# The 2012/2013 Influenza season in Portugal:
## an antigenic and genetic characterisation of circulating viruses


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## Background:

The continuous monitoring of the antigenic and genetic properties of circulating influenza viruses is essential in order to detect any changes that may justify the selection of different vaccine candidates or changes in antiviral recommendations. During the 2012/2013 season, the influenza activity in Portugal was characterized by the co-circulation of influenza B/Yamagata and A(H1)pdm09 viruses with sporadic detections of influenza A(H3) and B/Victoria viruses. This study reports the antigenic and genetic characterisation of influenza viruses isolated in Portugal during the 2012/2013 influenza season.

## Material and Methods:

During the 2012/2013 influenza season, nasopharyngeal swabs were obtained through the National Influenza Surveillance Programme (ILI cases from primary health care-based surveillance, n=1262) and from the Portuguese Laboratory Network for the Influenza Diagnosis (hospital-based surveillance, n=165). The National Influenza Reference Laboratory has characterised antigenically by hemagglutination-inhibition assays 388 influenza strains after isolation on MDCK-Siat1 cell culture (233 B/Yamagata, 138 AH1pdm09, 10 B/Victoria and 7 AH3 viruses). Ninety influenza viruses were selected from the beginning, middle and the end of the season (including severe and immunized cases) for HA1 genetic characterisation (43 B/Yamagata, 33 AH1pdm09, 8 AH3 and 6 B/Victoria).

## Results:

In this season, the 145 isolated influenza A viruses showed a good reactivity with antisera raised against vaccine viral strains (A/California/7/2009 and A/Victoria/361/2011) (Figure 1). Genetically, most A(H1) pandemic viruses (28 of 33) were from clade 6 (A/St. Petersburg/27/2011) and only 5 from clade 7 (A/St. Petersburg/100/2011) (Figure 2A). Most of clade 6 viruses presented 7 amino acid substitutions comparing with the vaccine strain, while clade 7 hemagglutinins shared 8 substitutions (Table I). There are only two substitutions located in antigenic sites and shared by both clades: S185T and S203T (Table I). However, there are a few strains from both clades with occasional substitutions in antigenic sites Ca and Sb (Table I).

Seven AH3 viruses clustered in the group 3C (A/Victoria/361/2011) and one virus clustered in the group 5 (A/Alabama/05/2010).

Isolated viruses from B/Victoria lineage antigenically (Figure 1) and genetically were similar to B/Brisbane/60/2008 (genetic clade 1A).

The majority of influenza B/Yamagata viruses (which predominated in this season) showed higher reactivity with B/Massachusetts/02/2012 antiserum than with antiserum raised against the vaccine strain B/Wisconsin/1/2010 (Figure 1). Sequence analysis (Figure 2B) showed that isolated B/Yamagata viruses were distributed between the clade 2 (B/Estonia/55669/2011) and clade 3 (B/Wisconsin/1/2010). Comparing with the vaccine strain B/Wisconsin/1/2010, most B/Yamagata viruses from clade 3 presented 6 amino acid changes (only one of these substitutions was located in an antigenic site of the HA: S202N) while all B/Yamagata from clade 2 revealed 7 amino acid substitutions (including 3 in antigenic sites: I150S, Y165N and S202N) (Table II). Some Yamagata strains revealed occasional amino acid changes in the 120-loop.

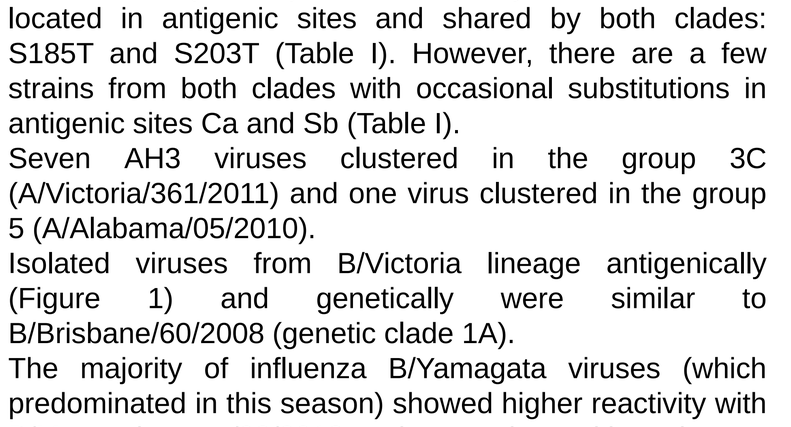


Figure 1 – Antigenic characterisation of influenza A and B viral strains, isolated during the 2012/2013 season.

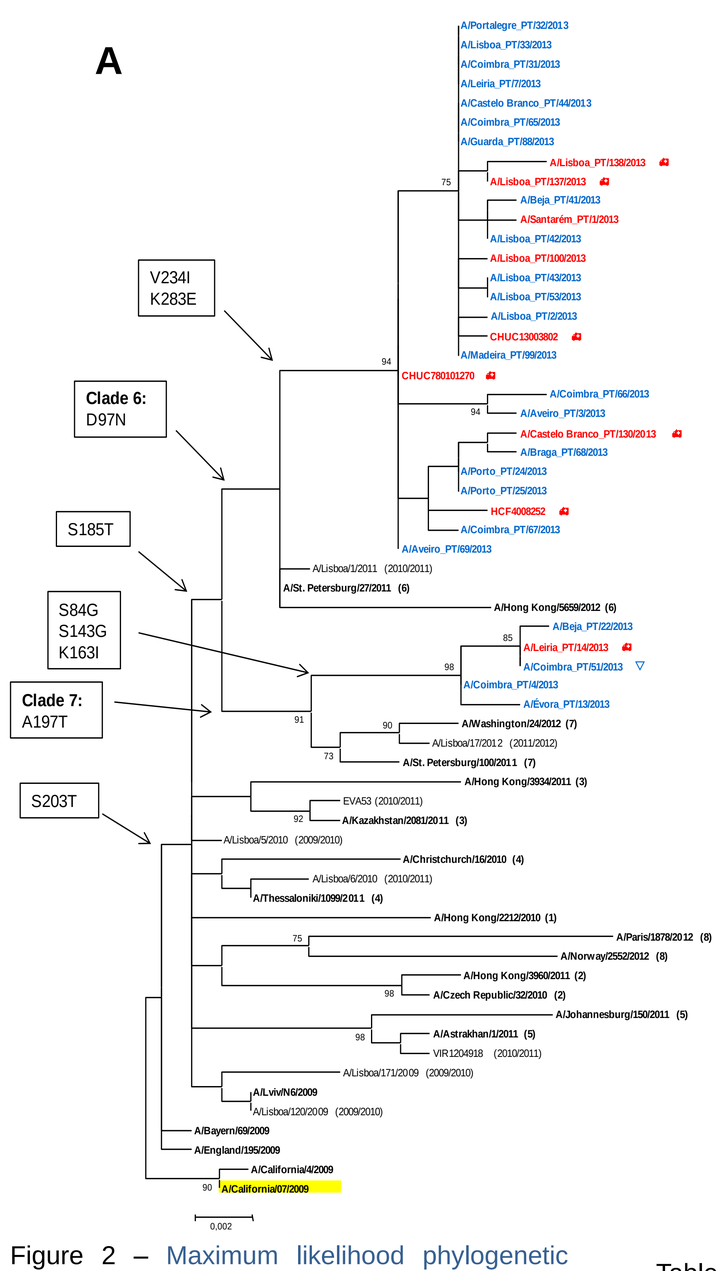


Figure 2 – Maximum likelihood phylogenetic trees of HA1 nucleotide sequences from influenza viruses, which predominated in Portugal during 2012/2013. Bootstrap values above 70 are shown (500 replicates). 2A – influenza A(H1)pdm09 viruses; 2B – influenza B/Yamagata viruses. Strains analysed are represented in blue (ILI cases from primary health care-based surveillance) and red (hospital-based surveillance). The 2012/2013 and 2013/2014 vaccine strains are highlighted in yellow and green, respectively. Reference strains in bold. ▽ - immunized cases; 🚑 - severe cases.

## Conclusions:

Influenza A viruses, although genetically grouped in different genetic clades, all remained antigenically similar to the vaccine strains. Pandemic A(H1)pdm09 viruses present 2 amino acid changes in antigenic sites since A/California/7/2009 (the vaccine strain).

This season influenza B/Yamagata viruses have co-circulated with influenza A(H1)pdm09, and showed a greater antigenic and genetic variability. Most of these viruses have diverged antigenically from the vaccine strain and genetically belong now to the clade 2 (represented by B/Estonia/55669/2011 and B/Massachusetts/02/2012). Comparing with the vaccine strain, viruses from clade 2 presented, at least, 3 amino acid substitutions located at antigenic sites of B hemagglutinin.

The few B/Victoria viruses detected in circulation remain similar to the former vaccine strain B/Brisbane/60/2008.

This antigenic and genetic scenario was very similar to the one observed at the European level (WHO - Weekly Epidemiological Record, 31 May 2013).

All the genetic clades of influenza viruses detected in circulation were uniformly distributed throughout the 2012/2013 season (data not shown).

Monitoring of amino acid substitutions in viral hemagglutinins did not found any substitution, exclusively associated with severe or immunized cases.

Table I - Amino acid substitutions observed in the HA1 subunit of influenza A(H1)pdm09 viruses comparing to the vaccine strain A/California/7/2009. ▽ - immunized cases; 🚑 - severe cases. Hemagglutinin antigenic sites are highlighted.

| | Antigenic Sites | | | | | | | | Ca | | Ca | | | Sb | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HA1 Amino acids | | 69 | 83 | 84 | 97 | 119 | 138 | 155 | 163 | 185 | 187 | 197 | 203 | 205 | 216 | 223 | 234 | 241 | 245 | 249 | 258 | 260 | 283 | 286 | 321 |
| Viral Strains | Obs. | Clade | | | | | | | | | | | | | | | | | | | | | | | | |
| A/California/07/2009 | | | S | P | S | D | K | H | S | G | K | S | D | A | S | R | I | Q | V | T | T | V | E | N | K | I | I |
| A/St. Petersburg/27/2011 | | 6 | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | R | . | . | . | . | . | . | . | . | V |
| A/Hong Kong/5659/2012 | | | . | S | . | N | N | R | . | . | . | T | . | . | T | . | . | . | . | . | . | L | . | . | . | . | V |
| A/St. Petersburg/100/2011 | | 7 | . | S | . | . | . | . | G | . | . | T | . | T | T | . | . | R | . | . | . | . | . | . | . | . | V |
| A/Washington/24/2012 | | | T | S | . | . | . | . | G | . | . | T | . | T | T | . | . | . | . | . | . | . | D | . | . | . | V |
| A/Santarém_PT/1/2013 | | | . | S | . | N | . | . | E | . | . | T | . | . | T | . | . | . | I | . | . | D | . | E | . | . | V |
| A/Lisboa_PT/2/2013 | | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Aveiro_PT/3/2013 | | | . | S | . | N | . | . | . | E | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Leiria_PT/7/2013 | | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Porto_PT/24/2013 | | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Porto_PT/25/2013 | | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Coimbra_PT/31/2013 | | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Portalegre_PT/32/2013 | | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Lisboa_PT/33/2013 | | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Beja_PT/41/2013 | | | . | S | . | N | . | . | E | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Lisboa_PT/42/2013 | | | . | S | . | N | . | . | E | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Lisboa_PT/43/2013 | | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Castelo Branco_PT/44/2013 | | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Lisboa_PT/53/2013 | | 6 | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Coimbra_PT/65/2013 | | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Coimbra_PT/66/2013 | | | . | S | . | N | . | . | . | E | . | T | . | . | T | . | . | . | I | . | . | . | . | E | V | . | V |
| A/Coimbra_PT/67/2013 | | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | . |
| A/Braga_PT/68/2013 | | | . | S | . | N | . | . | . | Q | . | T | . | . | T | . | M | . | I | . | . | . | . | E | . | . | V |
| A/Aveiro_PT/69/2013 | | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Guarda_PT/88/2013 | | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Madeira_PT/99/2013 | | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Lisboa_PT/100/2013 | | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Castelo Branco_PT/130/2013 | 🚑 | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | M | . | I | I | . | . | . | E | . | . | V |
| A/Lisboa_PT/137/2013 | 🚑 | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Lisboa_PT/138/2013 | 🚑 | | . | S | . | N | N | . | . | . | . | T | Y | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| HCF4008252 (cs) | 🚑 | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| CHUC780101270 (cs) | 🚑 | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| CHUC13003802 (cs) | 🚑 | | . | S | . | N | . | . | . | . | . | T | . | . | T | . | . | . | I | . | . | . | . | E | . | . | V |
| A/Coimbra_PT/4/2013 | | | . | S | G | . | . | . | G | . | I | T | . | T | T | . | . | . | . | . | . | . | . | . | . | . | V |
| A/Évora_PT/13/2013 | | | . | S | G | . | . | . | G | . | I | T | . | T | T | K | . | . | . | . | . | . | . | . | . | . | V |
| A/Leiria_PT/14/2013 | 🚑 | 7 | . | S | G | . | . | . | G | . | I | T | . | T | T | . | . | . | . | . | . | . | . | . | . | . | V |
| A/Beja_PT/22/2013 | | | . | S | G | . | . | . | G | . | I | T | . | T | T | . | . | . | . | N | . | . | . | . | . | . | V |
| A/Coimbra_PT/51/2013 | ▽ | | . | S | G | . | . | . | G | . | I | T | . | T | T | . | . | . | . | . | . | . | . | . | . | . | V |

Table II - Amino acid substitutions observed in the HA1 subunit of influenza B/Yamagata viruses comparing to the vaccine strain B/Wisconsin/7/2010. ▽ - immunized cases; 🚑 - severe cases. Hemagglutinin antigenic sites are highlighted.

| | Antigenic Sites | | | | | 120-loop | | | 120-loop | | 120-loop | | | 150-loop | 165-loop | | | | 190-loop | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HA1 Amino Acids (B/Wisconsin/1/2010 numbering) | | 29 | 30 | 37 | 48 | 75 | 88 | 108 | 116 | 121 | 122 | 123 | 144 | 150 | 165 | 172 | 181 | 182 | 196 | 202 | 220 | 223 | 229 | 234 | 246 | 251 | 253 | 266 | 282 | 298 | 312 | 326 |
| Viral Strains | Obs. | Clade | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| B/Wisconsin/1/2010 | | 3 | V | I | T | R | T | R | P | N | T | Q | N | P | I | Y | L | T | E | N | S | T | Y | D | T | V | M | K | V | I | K | E | T |
| B/Florida/4/2006 | | 1 | . | . | . | . | K | . | . | . | . | . | . | . | S | N | . | . | . | D | N | . | . | S | . | . | . | . | . | . | . | . | . | . |
| B/Brisbane/3/2007 | | 2 | . | . | . | K | . | A | . | . | . | . | . | . | S | N | . | . | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . | . |
| B/Estonia/55669/2011 | | | . | . | . | K | . | A | . | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . | . |
| B/Hong Kong/3577/2012 | | | . | . | . | K | . | A | . | . | . | . | . | . | S | N | . | . | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . | . |
| B/Massachusetts/02/2012 | | | . | . | . | K | . | A | . | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . | . |
| B/Bangladesh/3333/2007 | | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | D | N | . | . | . | . | . | . | . | . | . | . | . | . | . |
| B/Serbia/1894/2011 | | | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | . | . | N | . | . | . | . | . | V | R | . | . | . | . | . | . |
| B/Stockholm/12/2011 | | | A | . | . | . | . | . | . | . | . | . | . | . | . | . | Q | . | . | . | N | . | . | . | . | . | V | . | . | . | . | . | . | . |
| B/Novosibirsk/1/2012 | | | . | . | . | . | . | . | . | K | . | . | . | . | . | . | . | . | . | . | N | . | . | . | . | . | . | . | . | . | . | E | K | . |
| B/Lisboa_PT/9/2012 | | 2 | . | . | . | K | N | . | A | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | . | I | . | . | . | . | . | . | . |
| B/Lisboa_PT/6/2013 | | | . | . | . | K | . | . | A | . | . | . | K | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| B/Lisboa_PT/9/2013 | | | . | . | . | K | . | . | A | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | I | . | . | . |
| B/Portalegre_PT/10/2013 | | | . | . | . | K | . | . | A | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| B/Vila Real_PT/15/2013 | | | . | . | . | K | . | . | A | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| B/Braga_PT/17/2013 | | | . | . | . | K | . | . | A | . | S | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| B/Lisboa_PT/18/2013 | | | . | . | . | K | . | . | A | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| B/Lisboa_PT/35/2013 | | | . | . | . | K | . | . | A | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | K | . | . | . | . | . | . | . | . |
| B/Vila Real_PT/36/2013 | | | . | . | . | K | . | . | A | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| B/Aveiro_PT/43/2013 | | | . | . | . | K | . | . | A | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| B/Porto_PT/67/2013 | ▽ | | . | . | . | K | . | . | A | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| B/Lisboa_PT/72/2013 | | | . | . | . | K | . | . | A | . | . | . | . | . | S | N | . | A | G | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| B/Lisboa_PT/84/2013 | | | . | . | . | K | . | . | A | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| B/Açores_PT/108/2013 | | | . | . | . | K | . | . | A | . | S | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| B/Lisboa_PT/127/2013 | | | . | . | . | K | . | . | A | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| B/Lisboa_PT/135/2013 | | | . | . | . | K | . | . | A | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| B/Lisboa_PT/136/2013 | ▽ | | . | . | . | K | . | . | A | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| B/Lisboa_PT/161/2013 | | | . | . | . | . | . | . | A | . | . | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| CHLC-HSJ5150687 (cs) | | | . | . | . | K | . | . | A | . | S | . | . | . | S | N | . | A | . | . | N | . | . | G | . | . | . | . | . | . | . | . | . |
| B/Lisboa_PT/7/2012 | | 3 | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | . | E | K | . |
| B/Braga_PT/11/2012 | | | . | . | . | . | . | . | . | K | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | E | K | . |
| B/Braga_PT/13/2012 | | | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | H | . | . | . | . | . | . | V | E | K | . |
| B/Porto_PT/14/2012 | | | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | I | . | . | . | . | . | . | . | . | E | K | . |
| B/Braga_PT/15/2012 | | | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | V | E | K | . |
| B/Porto_PT/1/2013 | | | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | V | E | K | . |
| B/Braga_PT/2/2013 | | | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | V | E | K | . |
| B/Porto_PT/3/2013 | | | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | V | E | K | . |
| B/Braga_PT/7/2013 | | | . | . | . | . | . | . | . | K | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | E | K | . |
| B/Lisboa_PT/11/2013 | | | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | V | E | K | . |
| B/Porto_PT/12/2013 | | | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | V | E | K | . |
| B/Braga_PT/16/2013 | | | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | V | E | K | . |
| B/Madeira_PT/38/2013 | | | . | . | . | . | . | . | . | K | A | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | E | K | . |
| B/Porto_PT/46/2013 | ▽ | | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | V | E | K | . |
| B/Porto_PT/49/2013 | | | . | . | A | . | . | . | . | . | . | . | . | S | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | . | E | K | . |
| B/Leiria_PT/71/2013 | | | A | . | . | . | . | . | . | . | . | . | T | . | . | . | Q | . | . | . | N | . | . | . | . | . | V | . | . | . | . | . | . |
| B/Madeira_PT/155/2013 | | | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | V | E | K | . |
| B/Castelo Branco_PT/178/2013 | 🚑 | | . | . | V | K | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | V | E | K | . |
| B/Castelo Branco_PT/179/2013 | | | . | . | V | K | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | V | E | K | . |
| B/Madeira_PT/201/2013 | | | . | . | A | . | . | . | . | . | . | K | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | . | E | K | . |
| B/Beja_PT/205/2013 | | | . | . | A | . | . | . | . | . | . | K | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | . | E | K | . |
| CHLC-HSJ98028947 (cs) | 🚑 | | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | V | E | K | . |
| HDESPD9434 (cs) | 🚑 | | . | K | A | . | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | V | E | K | A |
| SU21+ (cs) | | | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | K | . | . | N | . | . | . | . | . | . | . | . | . | E | K | . |


**For further information on this poster please contact: Raquel Guiomar, Laboratório Nacional de Referência para o Vírus da Gripe, raquel.guiomar@insa.min-saude.pt; Tel.: 00351217519216, Fax.: 00351217526400**



**Options for the Control of Influenza VIII, Cape Town, South Africa, 5-10 September 2013**