TECHNICAL DOCUMENT

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

# Influenza virus characterisation

Summary Europe, June 2012

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## Summary

Since 01 January 2012, influenza A(H1N1)pdm09, influenza A(H3N2), and influenza B/Victoria- and B/Yamagata-lineage viruses have been detected in ECDC-affiliated countries.

- Type A viruses have predominated over type B.
- A(H3N2) viruses have predominated over A(H1N1)pdm09 viruses.
- A(H1N1)pdm09 viruses continue to show genetic drift from the vaccine virus, A/California/07/2009, but the vast majority are antigenically similar to A/California/07/2009.
- During this time period, all A(H3N2) viruses sequenced fell within five genetic groups. Test viruses isolated in mammalian cells show low titres with post-infection ferret antisera raised against egg-propagated viruses, including the new vaccine virus A/Victoria/361/2011. They react well with post-infection ferret antisera raised against A/Victoria/361/2011 and other current reference viruses exclusively propagated in tissue culture.
- Recent B/Victoria lineage viruses fell within the B/Brisbane/60/2008 genetic clade and were antigenically similar to reference cell-propagated viruses of the B/Brisbane/60/2008 genetic clade.
- Recent B/Yamagata-lineage viruses fell into two genetic clades, the B/Bangladesh/3333/2007 and B/Wisconsin/1/2010 genetic clade or into the B/Brisbane/3/2007 genetic clade; viruses in these clades are antigenically distinguishable.


Table 1 shows a summary of viruses from EU and EAA countries received by the WHO Collaborating Centre for Reference and Research on Influenza at the MRC National Institute for Medical Research in London from 1 January 2012. Viruses and/or clinical samples were submitted by 23 EU/EAA countries. Table 1 is an update of the table shown in the previous report (March 2012). The majority (72%) of viruses received were influenza A(H3N2) viruses. Influenza B viruses of the B/Yamagata lineage have predominated over those of the B/Victoria lineage at a ratio of approximately 3:2; influenza A(H1N1)pdm09 viruses were received from only a minority of countries.

## Influenza A(H1N1)pdm09 virus analyses

Influenza A(H1N1)pdm09 influenza viruses from Norway, Sweden and England have been analysed by HI assay since the previous report; the results are shown in Table 2. The analysed viruses show generally good reactivity with post-infection ferret antisera raised against the panel of reference viruses, including antiserum raised against the vaccine virus (A/California/7/2009); only two of the sixteen viruses analysed showed a reduction of eight-fold or more in reactivity with antiserum raised against A/California/7/2009, compared with the HI titre of the homologous virus.

Phylogenetic analysis of the HA1 coding region of a subset of the test viruses has been carried out (Figure 1), and further gene sequence analysis is underway. The HA genes of H1N1 viruses from EU/EAA countries cluster into two of

eight genetic groups that have been described previously. These viruses fall into genetic groups 6 and 7, which have the following amino acid substitutions in HA1:

- Group 6: **D97N and S185T,** e.g. A/St Petersburg/27/2011
- Group 7: **S143G, S185T and A197T,** e.g. A/St Petersburg/100/2011

The NA genes of the most recently analysed viruses fall into similar genetic groups (Figure 2).

# Influenza A(H3N2) virus analyses

As described before, A(H3N2) viruses have continued to be difficult to characterise antigenically by HI assay due to variable agglutination of red blood cells from guinea pigs, turkeys and humans. Approximately 70% of viruses gave sufficient titre in HA assays to be analysed by HI assay using guinea pig red blood cells in the presence of 20nM oseltamivir, added to circumvent the NA-mediated binding of H3N2 viruses to red blood cells (Lin et al. 2010).

The results of the HI assays carried out since the last report are shown in Tables 3 to 6. HI assays using post-infection ferret antiserum raised against the vaccine virus recommended for the 2011/2012 northern hemisphere influenza vaccine, A/Perth/16/2009, showed that nearly 90% of the test viruses had a reduction in HI titre of eight-fold or more compared with the titre of the homologous virus.

All tables show results using post infection antiserum raised against the newly recommended, egg-propagated vaccine virus for the northern hemisphere 2012/2013, A/Victoria/361/2011. With this antiserum, only three viruses of the 143 tested gave HI titres within four-fold of that of the homologous virus. Notably, of these three test viruses, two had been propagated in hens' eggs. In contrast, with antiserum raised against the cell-culture propagated A/Victoria/361/2011 only two of 80 test viruses showed a reduced reactivity of eight-fold or more compared with the titre of the homologous cell-propagated virus (Tables 5 and 6). The test viruses also showed good reactivity with post-infection ferret antisera raised against other reference viruses propagated exclusively in cell culture: only nine of the 143 viruses tested showed a reduction in titres of eight-fold or more with any of the antisera compared with the titres of the homologous viruses. These post-infection ferret antisera were against cell-propagated A/Alabama/5/2010, A/Hong Kong/3969/2011, A/Stockholm/18/2011, A/Finland/190/2011, A/Norway/1789/2011, A/Berlin/93/2011, and A/Athens/112/2012.

The results of HI analysis of the test viruses using antisera raised against other egg-propagated reference viruses, A/Victoria/208/2009 and A/Iowa/19/2010, showed low levels of reactivity with ferret antisera compared with the reactivity of the homologous egg-propagated viruses. The low reactivity of test viruses with antisera raised against each of the egg-adapted viruses, importantly including the new vaccine virus A/Victoria/361/2011, suggests that egg adaptation of the H3N2 reference viruses influences the immune response of the ferret. In light of these observations, results of HI tests and other serological assays with currently circulating A(H3N2) viruses continue to warrant careful consideration.

Phylogenetic analysis of the HA and NA gene sequences of representative viruses has been carried out (Figures 3 and 4). Viruses from the EU/EAA collected since January have HA genes that fall into HA gene genetic groups 3A, 3B and 3C, and group 5 and group 6 (Figure 3).

The amino acid substitutions that are associated with each of these groups are:

- Group 3A: **N144D** (resulting in the loss of a glycosylation site), **N145S and V223I,** e.g. A/Stockholm/18/2011;
- Group 3B: **N145S, A198S, V223I and N312S,** e.g. A/Athens/GR112/2012;
- Group 3C: **S45N, T48I, A198S, V223I and N312S,** e.g. A/Hong Kong/3969/2011 and the prototype vaccine virus A/Victoria/361/2011, with some viruses also carrying the substitutions **D53N**, or **Q33R and N278K**;
- Group 5: **D53N, Y94H, I230V and E280A,** e.g. A/Alabama/5/2010;
- Group 6: D53N, Y94H, S199A, I230V and E280A, e.g. A/Iowa/19/2010.

Phylogenetic analysis of the NA gene (Figure 4) shows a similar pattern of grouping but with a less distinct differentiation in those viruses which carry HA genes in genetic groups 5 and 6, although clustering of recent virus NA gene sequences in these groups seems to be emerging.

# Influenza B virus analyses

## B/Victoria-lineage viruses

B/Victoria-lineage viruses were received from nine EU/EAA Member States. The results of HI analyses of influenza B viruses of the B/Victoria lineage are shown in Table 7. All but four of the 23 test viruses showed reduced reactivity (eight-fold or more reduction in titre compared with the homologous titre) with post-infection ferret antiserum raised against the egg-propagated vaccine virus recommended for the northern hemisphere 2011/2012 season, B/Brisbane/60/2008, with the other four test viruses showing four-fold reductions in titre compared with the titre of the homologous virus. In contrast, all viruses reacted well with antisera raised against viruses genetically closely related to the vaccine virus but propagated in cells. In Table 7, these antisera are raised against B/Paris/1762/2008, B/Hong Kong/514/2009 and B/Odessa/3886/2010, which are surrogate cell-propagated antigens for the egg-propagated vaccine virus. The reactivity of test viruses with antiserum raised against B/Malta/MV636714/2011, another egg isolate, was low and similar to their reactivities with antiserum raised against B/Brisbane/60/2008.

Phylogenetic analysis of the HA1 coding region of the HA gene of representative B/Victoria lineage viruses is shown in Figure 5. The HA genes of all recently collected viruses from EU and EAA laboratories fell into Clade 1, the B/Brisbane/60 clade. The NA gene of recently isolated viruses cluster similarly with the exception of the NA gene of B/Norway/710/2012, which was representative of viruses in a group that has an intra-clade reassortment of the HA and NA genes (Figure 6).

## B/Yamagata-lineage viruses

Influenza B viruses of the B/Yamagata lineage were received from eight EU/EAA Member States. Table 8 shows the results of HI assays of the propagated viruses examined since the last report. Only five of 18 test viruses reacted within a four-fold titre of that of the homologous virus, with the post-infection ferret antiserum raised against the recommended egg-propagated vaccine virus for the northern hemisphere 2012/2013, B/Wisconsin/1/2010. However, all but one of the test viruses reacted well with post-infection ferret antiserum raised against the egg-propagated virus B/Stockholm/12/2011. Of the 18 test viruses, 12 showed good reactivity, within four-fold of the homologous titre, with post-infection ferret antiserum raised against B/Estonia/55669/2011, a virus from a distinct genetic clade.

Figure 7 shows a phylogenetic analysis of the HA1 coding region of B/Yamagata lineage viruses. The HA genes of these viruses fall into two genetic clades, one defined as Clade 3, represented by B/Bangladesh/3333/2007 (and includes B/Wisconsin/1/2010 and B/Stockholm/12/2011), and the other defined as Clade 2, represented by the reference strains B/Brisbane/3/2007 and B/Estonia/55669/2011. Viruses falling within these two clades are antigenically distinguishable.

The two clades are differentiated by substitutions at HA residues 48, 108, 150, 165, 181 and 229. The HA gene of viruses of Clade 2 encodes **K48**, **A108**, **S150**, **N165**, **A181** and **G229**; the HA gene of viruses in Clade 3 encodes **R48**, **P108**, **I150**, **Y165**, **T181** and **D229**.

Clade 2 seems genetically homogenous, but Clade 3 can be sub-divided into four genetic groups:

- a group defined by the amino acid substitution **N202S** similar to B/Wisconsin/1/2010;
- a group defined by the substitution **T181K** (e.g. B/Ireland/M1522/2012);
- a group defined by the substitution **M251V** with the substitutions **T181A** and **K253R** (e.g. B/Serbia/1894/2011);
- a group defined by the substitution **M251V** with the substitutions **V29A** and **L172Q** (e.g. B/Stockholm/12/2011).

Phylogenetic analysis of the NA gene of recently analysed and representative influenza B viruses of the B/Yamagata lineage are shown in Figure 8. The clustering of the NA gene is similar to that seen in the HA gene.

*The official 'Report prepared for the WHO annual consultation on the composition of influenza vaccine for the Northern Hemisphere', which presents the results of the WHO Vaccine Composition Meeting held at WHO Geneva from 20 to 22 February 2012, can be found at:* http://www.nimr.mrc.ac.uk/documents/about/interim-report-feb-2012.pdf

## *Note on figures*

The phylogenetic trees were constructed using RAxML and drawn using FigTree. The bars indicate the proportion of nucleotide changes in the sequence. Reference strains are viruses to which post-infection ferret antisera have been raised. The colours indicate the date of sample collection. Isolates from WHO NICs in ECDC countries are highlighted by an orange box. Sequences for some of the viruses from non-EU/EAA countries were recovered from GISAID, and we acknowledge all laboratories which submitted sequences directly to the London WHO CC.

Table 1. **Summary of clinical samples and isolates received from ECDC-affiliated countries, collection dates from 1 January 2012**

| MONTH | A Untyped * | H1N1pdm09 | | H3N2 | | B Untyped * | B Yamagata lineage | | B Victoria lineage | |
|---|---|---|---|---|---|---|---|---|---|---|
| Country | | Number received | Number propagated | Number received | Number propagated | | Number received | Number propagated | Number received | Number propagated |
| **JANUARY** | | | | | | | | | | |
| Austria | | | | 4 | 4 | | 1 | 1 | 1 | 1 |
| Bulgaria | | | | 4 | 3 | | | | | |
| Denmark | | | | 2 | 1 | | | | | |
| Estonia | | | | 1 | 0 | | | | | |
| Finland | | | | 3 | 2 | | | | | |
| France | | | | 4 | 4 | | | | | |
| Germany | | | | 14 | 13 | | | | 1 | 1 |
| Greece | | | | 8 | 7 | 5 | 1 | 1 | 4 | 4 |
| Iceland | | | | 9 | 7 | | | | | |
| Ireland | | | | 7 | 2 | | 1 | 1 | 1 | 1 |
| Italy | | | | 13 | 12 | | | | 1 | 1 |
| Latvia | | | | 6 | 5 | | 1 | 1 | | |
| Netherlands | | | | 2 | 2 | | | | | |
| Norway | | 5 | 2 | 11 | 11 | | | | | |
| Portugal | | | | 7 | 4 | | | | | |
| Romania | | | | 3 | 3 | | | | | |
| Slovenia | | | | 3 | 3 | | | | | |
| Spain | | 1 | 0 | 18 | 10 | | 2 | 2 | | |
| Sweden | | 2 | 2 | 8 | 7 | | 1 | 1 | | |
| United Kingdom | | | | 3 | 3 | | 1 | 1 | | |
| **FEBRUARY** | | | | | | | | | | |
| Bulgaria | | | | 8 | 8 | | | | | |
| Denmark | | | | 7 | 4 | | 3 | 3 | 2 | 2 |
| Estonia | | | | 18 | 2 | | | | | |
| Finland | | | | 4 | 2 | | | | 1 | 1 |
| Greece | | | | 14 | 13 | 4 | 4 | 4 | 2 | 2 |
| Iceland | | | | 11 | 11 | | | | | |
| Ireland | | 1 | 1 | 1 | 1 | | | | 1 | 1 |
| Italy | | | | 6 | 5 | | 5 | 5 | 1 | 1 |
| Norway | | 10 | 6 | 14 | 14 | | 3 | 3 | 1 | 1 |
| Portugal | | | | 1 | 1 | | 2 | 1 | | |
| Slovenia | | 1 | 1 | 9 | 7 | | 1 | 0 | 1 | 0 |
| Sweden | | 3 | 3 | | | | 1 | 1 | 1 | 1 |
| United Kingdom | | | | 5 | 4 | | 1 | 1 | 1 | 1 |
| **MARCH** | | | | | | | | | | |
| Denmark | | 3 | 0 | 8 | 7 | | 2 | 1 | 1 | 0 |
| Estonia | | | | 11 | 7 | | | | | |
| Finland | | | | 1 | 1 | | | | | |
| Iceland | | | | 3 | 2 | | | | | |
| Ireland | | | | 4 | 4 | | | | | |
| Italy | | | | 4 | 1 | | 6 | 6 | 1 | 0 |
| Norway | | | | | | | | | 1 | 0 |
| Portugal | | | | 6 | 4 | | 3 | 2 | 1 | 1 |
| Slovenia | | 1 | 0 | 9 | 7 | | 2 | 0 | 4 | 1 |
| Sweden | | | | 1 | 1 | | 1 | 1 | | |
| United Kingdom | | 1 | 1 | 3 | 3 | | 1 | 1 | 1 | 1 |
| **APRIL** | | | | | | | | | | |
| Denmark | | | | 4 | 3 | | 1 | 0 | 1 | 1 |
| Estonia | | | | 7 | 3 | | 1 | 1 | | |
| Iceland | | | | 1 | 1 | | | | | |
| Ireland | | | | 10 | 2 | | | | | |
| Slovenia | | | | 1 | 1 | 1 | | | | |
| Sweden | | 1 | 1 | 4 | 3 | | | | | |
| United Kingdom | | | | 4 | 4 | | 1 | 1 | 2 | 2 |
| **MAY** | | | | | | | | | | |
| Finland | | | | | | | | | 1 | 1 |
| **Total Received = 426** | **0** | **29** | **17** | **309** | **229** | **10** | **46** | **39** | **32** | **25** |
| | | **7%** | | **72.5%** | | **2%** | **11%** | | **7.5%** | |

## Table 2. **Antigenic analysis of A(H1N1)pdm viruses by HI (turkey RBCs)**

| | | | Haemagglutination inhibition titre[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Post infection ferret sera | | | | | | | |
| Viruses | Collection date | Passage History | A/Cal 7/09 F29/11 | A/Bayern 69/09 F11/11 | A/L.viv N6/2009 C4/34/09 | A/C'church 16/2010 F30/10 | A/HK 3934/2011 F21/11 | A/Astrak 1/2011 F22/11 | A/St. P'burg 27/11 F23/11 | A/St. P'burg 100/11 F24/11 |
| ***REFERENCE VIRUSES*** | | | | | | | | | | |
| A/California/7/2009 | 2009-04-09 | E1/E2 | 1280 | 2560 | 1280 | 1280 | 1280 | 1280 | 1280 | 2560 |
| A/Bayern/69/2009 | 2009-07-01 | MDCK5 | 160 | 640 | 320 | 160 | 80 | 160 | 160 | 80 |
| A/L.viv/N6/2009 | 2009-10-27 | M4/S1/MDCK2 | 1280 | 2560 | 1280 | 320 | 160 | 320 | 320 | 320 |
| A/Christchurch/16/2010 | 2010-07-12 | E2/E1 | 5120 | 5120 | 5120 | 5120 | 5120 | 5120 | 5120 | 5120 |
| A/Hong Kong/3934/2011 | 2011-03-29 | MDCK2/MDCK2 | 2560 | 640 | 640 | 1280 | 2560 | 2560 | 2560 | 5120 |
| A/Astrakhan/1/2011 | 2011-02-28 | MDCK1/MDCK5 | 2560 | 1280 | 1280 | 1280 | 2560 | 2560 | 2560 | 5120 |
| A/St. Petersburg/27/2011 | 2011-02-14 | E1/E2 | 2560 | 2560 | 2560 | 1280 | 2560 | 2560 | 2560 | 5120 |
| A/St. Petersburg/100/2011 | 2011-03-14 | E1/E2/E1 | 2560 | 1280 | 2560 | 2560 | 2560 | 2560 | 2560 | 5120 |
| ***TEST VIRUSES*** | | | | | | | | | | |
| A/Slovenia/421/2012 | 20/02/2012 | Mx/MDCK3 | 1280 | 640 | 640 | 640 | 1280 | 1280 | 640 | 2560 |
| A/Norway/418/2012 | 02/02/2012 | MDCK1/MDCK1 | 2560 | 1280 | 1280 | 2560 | 2560 | 2560 | 5120 | 5120 |
| A/Norway/421/2012 | 01/02/2012 | MDCK1/MDCK2 | 5120 | 2560 | 2560 | 2560 | 5120 | 5120 | 5120 | 5120 |
| A/Norway/446/2012 | 02/02/2012 | MDCK2/MDCK2 | 5120 | 2560 | 5120 | 2560 | 5120 | 5120 | 5120 | 5120 |
| A/Norway/560/2012 | 02/02/2012 | MDCK2/MDCK2 | 640 | 320 | 640 | 640 | 640 | 1280 | 1280 | 2560 |
| A/Norway/716/2012 | 17/02/2012 | MDCK1/MDCK2 | 1280 | 640 | 1280 | 1280 | 2560 | 2560 | 2560 | 5120 |
| A/Norway/717/2012 | 19/02/2012 | MDCK1/MDCK2 | 640 | 320 | 640 | 640 | 1280 | 1280 | 1280 | 2560 |
| A/Norway/771/2012 | 29/01/2012 | MDCK1/MDCK1 | 5120 | 2560 | 5120 | 5120 | 5120 | 5120 | 5120 | 5120 |
| A/Norway/301/2012 | 18/01/2012 | MDCK1/MDCK3 | 1280 | 640 | 1280 | 640 | 2560 | 1280 | 2560 | 5120 |
| A/Ireland/12M17525/2012 | 29/02/2012 | C1/SIAT1 | 1280 | 1280 | 1280 | 1280 | 1280 | 1280 | 1280 | 2560 |
| A/Stockholm/9/2012 | 2012-01-05 | C2/MDCK2 | 640 | 640 | 1280 | 640 | 1280 | 640 | 640 | 1280 |
| A/Stockholm/18/2012 | 2012-02-27 | C3/MDCK3 | 80 | 640 | 160 | 80 | 40 | 40 | 80 | 80 |
| A/Stockholm/17/2012 | 2012-02-17 | C2/MDCK2 | 160 | 160 | 160 | 160 | 320 | 160 | 160 | 320 |
| A/Stockholm/16/2012 | 2012-02-21 | C2/MDCK2 | 640 | 320 | 640 | 640 | 640 | 640 | 640 | 2560 |
| A/Stockholm/25/2012 | 2012-04-07 | C2/MDCK2 | 40 | 160 | 80 | 40 | < | < | < | < |
| A/England/344/2012 | 2012-03-01 | SIAT2/MDCK2 | 1280 | 640 | 640 | 640 | 1280 | 1280 | 1280 | 2560 |

1. < = <40

Vaccine virus

Sequence in phylogenetic tree

Figure 1. **Phylogenetic comparison of influenza A(H1N1)pdm HA genes (HA1 coding region)**

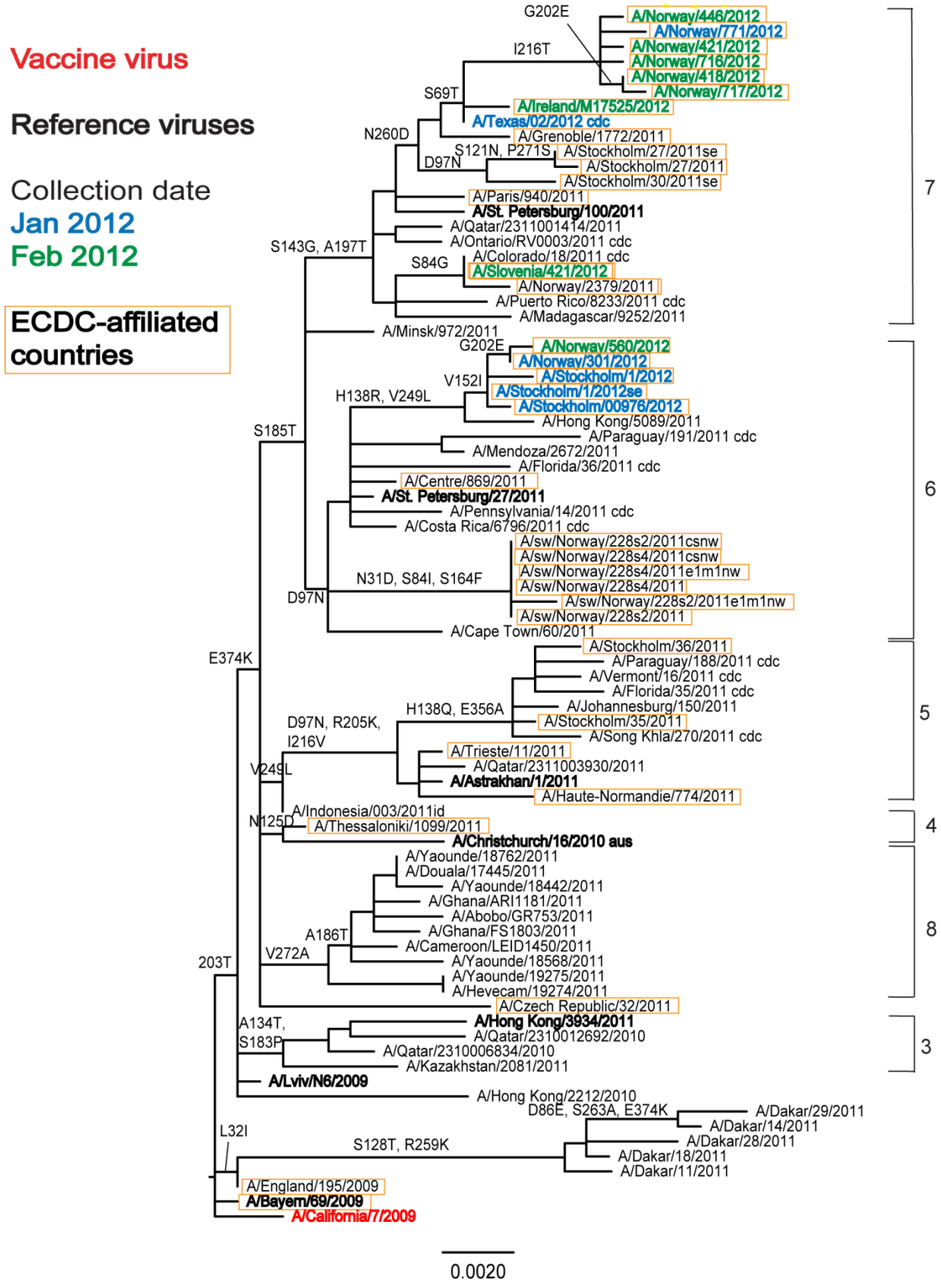
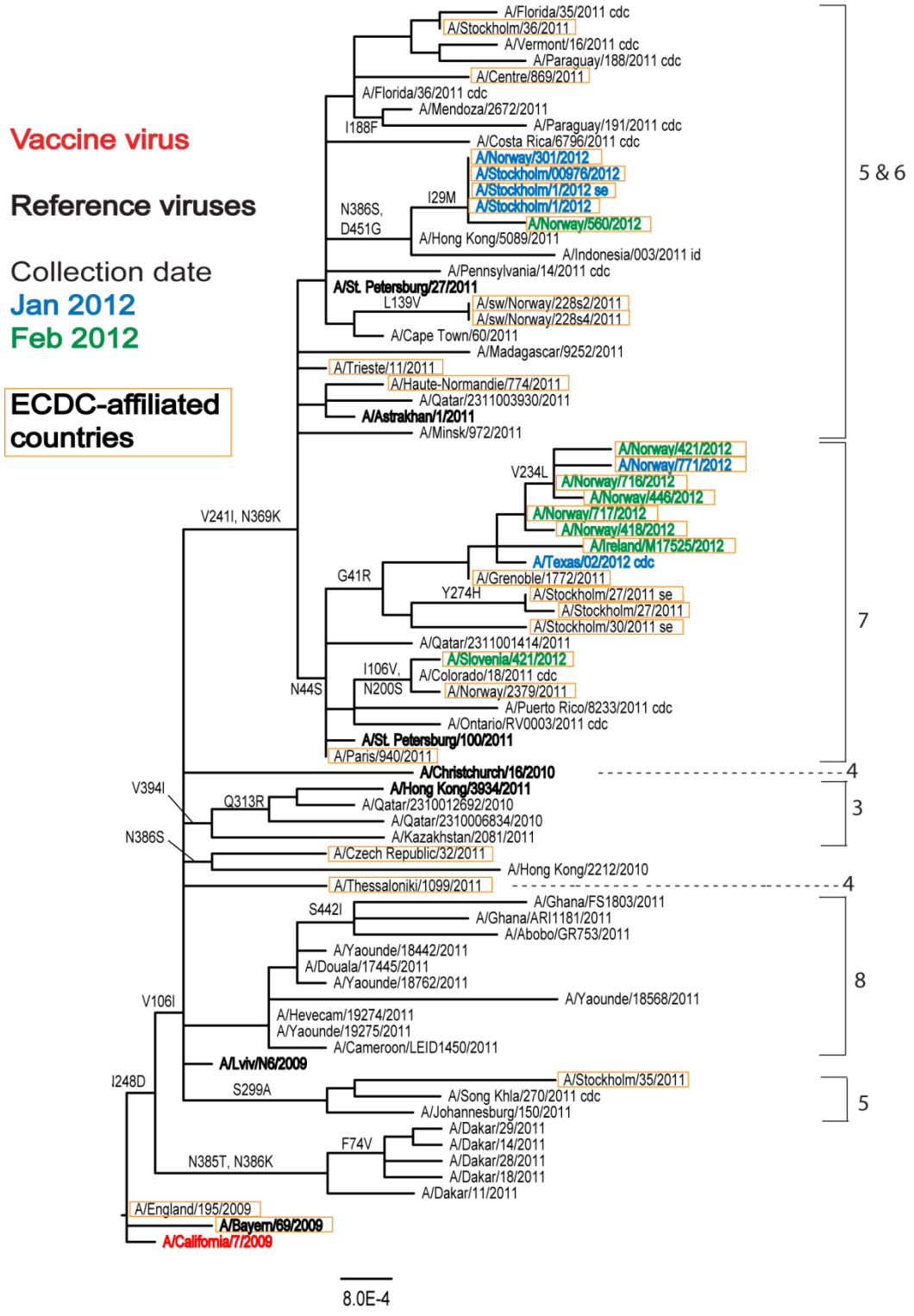


Figure 2. **Phylogenetic comparison of influenza A(H1N1)pdm NA genes**



## Table 3. **Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBCs with 20nM oseltamivir)**

| | | | Haemagglutination inhibition titre[1] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Post infection ferret sera | | | | | | | | | |
| Viruses | Collection Date | Passage History | A/Perth 16/09 | A/Vic 208/09 | A/Ala 5/10 | A/HK 3969/11 | A/Stock 18/11 | A/Iowa 19/10 | A/Fin 190/11 | A/Norway 1789/11 | A/Vic 361/11 | A/Berlin 93/11 |
| | | | F35/11 | F7/10 | F27/10 | F27/11 | F28/11 | F15/11 | F01/12 | F03/12 | Egg F05/12 | T/C F11/12 |
| Genetic group | | | | | group 5 | group 3C | group 3A | group 6 | group 3C | group 3C | group 3C | group 3C |
| ***REFERENCE VIRUSES*** | | | | | | | | | | | | |
| A/Perth/16/2009 | 2009-07-04 | E3/E2 | 1280 | 80 | 160 | 640 | 160 | 160 | 160 | 320 | 80 | 320 |
| A/Victoria/208/2009 | 2009-06-02 | E3/E1 | 1280 | 2560 | 1280 | 2560 | 1280 | 5120 | 2560 | 2560 | 2560 | 5120 |
| A/Alabama/5/2010 | 2010-07-13 | MK1/C2/SIAT2 | 40 | < | 160 | 320 | 80 | 160 | 80 | 160 | 40 | 160 |
| A/Hong Kong/3969/2011 | 2011-05-19 | MDCK2/SIAT4 | 160 | 160 | 160 | 640 | 320 | 320 | 640 | 640 | 160 | 1280 |
| A/Stockholm/18/2011 | 2011-03-28 | MDCK2/SIAT3 | 40 | 40 | 80 | 320 | 320 | 160 | 160 | 320 | 80 | 320 |
| A/Iowa/19/2010 | 2010-12-30 | E3/E1 | 640 | 2560 | 1280 | 2560 | 1280 | 5120 | 2560 | 2560 | 1280 | 5120 |
| A/Finland/190/2011 | 2011-11-25 | Cx/SIAT1 | 160 | 160 | 320 | 640 | 320 | 320 | 1280 | 640 | 160 | 1280 |
| A/Norway/1789/2011 | 2011-08-02 | Cx/SIAT1 | 160 | 160 | 160 | 640 | 160 | 320 | 640 | 640 | 160 | 1280 |
| A/Victoria/361/2011 | 2011-10-24 | E3/E1 | 320 | 1280 | 320 | 1280 | 160 | 1280 | 1280 | 1280 | 5120 | 1280 |
| A/Berlin/93/2011 | 2011-12-07 | NVD3/S2 | 160 | 160 | 320 | 1280 | 320 | 640 | 640 | 1280 | 320 | 1280 |
| ***TEST VIRUSES*** | | | | | | | | | | | | |
| A/Glasgow/407664/2012 | 2012-04-03 | SIAT2 | 80 | 160 | 160 | 640 | 320 | 320 | 640 | 640 | 80 | 1280 |
| A/Glasgow/407665/2012 | 2012-04-03 | SIAT2 | 160 | 160 | 320 | 640 | 320 | 320 | 640 | 640 | 160 | 1280 |
| A/Norway/326/2012 | 2012-01-30 | SIAT1/SIAT1 | 80 | 80 | 160 | 640 | 160 | 160 | 640 | 640 | 160 | 1280 |
| A/Norway/337/2012 | 2012-01-30 | SIAT1/SIAT2 | 80 | 80 | 160 | 320 | 160 | 160 | 320 | 320 | 160 | 640 |
| A/Norway/399/2012 | 2012-01-30 | SIAT1/SIAT1 | 80 | 160 | 160 | 640 | 320 | 320 | 640 | 640 | 160 | 1280 |
| A/Norway/428/2012 | 2012-01-31 | SIAT1/SIAT1 | 160 | 160 | 320 | 1280 | 320 | 640 | 1280 | 1280 | 320 | 1280 |
| A/Norway/433/2012 | 2012-02-05 | SIAT1/SIAT1 | 160 | 160 | 320 | 1280 | 640 | 640 | 1280 | 1280 | 320 | 1280 |
| A/Norway/479/2012 | 2012-02-08 | MDCK2/SIAT2 | 80 | 80 | 160 | 320 | 160 | 160 | 640 | 320 | 160 | 640 |
| A/Norway/528/2012 | 2012-02-13 | SIAT1/SIAT1 | 80 | 160 | 320 | 640 | 640 | 320 | 640 | 1280 | 160 | 1280 |
| A/Norway/531/2012 | 2012-02-13 | SIAT1/SIAT1 | 160 | 160 | 320 | 1280 | 320 | 640 | 1280 | 640 | 320 | 1280 |
| A/Norway/547/2012 | 2012-01-24 | SIAT1/SIAT2 | 80 | 80 | 160 | 320 | 320 | 160 | 320 | 320 | 160 | 640 |
| A/Norway/606/2012 | 2012-02-20 | SIAT1/SIAT1 | 60 | 160 | 320 | 640 | 320 | 320 | 640 | 640 | 160 | 1280 |
| A/Norway/624/2012 | 2012-02-20 | SIAT1/SIAT1 | 160 | 160 | 320 | 640 | 320 | 320 | 640 | 640 | 160 | 1280 |
| A/Slovenia/94/2012 | 2012-01-16 | MDCKx/SIAT2 | 80 | 160 | 320 | 640 | 320 | 320 | 640 | 640 | 160 | 640 |
| A/Slovenia/326/2012 | 2012-02-13 | MDCKx/SIAT2 | 80 | 160 | 160 | 320 | 320 | 160 | 320 | 320 | 80 | 640 |
| A/Slovenia/663/2012 | 2012-03-05 | MDCKx/SIAT2 | 80 | 160 | 160 | 640 | 320 | 320 | 640 | 640 | 160 | 640 |
| A/Slovenia/910/2012 | 2012-03-19 | MDCKx/SIAT2 | 40 | 80 | 160 | 640 | 160 | 160 | 640 | 640 | 80 | 640 |
| A/Slovenia/913/2012 | 2012-03-19 | MDCKx/SIAT2 | 40 | 80 | 160 | 640 | 160 | 160 | 320 | 640 | 80 | 640 |
| A/Glasgow/407581/2012 | 2012-03-30 | SIAT2 | 80 | 80 | 160 | 320 | 320 | 160 | 320 | 320 | 80 | 640 |
| A/Slovenia/646/2012 | 2012-03-05 | MDCKx/SIAT3 | 40 | 80 | 160 | 640 | 160 | 160 | 320 | 160 | 40 | 640 |
| A/Norway/342/2012 | 2012-01-26 | SIAT1/SIAT2 | 80 | 160 | 320 | 640 | 320 | 160 | 640 | 640 | 320 | 640 |
| A/Norway/410/2012 | 2012-02-01 | SIAT2/SIAT2 | 40 | 80 | 80 | 320 | 160 | 80 | 320 | 320 | 80 | 640 |
| A/Norway/564/2012 | 2012-02-10 | SIAT1/SIAT2 | 80 | 80 | 160 | 320 | 640 | 160 | 320 | 320 | 80 | 640 |
| A/Norway/596/2012 | 2012-02-09 | SIAT2/SIAT2 | 80 | 80 | 160 | 320 | 160 | 160 | 320 | 320 | 80 | 640 |
| A/Norway/625/2012 | 2012-02-20 | SIAT2/SIAT2 | 80 | 80 | 320 | 320 | 160 | 320 | 320 | 320 | 160 | 640 |
| A/Norway/657/2012 | 2012-02-22 | SIAT2/SIAT2 | 80 | 80 | 320 | 320 | 160 | 320 | 320 | 320 | 160 | 640 |
| A/Norway/722/2012 | 2012-02-15 | SIAT1/SIAT2 | 80 | 80 | 160 | 320 | 160 | 160 | 320 | 320 | 160 | 640 |
| A/Slovenia/274/2012 | 2012-02-06 | P1/SIAT1 | 40 | 160 | 160 | 160 | 160 | 160 | 160 | 640 | 80 | 640 |
| A/Estonia/66073/2012 | 2012-03-06 | MDCK2/SIAT1 | 40 | 80 | 40 | 80 | 160 | 80 | 320 | 320 | 160 | 640 |
| A/Estonia/66788/2012 | 2012-03-27 | MDCK1/SIAT1 | 160 | 160 | 320 | 320 | 320 | 640 | 320 | 640 | 320 | 1280 |
| A/Estonia/66234/2012 | 2012-03-09 | MDCK2/SIAT1 | 160 | 160 | 320 | 640 | 320 | 640 | 1280 | 1280 | 640 | 1280 |
| A/Estonia/66837/2012 | 2012-03-28 | MDCK1/SIAT1 | 40 | 40 | 80 | 160 | 80 | 320 | 160 | 320 | 160 | 320 |
| A/Ireland/12M28404/2012 | 2012-04-11 | SIAT3 | 160 | 320 | 160 | 640 | 640 | 320 | 640 | 640 | 160 | 640 |
| A/Estonia/66103/2012 | 2012-03-07 | MDCK2/SIAT2 | 80 | 160 | 160 | 1280 | 640 | 320 | 640 | 640 | 160 | 1280 |
| A/Estonia/66239/2012 | 2012-03-09 | MDCK2/SIAT2 | 40 | 160 | 80 | 640 | 320 | 160 | 640 | 320 | 80 | 640 |
| A/Estonia/66240/2012 | 2012-03-09 | MDCK1/SIAT2 | 40 | 80 | 80 | 160 | 320 | 160 | 160 | 320 | 80 | 640 |
| A/Estonia/67179/2012 | 2012-04-09 | MDCK1/SIAT3 | 160 | 320 | 640 | 1280 | 640 | 640 | 1280 | 1280 | 160 | 2560 |
| A/Estonia/67238/2012 | 2012-04-10 | MDCK1/SIAT3 | 160 | 160 | 640 | 1280 | 640 | 640 | 1280 | 1280 | 160 | 1280 |
| A/Estonia/67221/2012 | 2012-04-11 | MDCK1/SIAT2 | 160 | 320 | 640 | 1280 | 640 | 640 | 1280 | 640 | 160 | 1280 |

1. < = <40

Vaccine virus

Vaccine virus

Sequence in phylogenetic tree

## Table 4. **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/Perth 16/09 F35/11 | A/Vic 208/09 F7/10 | A/Ala 5/10 F27/10 | A/HK 3969/11 F27/11 | A/Stock 18/2011 F28/11 | A/Iowa 19/2010 F15/11 | A/Fin 190/11 F01/12 | A/Norway 1789/11 F03/12 | A/Vic 361/11 Egg F05/12 | A/Berlin 93/11 TC F11/12 |
| *REFERENCE VIRUSES* | | | | | | | | | | | | |
| A/Perth/16/2009 | 2009-07-04 | E3/E1 | 1280 | | 160 | 640 | 320 | 320 | 320 | 640 | 160 | 640 |
| A/Victoria/208/2009 | 2009-06-02 | E3/E1 | 1280 | 5120 | 2560 | 5120 | 2560 | 5120 | 5120 | 5120 | 5120 | 5120 |
| A/Alabama/5/2010 | 2010-07-13 | MK1/M2/SIAT1 | 40 | 40 | 160 | 320 | 160 | 160 | 160 | 320 | 40 | 320 |
| A/Hong Kong/3969/2011 | 2011-05-19 | MDCK3 | 160 | 160 | 160 | 640 | 320 | 320 | 640 | 1280 | 320 | 1280 |
| A/Stockholm/18/2011 | 2011-03-28 | MDCK2/SIAT2 | 40 | 80 | 80 | 320 | 320 | 80 | 160 | 320 | 160 | 640 |
| A/Iowa/19/2010 | 2010-12-30 | E3/E1 | 640 | 2560 | 1280 | 2560 | 2560 | 5120 | 5120 | 5120 | 2560 | 5120 |
| A/Finland/190/2011 | 2011-11-25 | Cx/SIAT3 | 160 | 160 | 160 | 640 | 320 | 320 | 1280 | 1280 | 320 | 1280 |
| A/Norway/1789/2011 | 2011-08-02 | Cx/SIAT3 | 160 | 320 | 160 | 1280 | 640 | 640 | 1280 | 1280 | 320 | 2560 |
| A/Victoria/361/2011 | 2011-10-24 | E3/E1 | 640 | 2560 | 320 | 1280 | 320 | 1280 | 2560 | 1280 | 5120 | 2560 |
| A/Victoria/361/2011 | 2011-10-24 | E3/E1S2 | 320 | 1280 | 160 | 640 | 160 | 640 | 1280 | 640 | 5120 | 1280 |
| A/Berlin/93/2011 | 2011-12-07 | NVD3/E3 | 160 | 640 | 160 | 640 | 160 | 640 | 1280 | 640 | 5120 | 2560 |
| A/Berlin/93/2011 | 2011-12-07 | NVD3/S2 | 160 | 640 | 160 | 640 | 320 | 640 | 1280 | 1280 | 320 | 2560 |
| *TEST VIRUSES* | | | | | | | | | | | | |
| A/Bulgaria/41/2012 | 2012-01-18 | C3/SIAT1 | 40 | 160 | 80 | 320 | 160 | 80 | 160 | 320 | 80 | 640 |
| A/Bulgaria/42/2012 | 2012-01-18 | C3/SIAT1 | 40 | 160 | 40 | 160 | 160 | 80 | 160 | 320 | 40 | 320 |
| A/Bulgaria/43/2012 | 2012-01-18 | C3/SIAT1 | 80 | 160 | 160 | 320 | 160 | 160 | 640 | 640 | 160 | 1280 |
| A/Bulgaria/217/2012 | 2012-02-09 | C3/SIAT1 | 40 | 320 | 80 | 320 | 160 | 160 | 320 | 640 | 80 | 640 |
| A/Bulgaria/218/2012 | 2012-02-09 | C3/SIAT1 | 40 | 320 | 80 | 160 | 160 | 160 | 320 | 320 | 80 | 640 |
| A/Bulgaria/280/2012 | 2012-02-20 | C3/SIAT1 | 80 | 160 | 320 | 320 | 320 | 320 | 640 | 640 | 160 | 1280 |
| A/Bulgaria/182/2012 | 2012-02-02 | C3/SIAT1 | 80 | 640 | 320 | 640 | 320 | 640 | 640 | 640 | 160 | 1280 |
| A/Bulgaria/311/2012 | 2012-02-24 | C3/SIAT1 | 40 | 320 | 160 | 320 | 160 | 160 | 320 | 320 | 80 | 1280 |
| A/Bulgaria/312/2012 | 2012-02-24 | C3/SIAT1 | 80 | 320 | 160 | 320 | 320 | 160 | 640 | 640 | 160 | 1280 |
| A/Bulgaria/313/2012 | 2012-02-24 | C3/SIAT1 | 80 | 320 | 160 | 640 | 320 | 320 | 640 | 640 | 160 | 1280 |
| A/Bulgaria/314/2012 | 2012-02-24 | C3/SIAT1 | 80 | 320 | 320 | 640 | 320 | 320 | 640 | 640 | 160 | 1280 |
| A/Stockholm/32/2011 | 2011-11-27 | NVD3 | 320 | 640 | 640 | 1280 | 640 | 640 | 2560 | 1280 | 320 | 2560 |
| A/Stockholm/32/2011 | 2011-11-27 | E3 | 160 | 640 | 160 | 640 | 160 | 640 | 1280 | 1280 | 5120 | 1280 |
| A/Stockholm/32/2011 | 2011-11-27 | E4 | 160 | 320 | 160 | 640 | 160 | 640 | 1280 | 640 | 2560 | 1280 |
| A/Slovenia/118/2012 | 2012-01-09 | Mx/SIAT1 | 40 | 80 | 40 | 160 | 80 | 80 | 320 | 320 | 40 | 640 |
| A/Slovenia/402/2012 | 2012-02-20 | Mx/SIAT1 | 40 | 160 | 80 | 320 | 160 | 160 | 640 | 640 | 160 | 640 |
| A/Slovenia/435/2012 | 2012-02-20 | Mx/SIAT1 | 40 | 160 | 80 | 640 | 160 | 160 | 640 | 640 | 80 | 1280 |
| A/Slovenia/459/2012 | 2012-02-20 | Mx/SIAT1 | 160 | 320 | 320 | 640 | 640 | 640 | 2560 | 1280 | 320 | 2560 |
| A/Slovenia/566/2012 | 2012-02-27 | Mx/SIAT1 | 40 | 160 | 80 | 320 | 160 | 160 | 320 | 640 | 80 | 640 |
| A/Slovenia/599/2012 | 2012-02-27 | Mx/SIAT1 | 80 | 320 | 320 | 640 | 640 | 640 | 1280 | 1280 | 320 | 2560 |
| A/Slovenia/637/2012 | 2012-03-05 | Mx/SIAT1 | 160 | 640 | 320 | 1280 | 640 | 640 | 1280 | 1280 | 320 | 2560 |
| A/Slovenia/815/2012 | 2012-03-12 | Mx/SIAT1 | 40 | 160 | 80 | 320 | 160 | 160 | 640 | 640 | 160 | 1280 |
| A/Slovenia/879/2012 | 2012-03-19 | Mx/SIAT1 | 160 | 320 | 160 | 1280 | 640 | 640 | 1280 | 1280 | 160 | 2560 |
| A/Slovenia/1049/2012 | 2012-04-02 | Mx/SIAT1 | 160 | 320 | 160 | 640 | 320 | 320 | 640 | 1280 | 160 | 1280 |
| | | | Vaccine | | | | | | | | Vaccine | |

## Table 5. **Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBCs with 20nM oseltamivir)**

| | | | Haemagglutination inhibition titre[1] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Post infection ferret sera | | | | | | | | | |
| Viruses | Collection Date | Passage History | A/Perth 16/09 | A/Vic 208/09 | A/Ala 5/10 | A/HK 3969/11 | A/Stock 18/11 | A/Iowa 19/10 | A/Vic 361/11 | A/Berlin 93/11 | A/Vic 361/11 | A/Athens 112/12 |
| | | | F35/11 | F7/10 | F27/10 | F27/11 | F28/11 | F15/11 | Egg F05/12 | T/C F11/12 | T/C F15/12 | F16/12 |
| Genetic group | | | | | group 5 | group 3C | group 3A | group 6 | group 3C | group 3C | group 3C | group 3B |
| ***REFERENCE VIRUSES*** | | | | | | | | | | | | |
| A/Perth/16/2009 | 2009-07-04 | E3/E2 | 1280 | 80 | 320 | 1280 | 320 | 320 | 320 | 640 | 640 | 1280 |
| A/Victoria/208/2009 | 2009-06-02 | E3/E1 | 1280 | 5120 | 2560 | 5120 | 2560 | 5120 | 5120 | 5120 | 5120 | 5120 |
| A/Alabama/5/2010 | 2010-07-13 | MK1/C2/SIAT2 | 40 | 40 | 160 | 640 | 160 | 160 | 80 | 320 | 160 | 640 |
| A/Hong Kong/3969/2011 | 2011-05-19 | MDCK2/SIAT4 | 80 | 160 | 320 | 1280 | 320 | 320 | 640 | 1280 | 2560 | 1280 |
| A/Stockholm/18/2011 | 2011-03-28 | MDCK2/SIAT4 | 40 | 40 | 80 | 640 | 160 | 80 | 160 | 320 | 160 | 640 |
| A/Iowa/19/2010 | 2010-12-30 | E3/E1 | 640 | 5120 | 1280 | 5120 | 1280 | 5120 | 2560 | 5120 | 5120 | 5120 |
| A/Victoria/361/2011 | 2011-10-24 | E3/E2 | 320 | 640 | 320 | 1280 | 160 | 640 | 5120 | 640 | 640 | 320 |
| A/Berlin/93/2011 | 2011-12-07 | NVD3/S2 | 160 | 160 | 320 | 1280 | 320 | 320 | 320 | 1280 | 640 | 1280 |
| A/Victoria/361/2011 | 2011-10-24 | M2/S2 | 160 | 160 | 320 | 1280 | 320 | 320 | 320 | 1280 | 640 | 1280 |
| A/Athens/112/2012 | 2012-02-01 | SIAT4 | 80 | 160 | 320 | 1280 | 320 | 320 | 320 | 1280 | 640 | 1280 |
| ***TEST VIRUSES*** | | | | | | | | | | | | |
| A/Denmark/38/2012 | 2012-03-05 | SIAT2 | 160 | 320 | 640 | 1280 | 320 | 640 | 320 | 1280 | 1280 | 2560 |
| A/Iceland/66/2011 | 2011-12-07 | MDCK1/SIAT1 | 80 | 160 | 320 | 640 | 160 | 320 | 160 | 640 | 320 | 640 |
| A/Iceland/67/2011 | 2011-12-08 | MDCK2/SIAT1 | 80 | 160 | 320 | 640 | 320 | 320 | 160 | 640 | 640 | 640 |
| A/Iceland/68/2011 | 2011-12-13 | MDCK3/SIAT1 | 40 | 80 | 160 | 160 | 80 | 160 | 80 | 160 | 160 | 320 |
| A/Iceland/69/2011 | 2011-12-22 | MDCK3/SIAT1 | 320 | 160 | 640 | 1280 | 320 | 640 | 320 | 1280 | 640 | 1280 |
| A/Iceland/70/2011 | 2011-12-28 | MDCK2/SIAT1 | 80 | 160 | 320 | 640 | 320 | 320 | 160 | 1280 | 640 | 640 |
| A/Iceland/02/2012 | 2012-01-13 | MDCK2/SIAT1 | 80 | 160 | 320 | 640 | 160 | 320 | 160 | 640 | 640 | 320 |
| A/Iceland/04/2012 | 2012-01-16 | MDCK3/SIAT1 | < | 80 | 80 | 160 | 80 | 80 | 80 | 320 | 160 | 320 |
| A/Iceland/05/2012 | 2012-01-18 | MDCK2/SIAT1 | < | 40 | 40 | 160 | 80 | 80 | 80 | 320 | 160 | 320 |
| A/Iceland/07/2012 | 2012-01-24 | MDCK2/SIAT1 | 160 | 320 | 640 | 1280 | 640 | 640 | 640 | 1280 | 1280 | 1280 |
| A/Iceland/10/2012 | 2012-02-08 | MDCK2/SIAT1 | 40 | 80 | 160 | 640 | 160 | 160 | 160 | 640 | 320 | 640 |
| A/Iceland/11/2012 | 2012-02-08 | MDCK3/SIAT1 | < | 40 | 80 | 80 | 80 | 80 | 80 | 320 | 160 | 320 |
| A/Iceland/12/2012 | 2012-02-09 | MDCK2/SIAT1 | 40 | 160 | 160 | 160 | 320 | 160 | 160 | 1280 | 640 | 1280 |
| A/Iceland/13/2012 | 2012-02-09 | MDCK2/SIAT1 | 40 | 80 | 160 | 160 | 320 | 160 | 160 | 1280 | 320 | 1280 |
| A/Iceland/14/2012 | 2012-02-12 | MDCK2/SIAT1 | 40 | 80 | 80 | 160 | 320 | < | 160 | 1280 | 320 | 1280 |
| A/Iceland/15/2012 | 2012-02-13 | MDCK1/SIAT1 | 40 | 80 | 80 | 160 | 160 | 80 | 160 | 640 | 320 | 6340 |
| A/Iceland/16/2012 | 2012-02-13 | MDCK2/SIAT1 | 40 | 160 | 80 | 160 | 320 | 160 | 160 | 640 | 320 | 640 |
| A/Iceland/17/2012 | 2012-02-17 | MDCK2/SIAT1 | 320 | 320 | 640 | 2560 | 320 | 1280 | 640 | 2560 | 640 | 1280 |
| A/Iceland/18/2012 | 2012-02-23 | MDCK3/SIAT1 | < | 40 | 80 | 320 | 80 | 80 | 80 | 320 | 160 | 320 |
| A/Iceland/19/2012 | 2012-02-24 | MDCK2/SIAT1 | 40 | 80 | 160 | 320 | 160 | 160 | 160 | 640 | 160 | 640 |
| A/Iceland/20/2012 | 2012-02-27 | MDCK2/SIAT1 | 160 | 160 | 160 | 320 | 160 | 160 | 320 | 640 | 160 | 1280 |
| A/Iceland/21/2012 | 2012-03-02 | MDCK2/SIAT1 | 80 | 160 | 320 | 640 | 320 | 320 | 160 | 1280 | 320 | 1280 |
| A/Iceland/23/2012 | 2012-03-27 | MDCK3/SIAT1 | 80 | 80 | 160 | 320 | 160 | 320 | 160 | 640 | 160 | 640 |
| A/Iceland/24/2012 | 2012-04-14 | MDCK2/SIAT1 | < | < | < | 80 | 40 | 40 | 40 | 160 | 40 | 160 |
| A/Lisboa/23/2012 | 2012-02-07 | SIAT1/SIAT1 | 40 | 80 | 160 | 160 | 160 | 160 | 160 | 320 | 320 | 160 |
| A/Lisboa/30/2012 | 2012-01-27 | SIAT1/SIAT1 | 40 | 160 | 320 | 640 | 160 | 160 | 160 | 640 | 320 | 640 |
| A/Lisboa/31/2012 | 2012-01-27 | SIAT1/SIAT1 | 80 | 160 | 320 | 640 | 320 | 320 | 160 | 640 | 640 | 1280 |
| A/Lisboa/32/2012 | 2012-01-27 | SIAT1/SIAT1 | < | 160 | 160 | 640 | 160 | 320 | 160 | 640 | 320 | 640 |
| A/Lisboa/38/2012 | 2012-03-15 | SIAT1/SIAT1 | < | 80 | 160 | 320 | 160 | 160 | 160 | 640 | 320 | 320 |
| A/Lisboa/58/2012 | 2012-03-20 | SIAT1/SIAT1 | < | 80 | 160 | 320 | 160 | 160 | 160 | 320 | 320 | 320 |
| A/Lisboa/59/2012 | 2012-03-27 | SIAT1/SIAT1 | < | 80 | 160 | 320 | 160 | 160 | 160 | 320 | 320 | 320 |
| A/Lisboa/61/2012 | 2012-03-05 | SIAT1/SIAT1 | 40 | 160 | 320 | 640 | 320 | 320 | 320 | 640 | 640 | 640 |
| A/Stockholm/11/2012 | 2012-01-21 | C2/SIAT1 | 80 | 160 | 320 | 640 | 320 | 1280 | 320 | 1280 | 640 | 640 |
| A/Stockholm/6/2012 | | C1/SIAT1 | 80 | 160 | 640 | 1280 | 320 | 640 | 320 | 1280 | 1280 | 1280 |
| A/Stockholm/14/2012 | 2012-03-16 | C2/SIAT1 | 80 | 160 | 160 | 1280 | 320 | 320 | 320 | 640 | 640 | 640 |
| A/Stockholm/19/2012 | 2012-04-14 | C1/SIAT1 | 80 | 160 | 320 | 1280 | 320 | 320 | 320 | 640 | 640 | 1280 |
| A/England/524/2012 | 2012-04-12 | SIAT1/SIAT3 | 40 | 80 | 160 | 160 | 320 | 160 | 80 | 640 | 320 | 320 |
| A/Iceland/65/2011 | 2011-11-19 | MDCK3/SIAT2 | < | 80 | 40 | 160 | 80 | 80 | 80 | 320 | 160 | 320 |
| A/Iceland/06/2012 | 2012-01-24 | MDCK2/SIAT2 | < | 40 | 80 | 160 | 80 | 80 | 80 | 320 | 160 | 160 |
| | | | Vaccine | | | | | | Vaccine | | Vaccine | |

1. < = <40

## Table 6. **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/Perth 16/09 | A/Vic 208/09 | A/Ala 5/10 | A/HK 3969/11 | A/Stock 18/11 | A/Iowa 19/10 | A/Vic 361/11 | A/Berlin 93/11 | A/Vic 361/11 | A/Athens 112/12 |
| | | | F35/09 | F7/10 | F27/10 | F27/11 | F28/11 | F15/11 | Egg F05/12 | T/C F17/12 | T/C F15/12 | F16/12 |
| Genetic group | | | | | group 5 | group 3C | group 3A | group 6 | group 3C | group 3C | group 3C | group 3B |
| ***REFERENCE VIRUSES*** | | | | | | | | | | | | |
| A/Perth/16/2009 | 2009-07-04 | E3/E2 | 640 | 80 | 160 | 640 | 160 | 160 | 160 | 640 | 640 | 640 |
| A/Victoria/208/2009 | 2009-06-02 | E3/E1 | 2560 | 5120 | 1280 | 5120 | 2560 | 5120 | 5120 | 5120 | 5120 | 5120 |
| A/Alabama/5/2010 | 2010-07-13 | MK1/C2/SIAT2 | 40 | 40 | 160 | 640 | 160 | 160 | 80 | 640 | 320 | 640 |
| A/Hong Kong/3969/2011 | 2011-05-19 | MDCK2/SIAT4 | 320 | 320 | 640 | 1280 | 640 | 640 | 640 | 2560 | 1280 | 2560 |
| A/Stockholm/18/2011 | 2011-03-28 | MDCK2/SIAT4 | 40 | 80 | 80 | 640 | 320 | 80 | 160 | 640 | 320 | 640 |
| A/Iowa/19/2010 | 2010-12-30 | E3/E1 | 1280 | 5120 | 2560 | 5120 | 5120 | 5120 | 5120 | 5120 | 5120 | 5120 |
| A/Victoria/361/2011 | 2011-10-24 | E3/E1 | 160 | 640 | 320 | 640 | 160 | 640 | 5120 | 1280 | 320 | 640 |
| A/Berlin/93/2011 | 2011-12-07 | NVD3/S2 | 160 | 160 | 320 | 1280 | 640 | 640 | 640 | 1280 | 1280 | 1280 |
| A/Victoria/361/2011 | 2011-10-24 | M2/S2 | 80 | 160 | 160 | 640 | 160 | 320 | 160 | 640 | 640 | 640 |
| A/Athens/112/2012 | 2012-02-01 | SIAT3 | 80 | 160 | 160 | 640 | 160 | 160 | 160 | 640 | 640 | 1280 |
| ***TEST VIRUSES*** | | | | | | | | | | | | |
| A/Pardubice/7/2012 | | MDCK5/SIAT2 | 160 | 160 | 640 | 1280 | 320 | 640 | 320 | 2560 | 1280 | 1280 |
| A/Ceska Lipa/81/2012 | | MDCK3/SIAT2 | 160 | 160 | 640 | 1280 | 320 | 640 | 320 | 1280 | 1280 | 1280 |
| A/Jihlava/83/2012 | | MDCK3/SIAT2 | 160 | 160 | 640 | 1280 | 320 | 640 | 320 | 1280 | 1280 | 1280 |
| A/Vsetin/85/2012 | | MDCK3/SIAT2 | 160 | 320 | 640 | 1280 | 640 | 640 | 320 | 2560 | 1280 | 1280 |
| A/Prerov/87/2012 | | MDCK3/SIAT2 | 160 | 160 | 320 | 640 | 320 | 320 | 160 | 1280 | 1280 | 640 |
| A/Praha/130/2012 | | MDCK3/SIAT2 | 160 | 320 | 640 | 2560 | 640 | 1280 | 640 | 2560 | 2560 | 2560 |
| A/England/294/2012 | 2012-02-20 | SIAT2/SIAT1 | 80 | 160 | 160 | 640 | 160 | 160 | 160 | 640 | 320 | 640 |
| A/England/49/2012 | 2012-02-26 | SIAT1/SIAT1 | 160 | 320 | 640 | 1280 | 640 | 640 | 320 | 2560 | 1280 | 1280 |
| A/England/316/2012 | 2012-02-28 | SIAT1/SIAT1 | 160 | 320 | 320 | 1280 | 640 | 640 | 320 | 2560 | 1280 | 1280 |
| A/England/392/2012 | 2012-03-13 | SIAT1/SIAT1 | 80 | 320 | 160 | 640 | 640 | 320 | 160 | 640 | 640 | 1280 |
| A/England/476/2012 | 2012-03-19 | SIAT1/SIAT1 | 160 | 320 | 320 | 1280 | 640 | 640 | 640 | 2560 | 1280 | 1280 |
| A/England/517/2012 | 2012-04-05 | SIAT1/SIAT1 | 80 | 160 | 160 | 640 | 320 | 320 | 160 | 1280 | 640 | 640 |
| A/England/531/2012 | 2012-04-16 | MDCK1/SIAT1 | 40 | 40 | 40 | 320 | 80 | 80 | 80 | 320 | 320 | 320 |
| A/Iceland/09/2012 | 2012-01-30 | MDCK3/SIAT3 | 80 | 80 | 160 | 640 | 160 | 320 | 160 | 640 | 320 | 640 |
| A/Ireland/12M28563/2012 | 2012-04-10 | SIAT4 | 160 | 160 | 320 | 1280 | 640 | 320 | 320 | 2560 | 640 | 1280 |
| A/Denmark/2/2012 | 2012-01-30 | SIAT3/SIAT1 | 80 | 160 | 160 | 640 | 320 | 160 | 160 | 1280 | 320 | 640 |
| A/Denmark/3/2012 | 2012-02-06 | SIAT1/MDCK2/SIAT1 | 40 | 160 | 80 | 640 | 320 | 160 | 160 | 1280 | 320 | 640 |
| A/Denmark/5/2012 | 2012-02-07 | SIAT1/MDCK2/SIAT1 | 160 | 320 | 640 | 1280 | 320 | 640 | 320 | 2560 | 640 | 1280 |
| A/Denmark/6/2012 | 2012-02-07 | SIAT1/MDCK2/SIAT1 | 40 | 80 | 80 | 320 | 160 | 160 | 160 | 640 | 320 | 320 |
| A/Denmark/12/2012 | 2012-02-14 | SIAT1/MDCK2/SIAT1 | 40 | 80 | 80 | 160 | 160 | 80 | 80 | 640 | 160 | 160 |
| A/Denmark/24/2012 | 2012-03-11 | SIAT1/MDCK1/SIAT1 | 80 | 320 | 160 | 640 | 640 | 320 | 320 | 1280 | 640 | 1280 |
| A/Denmark/25/2012 | 2012-03-13 | SIAT1/MDCK2/SIAT1 | 80 | 160 | 160 | 640 | 320 | 160 | 160 | 1280 | 640 | 640 |
| A/Denmark/26/2012 | 2012-03-18 | SIAT1/MDCK3/SIAT1 | 80 | 160 | 160 | 640 | 160 | 160 | 160 | 1280 | 640 | 640 |
| A/Denmark/28/2012 | 2012-03-14 | SIAT1/MDCK1/SIAT1 | 80 | 160 | 160 | 640 | 160 | 160 | 160 | 1280 | 320 | 640 |
| A/Denmark/31/2012 | 2012-03-21 | SIAT1/MDCK2/SIAT1 | 160 | 320 | 320 | 640 | 640 | 320 | 320 | 2560 | 640 | 1280 |
| A/Denmark/36/2012 | 2012-03-26 | SIAT1/MDCK2/SIAT1 | 320 | 640 | 640 | 1280 | 1280 | 640 | 640 | 5120 | 1280 | 2560 |
| A/Denmark/41/2012 | 2012-04-10 | SIAT1/MDCK2/SIAT1 | 80 | 320 | 320 | 1280 | 640 | 320 | 320 | 2560 | 640 | 640 |
| A/Denmark/42/2012 | 2012-04-21 | SIAT1/MDCK1/SIAT1 | 160 | 160 | 320 | 640 | 320 | 320 | 320 | 2560 | 640 | 640 |
| A/Denmark/45/2012 | 2012-04-24 | SIAT1/MDCK2/SIAT1 | 640 | 1280 | 1280 | 2560 | 1280 | 1280 | 1280 | 5120 | 2560 | 2560 |
| A/Karlovy Vary/46/2012 | | MDCK5/SIAT3 | 40 | 80 | 80 | 320 | 80 | 160 | 80 | 640 | 320 | 320 |
| A/Decin/42/2012 | | MDCK5/SIAT3 | 40 | 80 | 80 | 320 | 80 | 160 | 80 | 640 | 320 | 320 |
| A/Cercany/96/2012 | | MDCK3/SIAT3 | 40 | 80 | 160 | 320 | 160 | 160 | 160 | 640 | 640 | 320 |
| A/Praha/98/2012 | | MDCK3/SIAT3 | 40 | 80 | 80 | 320 | 160 | 160 | 160 | 640 | 320 | 320 |
| A/England/354/2012 | 2012-02-28 | SIAT2/SIAT2 | 40 | 80 | 80 | 320 | 160 | 160 | 80 | 320 | 160 | 320 |
| A/Scotland/1/2012 | 2012-03-30 | SIAT3/SIAT2 | < | 40 | 40 | 160 | 160 | 40 | 80 | 320 | 80 | 160 |
| A/England/509/2012 | 2012-04-04 | SIAT1/SIAT2 | 40 | 80 | 80 | 320 | 160 | 160 | 160 | 640 | 640 | 640 |
| A/Ireland/V1160/2012 | 2012-02-01 | C2/SIAT1 | 80 | 80 | 80 | 320 | 320 | 160 | 160 | 640 | 320 | 640 |
| A/Ireland/12V2608/2012 | 2012-03-22 | C1/SIAT1 | 40 | 80 | 80 | 320 | 320 | 160 | 160 | 640 | 320 | 640 |
| A/Ireland/12V2628/2012 | 2012-03-22 | C1/SIAT1 | 160 | 160 | 320 | 1280 | 320 | 640 | 320 | 2560 | 1280 | 1280 |
| A/Ireland/12V2734/2012 | 2012-03-26 | C1/SIAT1 | 40 | 80 | 80 | 640 | 320 | 160 | 160 | 640 | 320 | 640 |
| A/Ireland/12V2794/2012 | 2012-03-28 | C1/SIAT1 | 40 | 40 | 40 | 320 | 160 | 80 | 160 | 640 | 160 | 6340 |
| | | | Vaccine virus | | | | | | Vaccine virus | | Vaccine virus | |

1. < = <40

**Figure 3. Phylogenetic comparison of influenza A(H3N2) HA genes (HA1 coding region)**

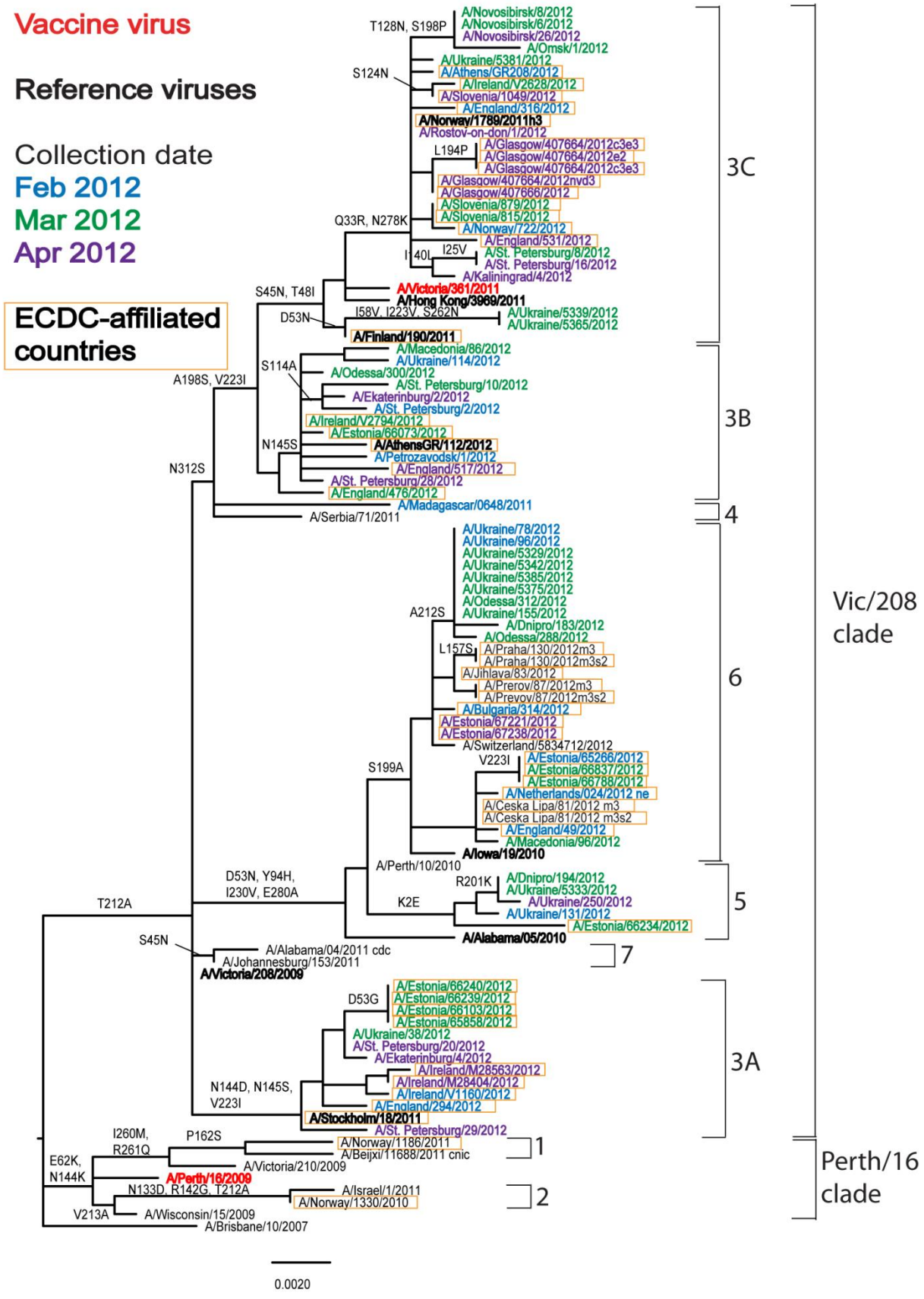
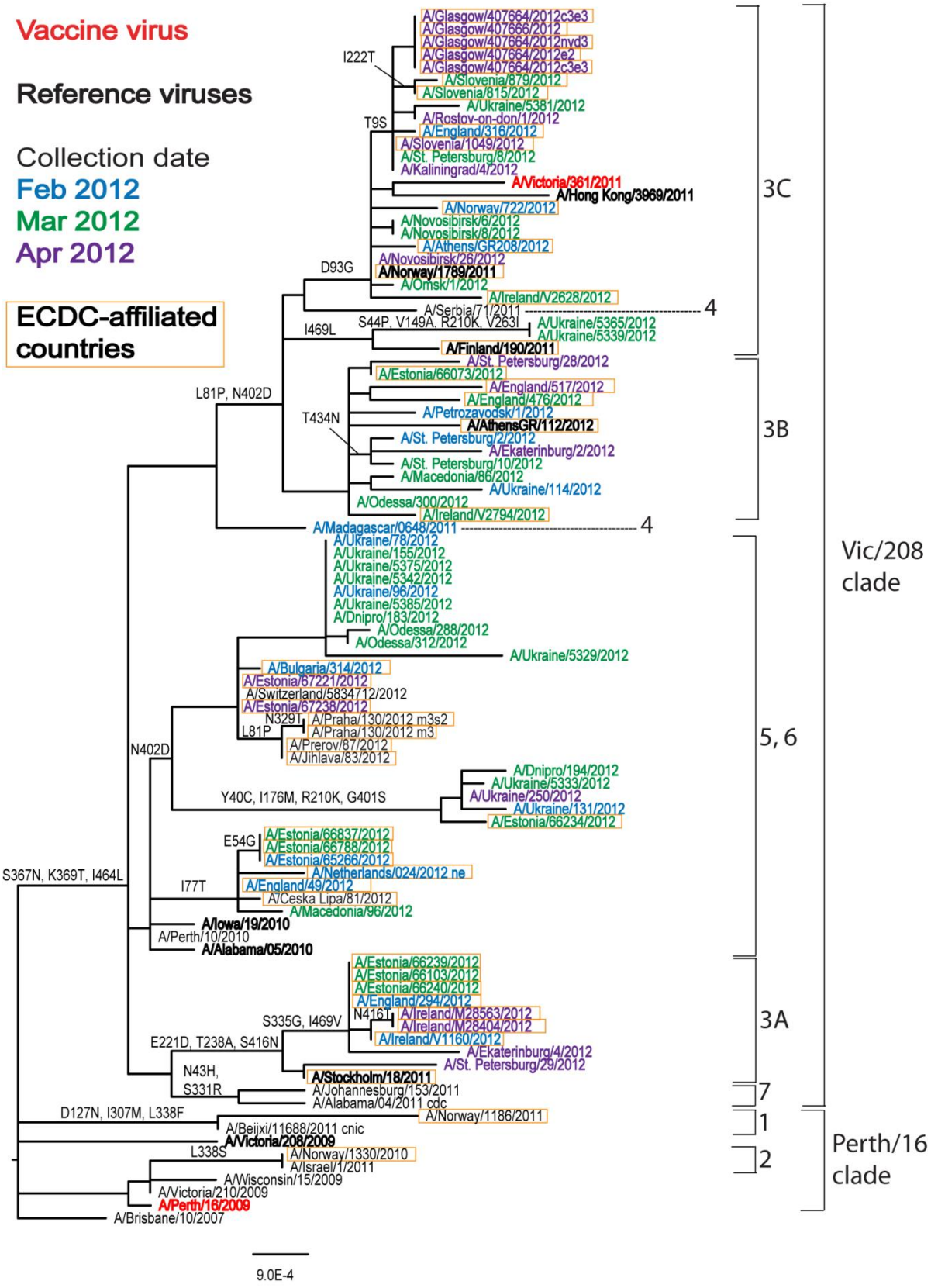


Figure 4. **Phylogenetic comparison of influenza A(H3N2) NA genes**



## Table 7. **Antigenic analysis of influenza B/Victoria-lineage viruses by HI (turkey RBCs)**

**Antigenic analyses of influenza B viruses (Victoria lineage)**

| Viruses | Collection date | Passage History | Haemagglutination inhibition titre[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Post infection ferret sera | | | | | | |
| | | | B/Bris[2] 60/08 Sh 523 | B/Mal 2506/04 F28/05 | B/England 393/08 F05/11 | B/Bris 60/08 F06/11 | B/Paris 1762/08 F07/11 | B/HK 514/09 F13/10 | B/Odessa 3886/10 F19/11 | B/Malta 636714/11 F33/11 |
| ***REFERENCE VIRUSES*** | | | | | | | | | | |
| B/Malaysia/2506/2004 | 2004-12-06 | E3/E5 | 2560 | 1280 | 40 | 80 | < | 40 | < | 160 |
| B/England/393/2008 | 2008-08-29 | E1/E6 | 2560 | 160 | 320 | 640 | 40 | 40 | 40 | 320 |
| B/Brisbane/60/2008 | 2008-08-04 | E4/E3 | 2560 | 160 | 160 | 320 | 40 | 40 | 40 | 320 |
| B/Paris/1762/2008 | 2009-02-09 | C2/MDCK4 | 2560 | 10 | 20 | 40 | 80 | 80 | 80 | 20 |
| B/Hong Kong/514/2009 | 2009-10-11 | MDCK1/MDCK3 | 5120 | 10 | 20 | 40 | 160 | 160 | 160 | 20 |
| B/Odessa/3886/2010 | 2010-03-19 | C2/MDCK4 | 5120 | 20 | 40 | 80 | 160 | 160 | 160 | 40 |
| B/Malta/636714/2011 | 2011-03-07 | E4/E1 | 2560 | 320 | 640 | 640 | 80 | 80 | 80 | 320 |
| ***TEST VIRUSES*** | | | | | | | | | | |
| B/Denmark/2/2012 | 2012-02-15 | MDCK2 | 5120 | 10 | 20 | 40 | 80 | 80 | 160 | 20 |
| B/Lisboa/3/2012 | 2012-03-13 | SIAT1/MDCK1 | 5120 | 20 | 40 | 80 | 160 | 160 | 80 | 40 |
| B/Sweden/3/2011 | 2011-12-30 | C2/MDCK1 | 5120 | 10 | 40 | 80 | 160 | 160 | 160 | 40 |
| B/Stockholm/5/2012 | 2012-02-09 | C1/MDCK1 | 5120 | 20 | 40 | 80 | 80 | 80 | 80 | 40 |
| B/Denmark/5/2012 | 2012-02-27 | MDCK2/MDCK1 | 2560 | 10 | 20 | 20 | 80 | 40 | 80 | 20 |
| B/Denmark/15/2012 | 2012-04-16 | MDCK2/MDCK1 | 2560 | 20 | 40 | 40 | 40 | 40 | 40 | 40 |
| B/Pardubice/21/2012 | | MDCK4/MDCK1 | 1280 | < | 10 | 10 | 40 | 40 | 20 | 10 |
| B/Karlovy Vary/24/2012 | | MDCK3/MDCK2 | 5120 | < | 10 | 40 | 80 | 80 | 40 | 20 |
| B/Vsetin/51/2012 | | MDCK3/MDCK1 | 2560 | < | 10 | 20 | 80 | 80 | 20 | 20 |
| B/Praha/27/2012 | | MDCK3/MDCK1 | 2560 | < | 10 | 20 | 80 | 80 | 20 | 10 |
| B/Praha/52/2012 | | MDCK4/MDCK1 | 2560 | < | 10 | 20 | 80 | 80 | 20 | 20 |
| B/Praha/90/2012 | | MDCK3/MDCK1 | 2560 | < | 10 | 20 | 80 | 80 | 40 | 20 |
| B/Karlovy Vary/93/2012 | | MDCK3/MDCK1 | 2560 | < | 20 | 20 | 80 | 80 | 40 | 20 |
| B/Karlovy Vary/23/2012 | | MDCK3/MDCK2 | 2560 | < | 20 | 40 | 40 | 80 | 20 | 20 |
| B/Prerov/55/2012 | | MDCK3/MDCK2 | 2560 | < | 20 | 20 | 80 | 80 | 40 | 20 |
| B/England/322/2012 | 2012-02-29 | SIAT1/MDCK1 | 2560 | < | 10 | 20 | 40 | 40 | 20 | 10 |
| B/England/507/2012 | 2012-04-04 | SIAT1/MDCK1 | 2560 | < | 10 | 20 | 80 | 80 | 20 | 20 |
| B/England/537/2012 | 2012-04-17 | SIAT2/MDCK1 | 2560 | < | 10 | 20 | 80 | 80 | 20 | 20 |
| B/Prerov/25/2012 | | MDCK3/MDCK3 | 5120 | 10 | 40 | 40 | 80 | 80 | 160 | 40 |
| B/England/345/2012 | 2012-03-08 | SIAT1/MDCK2 | 5120 | 10 | 40 | 80 | 80 | 160 | 160 | 40 |
| B/Ireland/12M17522/2012 | 2012-02-29 | C1/SIAT1 | 5120 | < | 20 | 40 | 40 | 40 | 80 | 20 |
| B/Slovenia/955/2012 | 2012-03-26 | MDCKx/MDCK1 | 5120 | 10 | 10 | 20 | 80 | 80 | 80 | 20 |
| B/Norway/710/2012 | 2012-02-13 | MDCK1/MDCK1 | 2560 | < | 10 | 40 | 40 | 20 | 40 | < |

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

Sequence in phylogenetic tree

## Table 8. **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 F21/07 | B/Bris[2] 3/07 F24/07 | B/Eng[2] 145/08 F9/08 | B/Bang[2] 3333/07 F21/08 | B/Wis[2] 1/10 F26/10 | B/Stock[2] 12/2011 F12/12 | B/Estonia[2] 55669/2011 F26/11 | B/Serbia[2] 1894/2011 F25/11 | B/Stock[2] 12/2011 T/C F8/12 |
| *REFERENCE VIRUSES* | | | | | | | | | | | | | |
| B/Egypt/144/2005 | 2005-05-01 | E3/E5 | 5120 | 640 | 1280 | 1280 | 320 | 640 | 640 | 1280 | 320 | 40 | 640 |
| B/Florida/4/2006 | 2006-12-15 | E3/E4 | 5120 | 320 | 1280 | 1280 | 320 | 320 | 640 | 1280 | 320 | 40 | 320 |
| B/Brisbane/3/2007 | 2007-09-03 | E2/E1 | 5120 | 160 | 640 | 640 | 80 | 320 | 320 | 640 | 160 | 10 | 320 |
| B/England/145/2008 | | Ex/E1 | 640 | < | 40 | 20 | 160 | 20 | 20 | 80 | < | < | 20 |
| B/Bangladesh/3333/2007 | 2007-08-07 | E4/E1 | 5120 | 80 | 160 | 160 | 80 | 320 | 320 | 1280 | 10 | 20 | 320 |
| B/Wisconsin/1/2010 | 2007-08-07 | E4/E1 | 2560 | 160 | 320 | 160 | 160 | 160 | 640 | 640 | 20 | 40 | 160 |
| B/Stockholm/12/2011 | 2007-08-07 | E4/E1 | 5120 | 80 | 160 | 160 | 80 | 320 | 320 | 640 | 20 | 40 | 320 |
| B/Estonia/55669/2011 | 2011-03-14 | MDCK2/MDCK2 | 2560 | 80 | 160 | 40 | 160 | 80 | 40 | 320 | 1280 | 80 | 40 |
| B/Serbia/1894/2011 | 2011-03-08 | MDCK1/MDCK4 | 2560 | 80 | 160 | 40 | 160 | 160 | 160 | 640 | 160 | 320 | 160 |
| B/Stockholm/12/2011 | 2011-03-28 | Cx/MDCK1 | 5120 | 160 | 320 | 80 | 640 | 320 | 320 | 320 | 320 | 640 | 320 |
| *TEST VIRUSES* | | | | | | | | | | | | | |
| B/Lisboa/1/2012 | 2012-02-28 | SIAT2/MDCK1 | 2560 | 40 | 160 | 80 | 160 | 40 | 40 | 160 | 1280 | 80 | 20 |
| B/Lisboa/5/2012 | 2012-03-19 | SIAT1/MDCK1 | 2560 | 40 | 80 | 40 | 160 | 160 | 160 | 320 | 80 | 160 | 160 |
| B/Lisboa/6/2012 | 2012-03-15 | SIAT1/MDCK2 | 5120 | 160 | 160 | 80 | 320 | 320 | 320 | 640 | 640 | 640 | 160 |
| B/Stockholm/2/2012 | 2012-02-02 | C1/MDCK1 | 5120 | 160 | 80 | 40 | 160 | 160 | 160 | 640 | 160 | 320 | 160 |
| B/Stockholm/8/2012 | 2012-03-06 | C0/MDCK1 | 2560 | 160 | 80 | 80 | 80 | 40 | 40 | 160 | 1280 | 80 | 20 |
| B/Denmark/3/2012 | 2012-02-22 | MDCK3/MDCK1 | 640 | 40 | 40 | 20 | 40 | 40 | 40 | 160 | 20 | 40 | 10 |
| B/Denmark/7/2012 | 2012-02-28 | MDCK2/MDCK1 | 640 | < | < | 10 | 40 | 20 | 40 | 160 | 10 | 40 | 20 |
| B/Denmark/8/2012 | 2012-02-29 | MDCK2/MDCK1 | 1280 | 40 | 40 | 40 | 80 | 40 | 80 | 320 | 80 | 80 | 40 |
| B/Hradec Kralove/26/2012 | | MDCK3/MDCK1 | 640 | < | 40 | 40 | 10 | 10 | 10 | 80 | 320 | 20 | ND |
| B/Hradec Kralove/29/2012 | | MDCK3/MDCK1 | 2560 | 40 | 160 | 80 | 80 | 160 | 80 | 160 | 640 | 160 | ND |
| B/England/267/2012 | 2012-02-22 | SIAT1/MDCK1 | 2560 | 40 | 40 | 40 | 40 | 80 | 40 | 160 | 80 | 160 | ND |
| B/England/386/2012 | 2012-03-13 | SIAT1/MDCK1 | 2560 | 80 | 80 | 80 | 40 | 40 | 40 | 160 | 640 | 40 | ND |
| B/England/538/2012 | 2012-04-10 | SIAT2/MDCK1 | 5120 | 80 | 80 | 80 | 80 | 160 | 160 | 320 | 320 | 320 | ND |
| B/Estonia/67205/2012 | 2012-04-09 | MDCK1/MDCK1 | 2560 | 80 | 160 | 160 | 80 | 160 | 160 | 640 | 160 | 160 | ND |
| B/Norway/579/2012 | 2012-02-14 | SIAT1/MDCK1 | 1280 | 40 | 80 | 20 | 20 | 20 | 10 | 160 | 640 | 80 | ND |
| B/Norway/773/2012 | 2012-02-23 | MDCK1/MDCK1 | 1280 | 40 | 80 | 20 | 20 | 20 | 10 | 160 | 640 | 80 | ND |
| B/Norway/777/2012 | 2012-02-20 | MDCK1/MDCK1 | 2560 | 40 | 80 | 40 | 40 | 40 | 10 | 160 | 640 | 160 | ND |
| B/Athens GR/199/2012 | 2012-02-07 | MDCK2 | 5120 | 160 | 80 | 80 | 320 | 160 | 80 | 640 | 1280 | 320 | ND |

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

ND = not determined

Sequence in phylogenetic tree

Figure 5. **Phylogenetic comparison of influenza B/Victoria-lineage HA genes (HA1 coding region)**

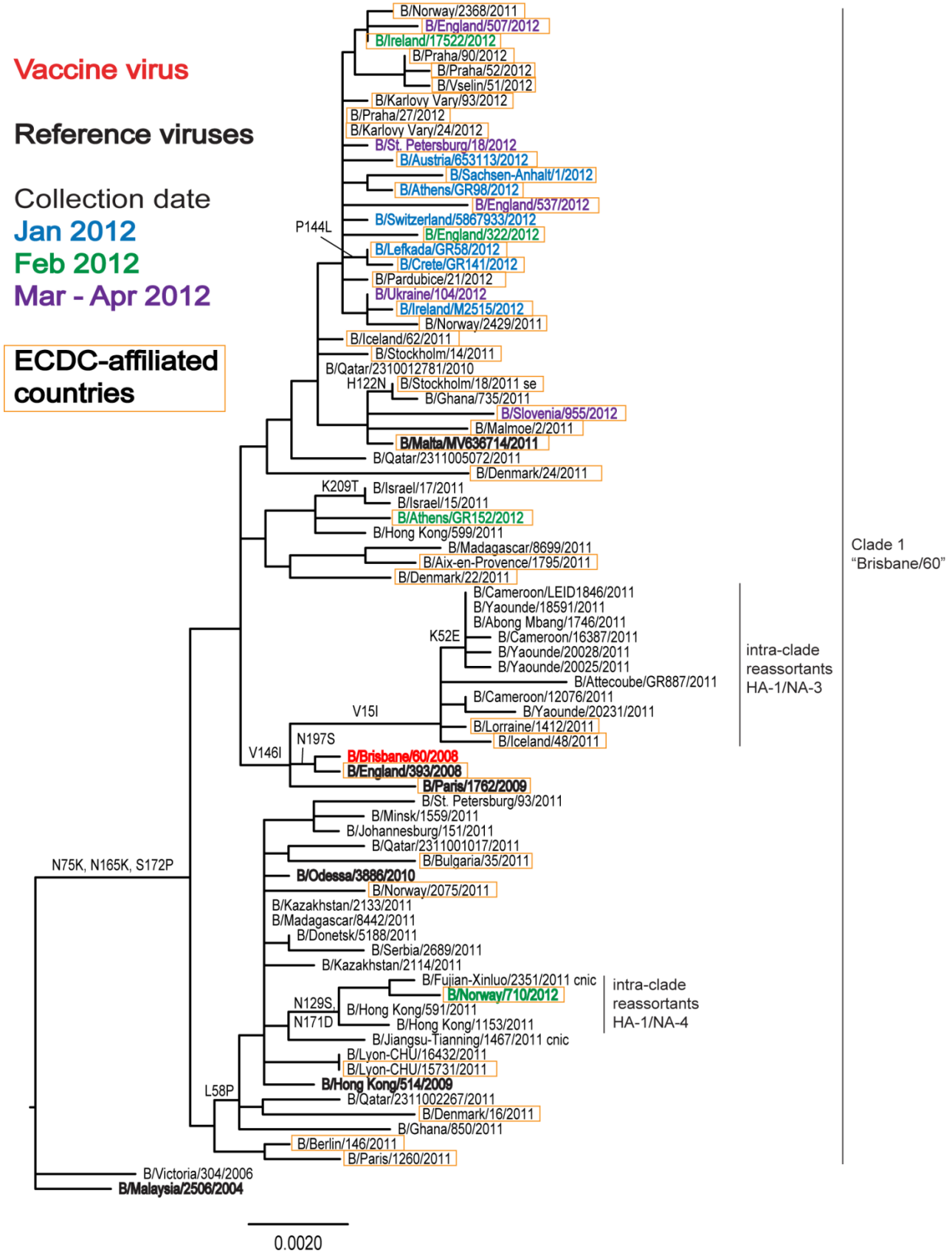


Figure 6. **Phylogenetic comparison of influenza B/Victoria-lineage NA genes**

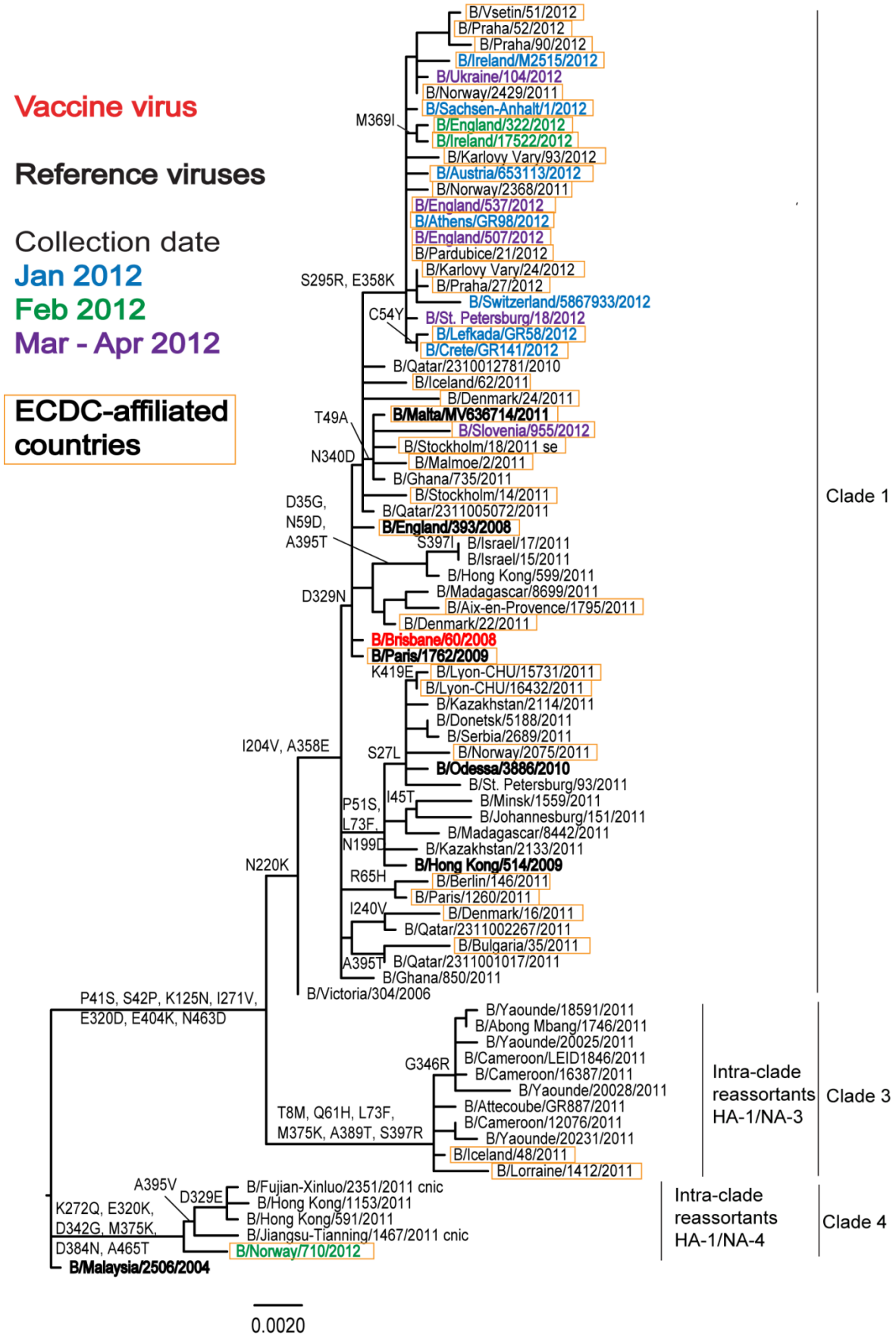


Figure 7. **Phylogenetic comparison of influenza B/Yamagata-lineage HA genes (HA1 coding region)**

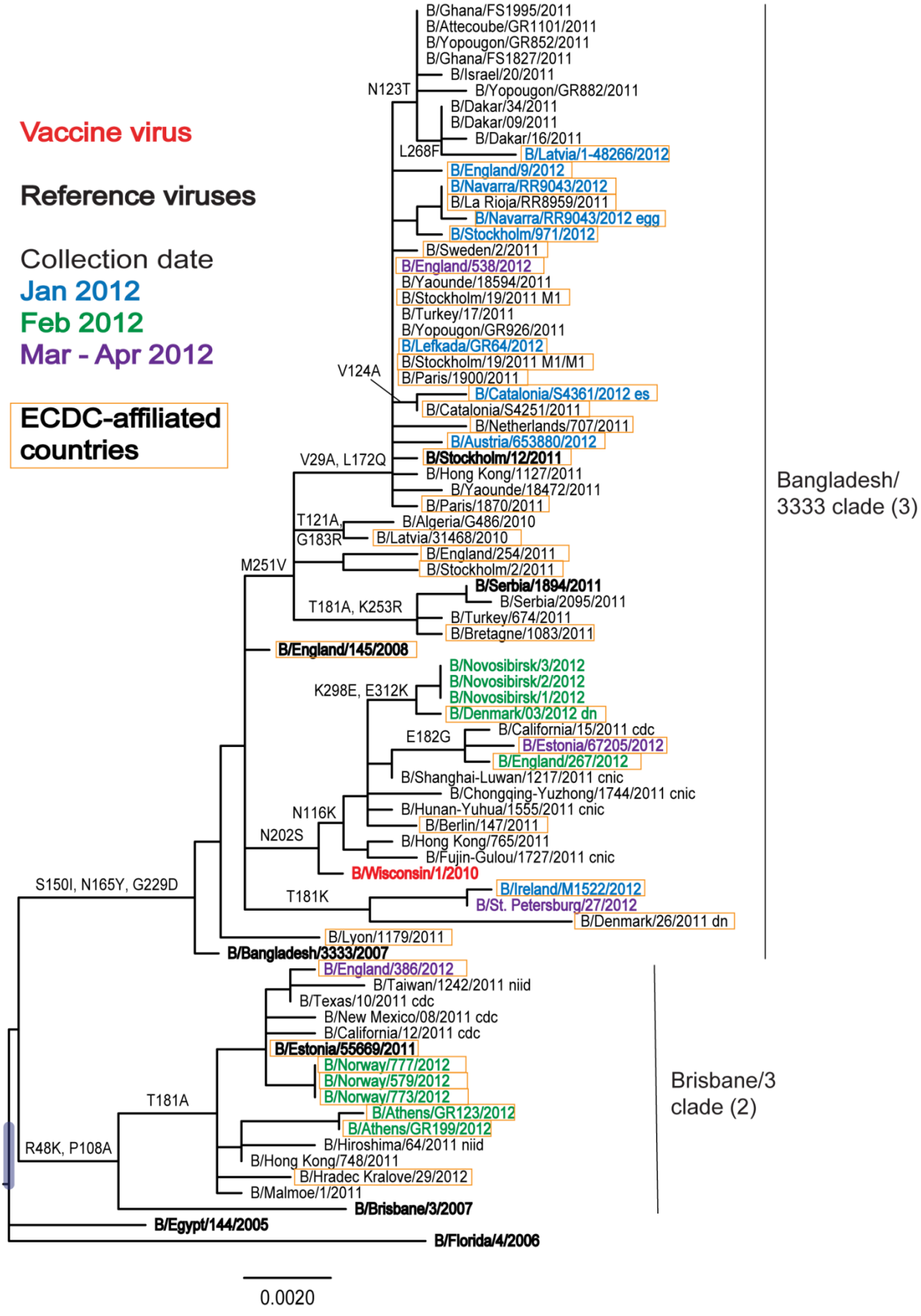


Figure 8. **Phylogenetic comparison of influenza B/Yamagata-lineage NA genes**