TECHNICAL DOCUMENT

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

Influenza virus characterisation

Summary Europe, February 2010

Influenza virus characterisation

2009 pandemic influenza A(H1N1) viruses have continued to predominate although at a lower level than earlier in the season. Circulation of H3N2 viruses and influenza B viruses has also remained low. There remains very little seasonal H1N1 virus activity.

Most of the viruses characterised by the WHO Collaborating Centre for Reference and Research on Influenza in London have been 2009 pandemic A(H1N1).

Table 1 shows the number of pandemic influenza A(H1N1) clinical samples or virus isolates received at the WHO CC from Europe since 1 September 2009 until 31 January 2010.

Table 1. Summary of WHOCC analyses conducted on pandemic A(H1N1) samples collected September to December 2009

		Clinical samples received		Isolates received	
Month Continent	**Number of countries**	**Number received**	**Number propagated***	**Number received**	**Number propagated***
September Europe	15	16	8	53	49
October Europe	16	93	29	72	59
November Europe	21	430	100	168	161
December Europe	11	42	21	56	56
Unknown Europe	3	178		21	21
Total		**759**	**158**	**370**	**346**

All propagated viruses have been antigenically characterised by HI assay.

Antigenic analysis of a representative set of viruses from Europe is shown in Table 2. The table shows haemagglutination inhibition (HI) results using a panel of reference post-infection ferret antisera. As reported in the previous ECDC Virus Characterisation Report, the viruses continued to react well with ferret antisera raised against the reference pandemic A(H1N1) viruses; notably all the viruses react well with the antiserum raised against the vaccine virus A/California/7/2009. A similar pattern has been observed with viruses from other countries assayed at the WHO CC in London and at the WHO Collaborating Centres elsewhere.

Table 2. Antigenic analyses of pandemic A(H1N1) influenza viruses conducted at WHOCC

		Passage history	Haemagglutination inhibition titre[1]					
			Post-infection ferret sera					
Viruses	Collection date		A/Cal 4/09 C4/F14/09	A/Cal 7/09 C4/31/09	A/Eng 195/09 NIBSC F17/09	A/Auck 3/09 C4/17/09	A/Bayern 69/09 C4/33/09	A/Lviv N6/2009 C4/34/09
Reference viruses								
A/California/4/2009		C1,E2	**2560**	2560	1280	2560	1280	2560
A/California/7/2009		E6	2560	**2560**	640	1280	1280	2560
A/England/195/2009		MDCK5	1280	2560	**1280**	2560	640	1280
A/Auckland/3/2009		Ex+3	2560	2560	1280	**2560**	1280	2560
A/Bayern/69/2009		MDCK4/SIAT1	80	320	40	80	**640**	320
A/Lviv/N6/2009		MDCK4/SIAT1	320	1280	160	320	1280	**1280**
Test viruses								
A/Bosnia/1226/2009	unknown	SIAT2	640	1280	1280	640	2560	640
A/Bosnia/1442/2009	unknown	SIAT3	640	1280	1280	640	1280	640
A/France/6808/09[2]	unknown	Cx/SIAT2	2560	2560	2560	2560	2560	1280
A/Paris/6232/09[2]	Nov-09	C1/SIAT2	1280	1280	1280	1280	1280	640
A/Lisboa/113/2009	16/11/2009	MCDK2/SIAT2	2560	2560	2560	1280	5120	1280
A/Barcelona/1935/2009	21/11/2009	SIAT2	1280	1280	1280	1280	2560	1280
A/Barcelona/1937/2009	22/11/2009	SIAT2	1280	2560	2560	1280	2560	1280
A/Linkoping/1/2009	22/11/2009	p0/SIAT1	1280	1280	1280	1280	5120	1280
A/Stockholm/106/2009	24/11/2009	p1/SIAT1	1280	1280	1280	2560	2560	1280
A/Norway/4619/2009	24/11/2009	MDCK1/SIAT2	640	1280	1280	640	2560	640
A/Belgium/G4455/2009	25/11/2009	Siat2	1280	1280	1280	640	2560	640
A/Belgium/G4461/2009	26/11/2009	Siat2	2560	2560	2560	2560	2560	1280
A/Kosovo/408/2009	26/11/2009	Siat2	5120	5120	5120	5120	2560	1280
A/Norway/4708/2009	27/11/2009	MDCK1/SIAT2	1280	2560	2560	1280	1280	1280
A/Kosovo/469/2009	30/11/2009	Siat2	5120	2560	2560	5120	5120	2560
A/Valladolid/35/2009	22/12/2009	MDCK1/SIAT1	1280	5120	5120	1280	5120	640
A/Albania/2338/2009	16/12/2009	P1 / SIAT1	1280	2180	2180	1280	2560	640
A/Albania/2376/2009	18/12/2009	P1 / SIAT1	1280	1280	1280	1280	2560	640
A/Mecklenburg-Vorp/6/2009	03/12/2009	MDCK/S3.P/SIAT1	1280	1280	1280	1280	2560	640
A/Berlin/164/2009	07/12/2009	MDCK/S3.P/SIAT1	1280	1280	1280	2560	2560	1280
A/Poland/917/2009	10/12/2009	SIAT3	1280	1280	1280	1280	2560	640
A/Poland/922/2009	02/12/2009	SIAT2	1280	1280	1280	2560	2560	1280
A/Austria/531141/2009	03/12/2009	1 pMDCK-SIAT1/SIAT2	2560	2560	2560	2560	5120	1280

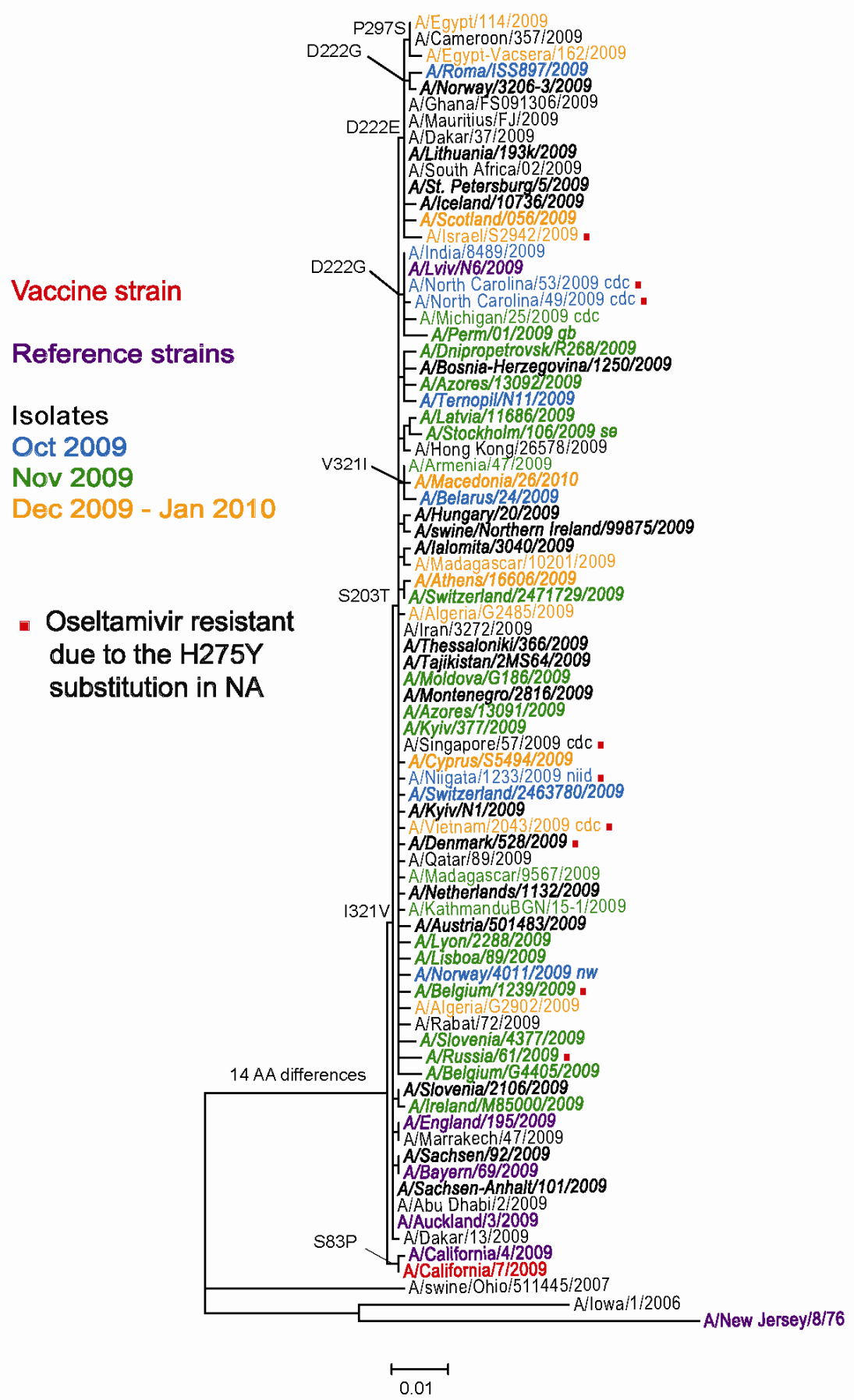
[1] <=40

		Passage history	Haemagglutination inhibition titre[1]					
			Post-infection ferret sera					
Viruses	Collection date		A/Cal 4/09 C4/F14/09	A/Cal 7/09 C4/31/09	A/Eng 195/09 NIBSC F17/09	A/Auck 3/09 C4/17/09	A/Bayern 69/09 C4/33/09	A/Lviv N6/2009 C4/34/09
A/Leivadeia/16624/2009	26/12/2009	SIAT3	2560	1280	1280	2560	2560	640
A/Patras/16656/2009	27/12/2009	SIAT4	2560	2560	2560	5120	5120	2560
A/Lisboa/116/2009	29/12/2009	MCDK2/SIAT1	2560	1280	1280	1280	2560	1280

Vaccine strain

Gene sequence analysis of a subset of recent viruses and clinical specimens shows that circulating viruses remain genetically similar to the prototype and vaccines viruses. Figure 1 shows a phylogenetic tree of the HA1 coding region of the haemagglutinin (HA) gene; viruses from Europe (highlighted in bold italics) are shown compared with viruses from other parts of the world. As described in the previous report, most viruses carry the amino acid substitution S203T in the HA glycoprotein, with a subset carrying substitutions at amino acid 222 as well. These substitutions do not affect the antigenicity of the viruses. Viruses resistant to oseltamivir, associated with the H275Y amino acid substitution in the neuraminidase (NA), remain unclustered in the HA1 phylogenetic tree.

Figure 1. Phylogenetic comparison of pandemic influenza A(H1N1) HA genes



The phylogenetic tree was constructed using maximum parsimony in PAUP (Sinauer Associates). The bar indicates the proportion of nucleotide changes in the sequence. Reference strains are viruses to which post-infection antisera have been developed. The colours indicate the date of sample collection. Isolates from Europe are in italics.

Table 3 shows the number of seasonal influenza viruses characterised at the WHO CC in London. No seasonal H1N1 virus isolates received have been collected after October 2009; very few H3N2 and influenza B viruses received have been collected in recent months.

Table 3. Summary of European seasonal influenza viruses received, collected between September 2009 and January 2010

Country and month collected	H1N1		H3N2		B			
					Yamagata		Victoria	
	received	**grown**	**received**	**grown**	**received**	**grown**	**received**	**grown**
September								
France	2	2	5	5				
Gibraltar			1	1				
Sweden			3	3				
United Kingdom			3	3				
October								
Finland			2	2				
France	1	1	1	1				
Gibraltar			1	1				
Norway			2	2			1	0
November								
France			7	4				
Sweden			2	2			1	1
December 2009								
United Kingdom							1	1
January 2010								
Germany			1	1				
Sweden							1	1
Total	**3**	**3**	**28**	**25**	**0**	**0**	**4**	**3**

Table 4 shows antigenic analysis, based on HI assays carried out in the presence of oseltamivir carboxylate, of representative H3N2 viruses recovered from samples collected in Europe since September 2009 until January 2010. As described, two patterns of reactivity can be seen which differentiate viruses that have been emerging since early 2009 and the viruses that predominated previously. The patterns can be seen in the panel of reference viruses: those from 2007 and 2008 are antigenically distinct from the reference viruses collected in 2009. The H3N2 viruses in the trivalent seasonal influenza vaccine for use in the northern hemisphere in the 2009–2010 influenza season (A/Brisbane/10/2007 or A/Uruguay/716/2007) differ in their antigenic profile from the virus (A/Perth/16/2009) recommended for use in vaccines for the southern hemisphere 2010. Only one virus (A/Gothenburg/3/2009) displayed antigenic reactivity similar to the A/Brisbane/10/2007 and A/Uruguay/716/2007 pattern; all other viruses from Europe were antigenically similar to A/Perth/16/2009. This is a similar pattern to that observed for H3N2 viruses from other countries with circulating H3N2 virus.

Table 4. Antigenic analyses of influenza A(H3N2) viruses — guinea pig RBC — in 20nM oseltamivir

				Haemagglutination inhibition titre[2]								
				Post infection ferret sera								
Viruses	Collection month	Passage history		A/Wis 67/05 F1/06	A/Bris 10/07 F29/08	A/Uru 716/07 F26/08	A/Fin 9/08 F12/088	A/Jhb 15/08 F22/08	A/HK 1952/09 F22/09	A/HK 1985/09 F21/09	A/Perth 16/09 F25/09	A/Wis 15/09 F24/09
Reference viruses												
A/Wisconsin/67/2005		SpfCk3E3	E7	**1280**	640	1280	320	320	<	<	<	<
A/Brisbane/10/2007		E2	E3	640	**1280**	1280	640	320	<	<	<	<
A/Uruguay/716/2007		spfck1, E3	E3	1280	1280	**2560**	1280	640	<	<	40	<
A/Finland/9/2008		MDCK2	Siat2	1280	1280	1280	**640**	640	<	80	80	<
A/Johannesburg/15/2008		MDCKx	Siat6	1280	1280	1280	1280	**2560**	40	80	80	40
A/Hong Kong/1952/2009		MDCKx2	Siat7	<	80	80	<	<	**160**	1280	640	320
A/Hong Kong/1985/2009		MDCKx2	Siat5	<	80	80	<	<	160	**1280**	640	320
A/Perth/16/2009		E3	E2	<	<	<	<	<	40	320	**640**	80
A/Wisconsin/15/2009		E2	E2	<	<	<	<	<	40	640	320	**160**
Test viruses												
A/Gothenburg/3/2009	27/11/2009			160	640	640	320	640	40	80	40	<
A/Paris/3873/09	Sep-09	C1	Siat1	<	80	160	40	40	80	320	160	160
A/Sweden/2/2009	01/09/2009	C1	Siat1	80	160	160	160	160	320	2560	1280	640
A/Umeå/4/2009	02/09/2009	C2	Siat1	40	160	160	80	160	160	640	1280	320
A/Stockholm/89/2009	06/09/2009	C1	Siat1	40	160	160	80	80	80	320	640	160
A/England/815/2009	15/09/2009	Siat1	Siat1	40	160	160	80	80	160	640	320	160
A/Lyon/1715/2009	17/09/2009	MDCK2	Siat2	80	160	80	40	80	40	640	640	80
A/Bordeaux/1942/2009	18/09/2009	MDCK2	Siat3	<	160	80	<	40	160	1280	1280	640
A/Gibraltar/SB226/09	22/09/2009	Siat1	Siat1	40	160	160	80	80	80	320	320	160
A/Paris/5870/09	Oct-09	C1	Siat1	40	<	80	<	<	80	320	160	160
A/Gibraltar/SB252/09	07/10/2009	Siat1	Siat1	40	160	160	40	80	80	320	320	160
A/Norway/3789/2009	12/10/2009	MDCK1	Siat1	<	40	40	40	40	40	80	160	40
A/Norway/3790/2009	13/10/2009	MDCK1	Siat1	<	80	80	40	40	40	160	320	40
A/Finland/638/2009	20/10/2009	MDCK3	Siat1	40	80	80	40	40	40	160	320	80
A/Finland/640/2009	26/10/2009	MDCK3	Siat1	40	160	160	80	80	40	320	320	80
A/Paris/6047/2009	Nov-09	C1	Siat2	80	320	320	160	160	320	1280	1280	160
A/Lyon/Cx-R/3120/2009	Nov-09	p2MDCK	Siat1	40	80	80	40	40	40	160	320	80
A/Lyon/3670/2009	Nov-09	p2MDCK	Siat1	80	160	160	80	160	80	640	640	320
A/Lyon/CHU/52.384/09	Nov-09	p2MDCK	Siat1	160	320	320	160	160	160	1280	1280	320
A/Turkey/TR-26/2009	02/12/2009	Siat1	Siat1	<	40	80	40	40	NT	2560	1280	640

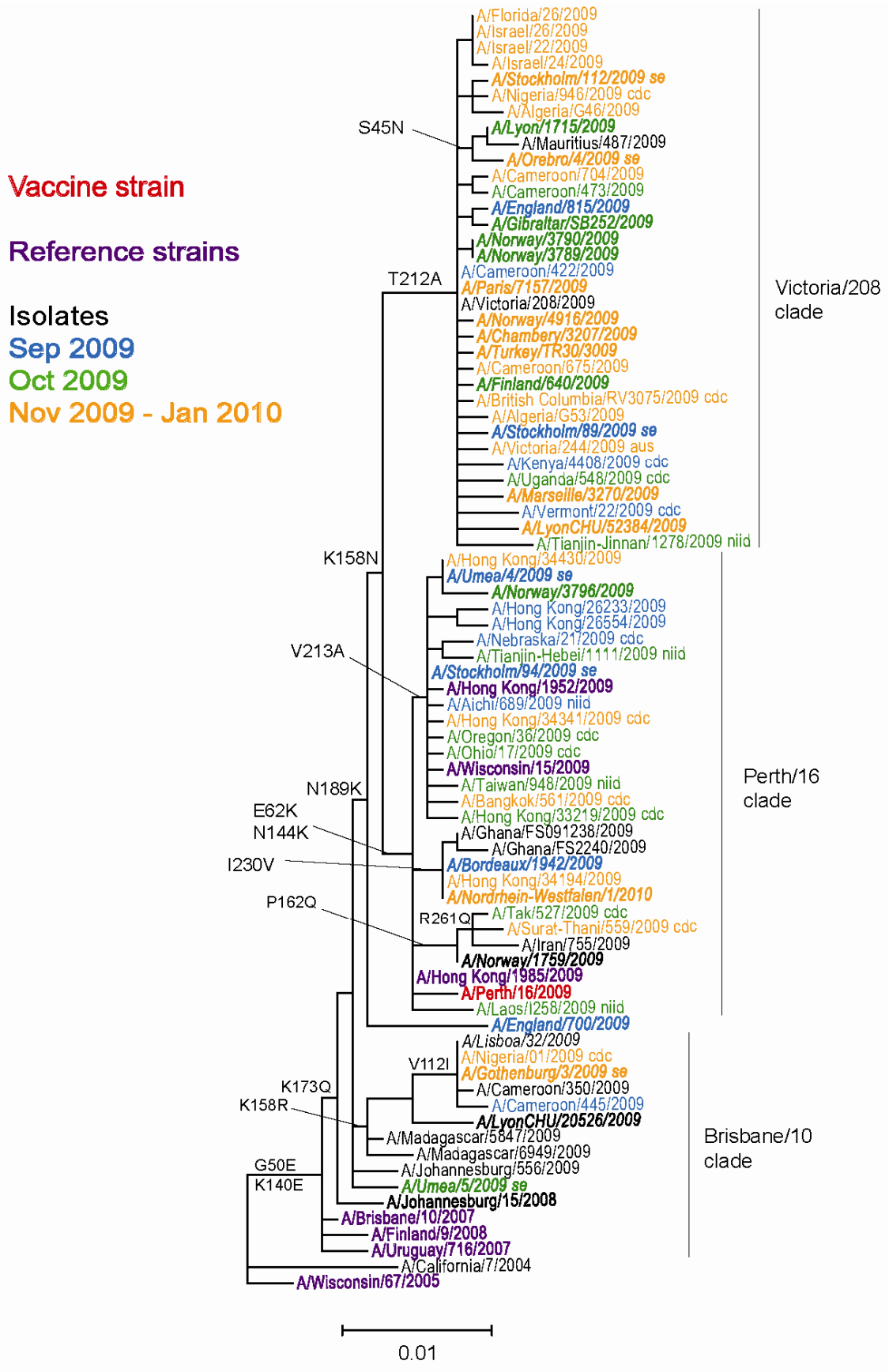
[2] <=40

				Haemagglutination inhibition titre[2]								
				Post infection ferret sera								
Viruses	Collection month	Passage history		A/Wis 67/05 F1/06	A/Bris 10/07 F29/08	A/Uru 716/07 F26/08	A/Fin 9/08 F12/088	A/Jhb 15/08 F22/08	A/HK 1952/09 F22/09	A/HK 1985/09 F21/09	A/Perth 16/09 F25/09	A/Wis 15/09 F24/09
A/Turkey/TR-28/2009	12/12/2009	Siat1	Siat1	<	40	80	<	40	NT	2560	1280	320
A/Nordrhein-Westfalen/1/2010	Jan-10	Siat1	Siat1	80	160	160	160	80	640	2560	2560	1280
A/Turkey/TR-33/2009	05/01/2010	Siat1	Siat1	<	40	40	<	<	NT	1280	640	160
				Vaccine strain Northern hemisphere 2010–2011					**Vaccine strains Northern hemisphere 2010–2011**			

NT = not tested

Genetic analysis of the HA gene of a representative set of H3N2 viruses is shown in Figure 2. As in Figure 1, viruses from Europe are highlighted in bold italics in a phylogenetic tree of the HA1 coding region of the HA gene. Three distinct clades are identified: the Brisbane/10 clade, the Perth/16 clade, and a clade with A/Victoria/208/2009 as a prototype. Viruses from the Perth/16 clade and the Victoria/208 clade are antigenically indistinguishable and share the amino acid substitutions K158N and N189K in comparison with viruses of the Brisbane/10 clade. Viruses from both the Perth/16 and the Victoria/208 genetic clades have been isolated in recent months in Europe. A similar pattern has been observed in H3N2 isolates from countries outside of Europe.

Figure 2. Phylogenetic comparison of H3N2 HA genes



The phylogenetic tree was constructed using maximum parsimony in PAUP (Sinauer Associates). The bar indicates the proportion of nucleotide changes in the sequence. Reference strains are viruses to which post-infection antisera have been developed. The colours indicate the date of sample collection. Isolates from Europe are in italics.

Only four influenza B viruses from Europe, collected since September, were received at the WHO CC in London (Table 3). All viruses were of the B/Victoria lineage and, with such a small number of viruses from Europe available for analysis, no pattern of reactivity can be adduced. Results from other WHO Collaborating Centres lead to the conclusion that the majority of viruses are of the B/Victoria lineage and are antigenically similar to the current vaccine virus B/Brisbane/60/2008. For recently isolated viruses in the B/Yamagata/16/88 lineage, those isolated in China have been closely related to B/Hubei-Wujiagang/158/2009, while in Bangladesh and elsewhere the majority of B/Yamagata/16/88 lineage viruses were closely related to the previous vaccine virus B/Florida/4/2006.

The recent vaccine strain recommendations for the northern hemisphere 2010–2011 influenza season can be found at: http://www.who.int/csr/disease/influenza/201002_Recommendation.pdf.

Note

Prepared by WHO Collaborating Centre, Mill Hill, London, on behalf of the
Community Network of Reference Laboratories for Human Influenza in Europe (CNRL) Coordination Group.