



## TECHNICAL DOCUMENT

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

# Influenza virus characterisation

Summary Europe, January 2010

## Influenza virus characterisation

A(H1N1pdm) viruses have continued to predominate. Surveillance data indicate that H3N2 viruses and influenza B viruses have circulated at lower levels with very little seasonal H1N1 circulation.

The majority of work on the genetic and antigenic characteristics of influenza viruses isolated in Europe by the CNRL and the WHO Collaborating Centre for Reference and Research on Influenza (WHO CC) in London has been carried out on pandemic A/H1N1 viruses and on seasonal H3N2 viruses.

Table 1 shows the number of pandemic influenza H1N1 clinical samples or virus isolates received at the WHO CC from 1 September 2009 until 31 December 2009 and the number of countries in Europe that supplied isolates or samples.

**Table 1. Summary of H1N1 pandemic analyses conducted on samples collected September to December 2009**

| Month of collection | Number of countries | Clinical samples received |                   | Isolates received |                   |
|---------------------|---------------------|---------------------------|-------------------|-------------------|-------------------|
|                     |                     | Number received           | Number propagated | Number received   | Number propagated |
| September Europe    | 12                  | 16                        | 8                 | 50                | 46                |
| October Europe      | 12                  | 92                        | 29                | 46                | 33                |
| November Europe     | 14                  | 412                       | 80                | 96                | 92                |
| December Europe     | 3                   | 38                        | 18                | 0                 | 0                 |
| <b>Total</b>        |                     | <b>558</b>                | <b>135</b>        | <b>192</b>        | <b>171</b>        |

Antigenic analysis of a representative set of viruses from Europe is shown in Table 2. The table shows the haemagglutination inhibition (HI) results with a panel of reference H1N1 post-infection ferret antisera of recently isolated viruses and one rabbit hyperimmune serum. All viruses react well with the ferret antisera raised against pandemic H1N1 viruses and appear antigenically homogeneous. All the viruses react well with the antiserum to the vaccine virus A/California/7/2009.

**Table 2. Antigenic analyses of pandemic influenza A(H1N1) viruses**

|                             |                 | Haemagglutination inhibition titre <sup>1</sup> |                        |                            |                           |                               |                            |                               |                               |
|-----------------------------|-----------------|---|------------------------|----------------------------|---------------------------|-------------------------------|----------------------------|-------------------------------|-------------------------------|
|                             |                 | Post infection ferret sera                      |                        |                            |                           |                               |                            |                               |                               |
| Viruses                     | Collection date | A/NJ <sup>2</sup><br>8/76<br>R15/79             | A/NJ<br>8/76<br>F34/82 | A/Cal<br>4/09<br>C4/F14/09 | A/Cal<br>7/09<br>C4/31/09 | A/Eng<br>195/09<br>NIB F18/09 | A/Auck<br>3/09<br>C4/17/09 | A/Bayern<br>69/09<br>C4/33/09 | A/Lviv<br>N6/2009<br>C4/34/09 |
| <b>Reference viruses</b>    |                 |   |                        |                            |                           |                               |                            |                               |                               |
| A/New Jersey/8/76           |                 | >5120   | 1280                   | 320                        | 640                       | 640                           | 1280                       | 320                           | 640                           |
| A/California/4/2009         |                 | 5120  | 320                    | 2560                       | 2560                      | 2560                          | 2560                       | 1280                          | 2560                          |
| A/California/7/2009         |                 | 2560  | 320                    | 2560                       | 2560                      | 2560                          | 2560                       | 1280                          | 5120                          |
| A/England/195/2009          |                 | 2560  | 320                    | 2560                       | 2560                      | 2560                          | 2560                       | 1280                          | 2560                          |
| A/Auckland/3/2009           |                 | 5120  | 640                    | 2560                       | 2560                      | 5120                          | 2560                       | 2560                          | 5120                          |
| A/Bayern/69/2009            |                 | 640   | <                      | 80                         | 320                       | 160                           | 160                        | 640                           | 640                           |
| A/Lviv/N6/2009              |                 | 640   | <                      | 320                        | 640                       | 160                           | 160                        | 1280                          | 1280                          |
| <b>Test viruses</b>         |                 |   |                        |                            |                           |                               |                            |                               |                               |
| A/Bosnia-Herzegov/28Sw/2009 | Aug-09          | 2560  | 160                    | 1280                       | 1280                      | 1280                          | 1280                       | NT                            | NT                            |
| A/Lisboa/64/2009            | Sep-09          | ≥5120   | 160                    | 5120                       | 5120                      | 5120                          | 5120                       | 5120                          | 5120                          |
| A/St. Petersburg/5/2009     | Sep-09          | 5120  | 320                    | 2560                       | 2560                      | 2560                          | 5120                       | NT                            | NT                            |
| A/Norway/4158/2009          | Oct-09          | 1280  | 80                     | 1280                       | 1280                      | 1280                          | 1280                       | 640                           | 1280                          |
| A/Slovenia/4221/2009        | Nov-09          | 2560  | 160                    | 1280                       | 2560                      | 2560                          | 2560                       | 640                           | 1280                          |
| A/Switzerland/2471729/2009  | Nov-09          | 640   | 80                     | 1280                       | 1280                      | 2560                          | 2560                       | NT                            | NT                            |
| A/Kosovo/578/2009           | Nov-09          | 2560  | 160                    | 5120                       | 5120                      | 5120                          | 5120                       | 2560                          | 2560                          |
| A/Belarus/226/2009          | Nov-09          | 5120  | 160                    | 2560                       | 2560                      | 2560                          | 2560                       | 1280                          | 2560                          |
| A/Lyon/2490/2009            | Nov-09          | 2560  | 80                     | 2560                       | 2560                      | 2560                          | 2560                       | 1280                          | 2560                          |
| A/Belgium/G4363/2009        | Nov-09          | 2560  | 80                     | 1280                       | 2560                      | 2560                          | 2560                       | 1280                          | 2560                          |
| A/Ireland/M85000/2009       | Nov-09          | 2560  | 160                    | 1280                       | 1280                      | 1280                          | 2560                       | 640                           | 1280                          |
| A/Poland/923/2009           | Dec-09          | 2560  | NT <sup>3</sup>        | 2560                       | 2560                      | 2560                          | 5120                       | 1280                          | 2560                          |
| A/Berlin/164/2009           | Dec-09          | 2560  | NT                     | 1280                       | 1280                      | 2560                          | 2560                       | 1280                          | 2560                          |
| A/Athens/16586/2009         | Dec-09          | 2560  | NT                     | 2560                       | 2560                      | 2560                          | 5120                       | 2560                          | 2560                          |
| A/Leivadeia/16623/2009      | Dec-09          | 2560  | NT                     | 1280                       | 1280                      | 2560                          | 2560                       | 1280                          | 1280                          |

Vaccine strain

<sup>1</sup> <=40

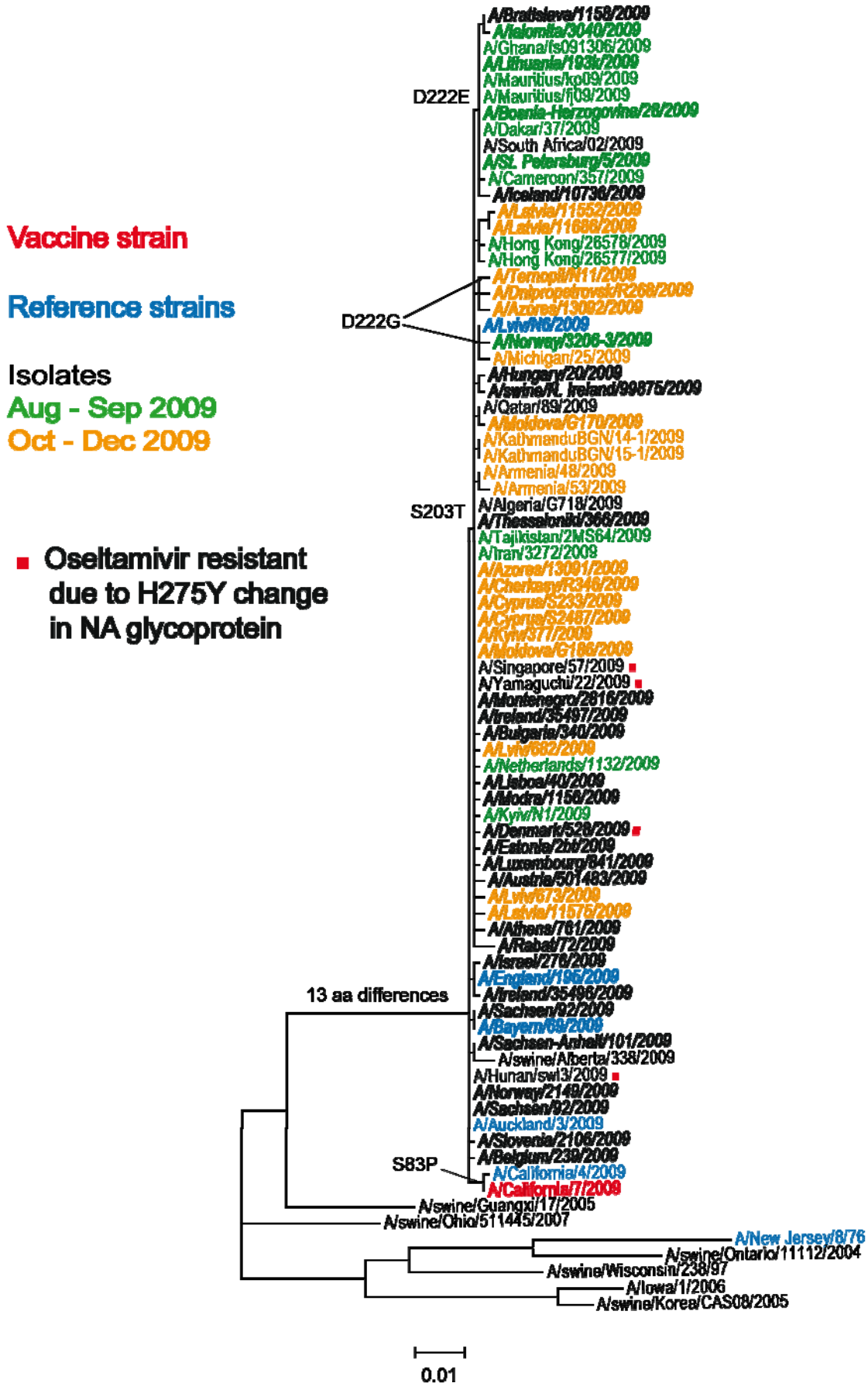
<sup>2</sup> Hyperimmune rabbit serum

<sup>3</sup> NT = not tested

Gene sequence analyses of a subset of viruses and clinical samples have also been carried out. A phylogenetic tree of the HA1 coding region of the HA gene sequences is shown in Figure 1. Viruses from Europe are highlighted in *Italic script* and the dates of sample collection are ordered by colour. It can be seen from the representative tree that the majority of recent pandemic H1N1 viruses are genetically closely related. Most recent viruses carry the amino acid substitution in the HA protein S203T. The significance of this substitution is not known but it seems not to affect the antigenicity of the virus. Amino acid substitutions at residue 222 can also be seen in the tree. This substitution has been noted previously and its significance is under appraisal by WHO ([http://www.who.int/csr/resources/publications/swineflu/cp165\\_2009\\_2812\\_review\\_d222g\\_amino\\_acid\\_substitution\\_in\\_ha\\_h1n1\\_viruses.pdf](http://www.who.int/csr/resources/publications/swineflu/cp165_2009_2812_review_d222g_amino_acid_substitution_in_ha_h1n1_viruses.pdf))

Viruses resistant to oseltamivir have been detected in low numbers and do not cluster on the phylogenetic tree and are associated with prophylactic or therapeutic use of the drug.

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



The phylogenetic tree was constructed using the 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 antiserum 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 virus isolates characterised at the WHO CC, following submission from CNRL members for the period from September 1 2009 to mid-January 2010.

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

| Country and month collected | H1N1     |          | H3N2      |           | B                    |          |                      |          |
|-----------------------------|----------|----------|-----------|-----------|----------------------|----------|----------------------|----------|
|                             | received | grown    | received  | grown     | Yamagata<br>received | grown    | Victoria<br>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                    | 1        |
| November                    |          |          |           |           |                      |          |                      |          |
| France                      |          |          | 2         | 2         |                      |          |                      |          |
| Sweden                      |          |          | 2         | 2         |                      |          | 1                    | 1        |
| December                    |          |          |           |           |                      |          |                      |          |
| January                     |          |          |           |           |                      |          |                      |          |
| Germany                     |          |          | 1         | 1         |                      |          |                      |          |
| <b>Total</b>                | <b>3</b> | <b>3</b> | <b>23</b> | <b>23</b> | <b>0</b>             | <b>0</b> | <b>2</b>             | <b>2</b> |

Antigenic analysis of representative H3N2 viruses is shown in Table 4. This HI assay was carried out on guinea pig red cells in the presence of oseltamivir phosphate. The HI results show two clear patterns of reactivity. One pattern is represented by reactivity with sera raised against reference viruses isolated from 2005 to 2008; including A/Brisbane/10/2007 and A/Uruguay/716/2007, the virus used in the 2009-2010 Northern Hemisphere seasonal vaccine. Two viruses shown in the table collected in late 2009 were antigenically similar to A/Brisbane/10/2007. The second pattern of reactivity is distinct and can be seen when viruses react well with sera raised against 2009 reference strains, including A/Perth/16/2009, the virus recommended for inclusion in the Southern Hemisphere 2010 vaccine. The Perth/16 antigenic group of viruses was first detected in early 2009. The majority of H3N2 viruses isolated recently in Europe were antigenically similar to the A/Perth/16/2009 virus and now appear to represent the predominant H3N2 virus currently circulating in Europe.

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

|                              |                  | Haemagglutination inhibition titre <sup>4</sup> |                        |                        |                       |                       |                        |                        |                         |                       |  |
|------------------------------|------------------|---|------------------------|------------------------|-----------------------|-----------------------|------------------------|------------------------|-------------------------|-----------------------|--|
|                              |                  | Post infection ferret sera                      |                        |                        |                       |                       |                        |                        |                         |                       |  |
| Viruses                      | Collection Month | A/Wis 67/05<br>F1/06                            | A/Bris 10/07<br>F29/08 | A/Uru 716/07<br>F26/08 | A/Fin 9/08<br>F12/088 | A/Jhb 15/08<br>F22/08 | A/HK 1952/09<br>F22/09 | A/HK 1985/09<br>F21/09 | A/Perth 16/09<br>F25/09 | A/Wis 15/09<br>F24/09 |  |
| Reference viruses            |                  |   |                        |                        |                       |                       |                        |                        |                         |                       |  |
| A/Wisconsin/67/2005          |                  | 1280  | 640                    | 1280                   | 320                   | 320                   | <                      | <                      | <                       | <                     |  |
| A/Brisbane/10/2007           |                  | 640   | 1280                   | 1280                   | 640                   | 320                   | <                      | <                      | <                       | <                     |  |
| A/Uruguay/716/2007           |                  | 1280  | 1280                   | 2560                   | 1280                  | 640                   | <                      | <                      | 40                      | <                     |  |
| A/Finland/9/2008             |                  | 1280  | 1280                   | 1280                   | 640                   | 640                   | <                      | 80                     | 80                      | <                     |  |
| A/Johannesburg/15/2008       |                  | 1280  | 1280                   | 1280                   | 1280                  | 2560                  | 40                     | 80                     | 80                      | 40                    |  |
| A/Hong Kong/1952/2009        |                  | <   | 80                     | 80                     | <                     | <                     | 160                    | 1280                   | 640                     | 320                   |  |
| A/Hong Kong/1985/2009        |                  | <   | 80                     | 80                     | <                     | <                     | 160                    | 1280                   | 640                     | 320                   |  |
| A/Perth/16/2009              |                  | <   | <                      | <                      | <                     | <                     | 40                     | 320                    | 640                     | 80                    |  |
| A/Wisconsin/15/2009          |                  | <   | <                      | <                      | <                     | <                     | 40                     | 640                    | 320                     | 160                   |  |
| Test viruses                 |                  |   |                        |                        |                       |                       |                        |                        |                         |                       |  |
| A/England/700/2009           | Sep-09           | 80  | 160                    | 320                    | 160                   | 320                   | <                      | 80                     | 320                     | <                     |  |
| A/Gothenburg/3/2009          | Nov-09           | 160   | 640                    | 640                    | 320                   | 640                   | 40                     | 80                     | 40                      | <                     |  |
| A/Scotland/28/2009           | Aug-09           | 40  | 40                     | 160                    | 40                    | 40                    | 160                    | 1280                   | 640                     | 320                   |  |
| A/Paris/3873/2009            | Sep-09           | <   | 80                     | 160                    | 40                    | 40                    | 80                     | 320                    | 160                     | 160                   |  |
| A/England/815/2009           | Sep-09           | 40  | 160                    | 160                    | 80                    | 80                    | 160                    | 640                    | 320                     | 160                   |  |
| A/Lyon/1715/2009             | Sep-09           | 80  | 160                    | 80                     | 40                    | 80                    | 40                     | 640                    | 640                     | 80                    |  |
| A/Bordeaux/1942/2009         | Sep-09           | <   | 160                    | 80                     | <                     | 40                    | 160                    | 1280                   | 1280                    | 640                   |  |
| A/Umeå/4/2009                | Sep-09           | 40  | 160                    | 160                    | 80                    | 160                   | 160                    | 640                    | 1280                    | 320                   |  |
| A/Gibraltar/SB226/2009       | Sep-09           | 40  | 160                    | 160                    | 80                    | 80                    | 80                     | 320                    | 320                     | 160                   |  |
| A/Paris/5870/2009            | Oct-09           | 40  | <                      | 80                     | <                     | <                     | 80                     | 320                    | 160                     | 160                   |  |
| A/Gibraltar/SB252/2009       | Oct-09           | 40  | 160                    | 160                    | 40                    | 80                    | 80                     | 320                    | 320                     | 160                   |  |
| A/Norway/3790/2009           | Oct-09           | <   | 80                     | 80                     | 40                    | 40                    | 40                     | 160                    | 320                     | 40                    |  |
| A/Finland/640/2009           | Oct-09           | 40  | 160                    | 160                    | 80                    | 80                    | 40                     | 320                    | 320                     | 80                    |  |
| A/Paris/6047/2009            | Nov-09           | 80  | 320                    | 320                    | 160                   | 160                   | 320                    | 1280                   | 1280                    | 160                   |  |
| A/Stockholm/112/2009         | Nov-09           | 80  | 320                    | 320                    | 160                   | 160                   | 320                    | 1280                   | 1280                    | 320                   |  |
| A/Lyon/3670/2009             | Nov-09           | 80  | 160                    | 160                    | 80                    | 160                   | 80                     | 640                    | 640                     | 320                   |  |
| A/Nordrhein-Westfalen/1/2010 | Jan-10           | 80  | 160                    | 160                    | 160                   | 80                    | 640                    | 2560                   | 2560                    | 1280                  |  |

Vaccine strain

<sup>4</sup> < =40

A/Uruguay/716/2008 was the virus used in vaccines in Europe for the 2009/2010 season, A/Perth/16/2009 is the virus recommended for use in the vaccine for the southern hemisphere 2010.

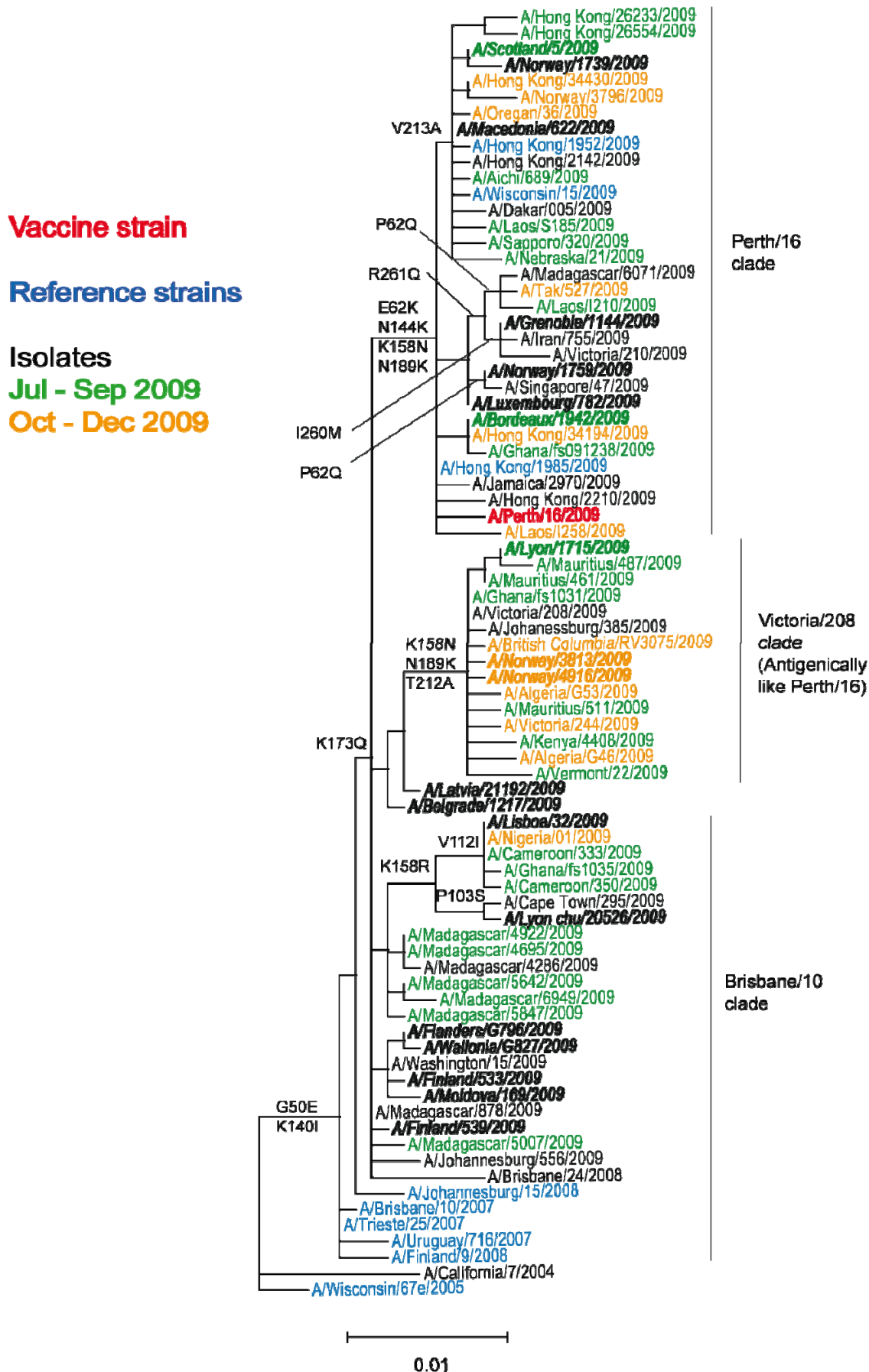
Genetic analysis of the HA gene of a group of H3N2 viruses is shown in Figure 2. Again viruses from Europe are highlighted in *Italic script* and the dates of sample collection are ordered by colour. Three distinct clades are highlighted: the Brisbane/10 clade, the Perth/16 clade and a clade with A/Victoria/208/2009 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 genetic clade and the Victoria/208 genetic clade have been isolated in recent months in Europe.

Very few H1N1 viruses have been received and genetic and antigenic analysis of these isolates is ongoing. Initial antigenic studies indicate that these viruses react less well with ferret antiserum against the previous H1N1 vaccine (A/Brisbane/59/2007) but better against ferret antiserum raised against more recently isolated reference viruses of the same genetic group.

Only two influenza B viruses from Europe collected since September were received; both were of the B/Victoria lineage of virus. Analysis is ongoing.



Figure 2. Phylogenetic comparison of H3N2 HA genes



The phylogenetic trees was constructed using the 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 antiserum have been developed. The colours indicate the date of sample collection. Isolates from Europe are in italics.

## 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.