 HA phylogeny. Figure 1 shows an updated HA phylogeny in a global context: all recently circulating viruses fall within the A/Victoria/208/2009 clade, with the most recently circulating viruses (August–November) located in genetic groups 3, 5 and 6. The seven EU/EAA virus-containing specimens with collection dates in October and November 2011, received at the London WHO CC and represented in the phylogeny, all fall within genetic group 3 in a sub-group defined by A198S and N312S amino acid substitutions, together with viruses from Alaska, American Samoa, Norway and Sweden, and earlier viruses from Hong Kong, Slovenia, South Africa and Thailand.

Influenza B virus analyses

While the number of influenza B virus detections has been low, they appear to be present in approximately equal proportions, and to date only a single B/Yamagata lineage virus has been characterised at the WHO CC.

B/Yamagata-lineage viruses

With HI assay, the B/England/254/2011 virus showed 8-fold or greater reductions with all post-infection ferret sera used, with the exception of that raised against B/Florida/4/2006, a previous vaccine virus (Table 3). Phylogeny based on the HA1-coding region of the HA gene shows the virus to fall in the B/Bangladesh/3333/2007 and B/Wisconsin/01/2010 genetic clade, defined by amino acid substitutions S150I, N165Y and G229D, as do the majority of recently collected B/Yamagata-lineage viruses. A number of genetic groups exist within this clade and the B/England/254/2011 virus falls within a group defined by M251V amino acid substitution, with other recently circulating viruses (August–November) from Spain, Sweden and the USA, falling in a sub-group with the B/Indiana/10/2011 virus (Figure 2).

Note on the figures

The phylogenetic trees were constructed using neighbour-join in MEGA4. The bars indicate the proportion of nucleotide changes in the sequence. Reference strains are viruses against which post-infection ferret antisera have been raised. The colours indicate the date of sample collection. Isolates from ECDC countries are highlighted in yellow. Sequences for some of the viruses from non-European countries were recovered from GISAID and we acknowledge all laboratories who submitted sequences directly to the London WHO CC.

Table 1 Summary of specimens collected since 1 October 2011 and received by 31 December 2011.

MONTH / Country	H1N1pdm		H3N2		B Yamagata lineage		B Victoria lineage	
	Number received	Number recovered	Number received	Number recovered	Number received	Number recovered	Number received	Number recovered
OCTOBER								
Germany							1	in process
Norway			3	in process			2	in process
Sweden	1	in process	2	in process				
United Kingdom			3	3	1	1		
NOVEMBER								
Finland			1	1				
Germany			1	in process				
Netherlands				in process	1	in process		
Norway			2	in process				
Slovakia			2	2				
Sweden	3	in process	4	in process	3	in process		
United Kingdom			2	2				
DECEMBER								
Germany			3	in process				
Netherlands			1	in process				
Norway			1	in process				
Total Received = 37	4	0	25	8	5	1	3	0
% of total	11%		68%		13%		8%	

Table 2 Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBCs with 20nM oseltamivir).

Viruses	Collection Date	Passage History	Haemagglutination inhibition titre[1] Post infection ferret sera								
			A/Bris 10/07 F18/07	A/Perth 16/09 F35/11	A/Vic 208/09 F7/10	A/Vic 210/09 F11/10	A/Ala 5/10 F27/10	A/Perth 10/10 F03/11	A/HK 3969/11 F27/11	A/Stock 18/11 F28/11	A/Iowa 19/10 F15/11
Genetic group						group 1	group 5	group 5	group 3	group 3	group 6
REFERENCE VIRUSES											
A/Brisbane/10/2007	2007-02-06	E2/E1	2560	40	<	<	<	80	160	<	<
A/Perth/16/2009	2009-07-04	E3/E1	<	640	40	160	160	160	640	160	160
A/Victoria/208/2009	2009-06-02	E3/E1	640	640	2560	2560	1280	2560	2560	2560	2560
A/Victoria/210/2009	2009-06-02	E2/E3	640	2560	1280	2560	320	1280	1280	640	1280
A/Alabama/5/2010	2010-07-13	MK1/M2/SIAT5	80	80	40	40	160	160	640	320	320
A/Perth/10/2010	2010-05-25	E2/E1	640	640	1280	2560	320	2560	5120	1280	2560
A/Hong Kong/3969/2011	2011-05-19	MDCK3	160	160	160	160	320	640	640	320	640
A/Stockholm/18/2011	2011-03-28	MDCK2/SIAT2	160	80	80	40	160	160	640	640	160
A/Iowa/19/2010	2010-12-30	E3/E1	160	640	1280	1280	1280	2560	2560	1280	5120
TEST VIRUSES											
A/England/257/2011	2011-10-10	SIAT P1/SIAT1	160	80	160	160	320	320	640	640	320
A/England/256/2011	2011-10-12	SIAT P1/SIAT1	160	80	160	160	160	320	640	320	320
A/England/255/2011	2011-10-14	SIAT P1/SIAT1	80	40	80	160	160	160	320	320	160
A/Bratislava/31/2011	2011-11-03	SIAT2	160	160	320	640	640	640	1280	640	640
A/Bratislava/31/2011	2011-11-03	MDCK2/SIAT1	160	160	160	160	320	320	640	320	320
A/England/258/2011	2011-11-07	SIAT P1/SIAT1	80	80	80	80	160	320	640	320	320
A/England/259/2011	2011-11-16	SIAT P1/SIAT1	80	80	80	80	160	320	640	320	160
A/Finland/190/2011	2011-11-25	SIAT3/SIAT3	160	80	80	80	160	160	640	320	160

1. < = <40

Vaccine virus

Sequences included in HA phylogeny

Table 3 Antigenic analysis of influenza B/Yamagata-lineage viruses by HI (turkey RBCs)

Viruses	Collection date	Passage History	Haemagglutination inhibition titre Post infection ferret sera						
			B/Fl[3] 4/06 SH479	B/Eg[1] 144/05 F3/07	B/Fl[1] 4/06 F20/07	B/Bris[2] 3/07 F24/07	B/Eng[2] 145/08 F09/08	B/Bang[2] 3333/07 F21/08	B/Wis[2] 1/10 F26/10
REFERENCE VIRUSES									
B/Egypt/144/2005	2005-05-01	E3/E5	5120	320	2560	1280	80	160	320
B/Florida/4/2006	2006-12-15	E3/E4	1280	40	640	320	20	40	40
B/Brisbane/3/2007	2007-09-03	E2/E4	2560	80	640	320	20	80	40
B/England/145/2008		Ex/E5	320	<	80	10	160	10	<
B/Bangladesh/3333/2007	2007-08-07	E4/E4	2560	40	320	80	20	160	40
B/Wisconsin/1/2010	2010-02-20	E3/E3	1280	80	320	80	20	80	160
TEST VIRUSES									
B/England/254/2011	2011-10-04	SIAT1/MDCK1	1280	<	160	<	20	20	10

1. < = <40; 2. < =<10 ; 3. hyperimmune sheep serum

Sequence included in HA phylogeny

Figure 1 Phylogenetic comparison of influenza A(H3N2) HA genes.

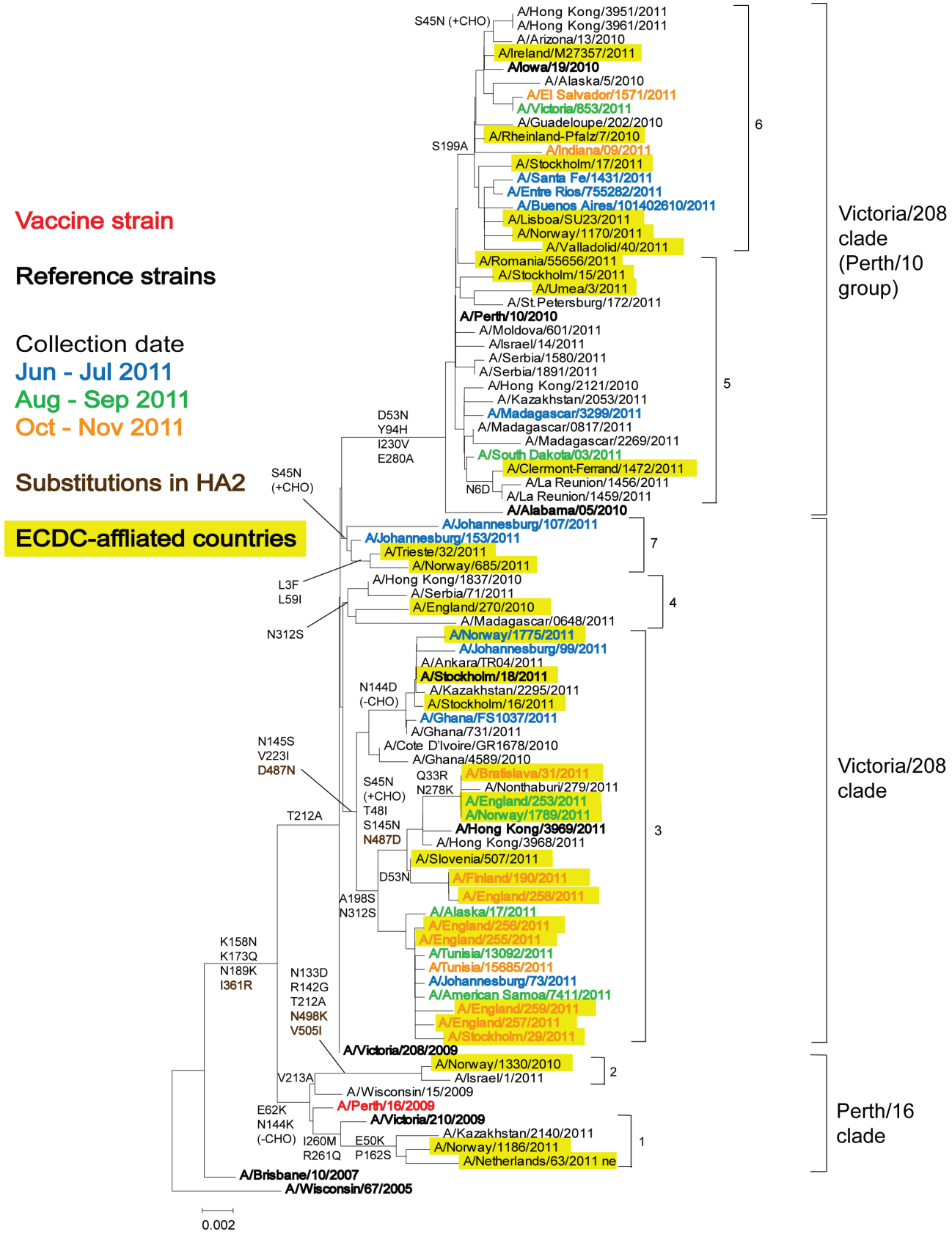

Figure 2 Phylogenetic comparison of influenza B/Yamagata-lineage HA genes (HA1 coding region).