# CHARACTERISATION OF INFLUENZA B VIRUSES CIRCULATING IN PORTUGAL DURING THE 2010/2011 INFLUENZA SEASON

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## Objectives:

The continuous monitoring of the antigenic and genetic properties of circulating *influenza* viruses is essential in order to detect any amino acid substitution that may justify the selection of different vaccine candidates or changes in antiviral recommendations. During the 2010-2011 season, the *influenza* activity in Portugal was characterized by the co-circulation of *influenza* B/Victoria and pandemic A(H1N1)2009 viruses with sporadic detections of *influenza* A(H3) and B/Yamagata viruses. This study reports the antigenic and genetic characterisation of *influenza* B viruses isolated in Portugal during the 2010/2011 *influenza* season.

## Methods:

During the 2010/11 *influenza* season, 1017 nasopharyngeal swabs were collected from patients showing symptoms of *influenza*-like illness in the context of virological surveillance. The clinical specimens were collected by General Practitioners participating in the National Influenza Surveillance Programme and were tested at the National Influenza Reference Laboratory for the presence of *influenza* virus (RT-PCR). Seventy one B/Victoria positive specimens were inoculated on MDCK cell cultures and the isolates were characterised antigenically by hemagglutination-inhibition assays (Manual for the Laboratory Diagnosis and Virology, Surveillance of Influenza, 2011). Nineteen *influenza* B/Victoria (18 isolates and 1 clinical specimen designated by EE98) and two *influenza* B/Yamagata (one isolate and one clinical specimen designated by EE222) viruses were selected for sequence analysis of the HA1 gene segment (Pechirra *et al.*, 2005). Phylogenetic analysis was performed using MEGA Software 5.05, phylogenetic trees were obtained by the Maximum Likelihood algorithm using Tamura-Nei nucleotide substitution model (Tamura *et al.*, 2011).

## Results:

Isolated *influenza* B/Victoria viruses showed high reactivity with serum raised against the vaccine strain B/Brisbane/60/2008 **(Table I)**. Antigenic analysis performed at the WHO collaborating centre (National Institute for Medical Research, London) showed that the majority of the isolated viruses presented a low reactivity with antisera raised against the egg-propagated vaccine virus B/Brisbane/60/2008.

They showed, however, a good reactivity with antisera raised against viruses genetically closed related with vaccine viruses and propagated in cells (B/Hong Kong/514/2009, B/Paris/1762/2008 and B/Odessa/3886/2010) **(Table I)**.

Sequence analysis showed that all B/Victoria viruses clustered into the B/Brisbane/60/2008 genetic clade **(Figure 1)**, presenting the characteristic amino acid substitutions N75K, N165K and S172P **(Figure 1 and Table II)**. All *influenza* B/Victoria isolates (except B/Lisboa/4/2011) shared the amino acid mutation I146V **(Table II)** and clustered into 2 different subgroups: a subgroup of 5 strains (the only common substitution was I146V) and a second subgroup of fourteen viruses which additionally presented the amino acid substitution L58P (B/Hong Kong/514/2009 subgroup) **(Figure 1 and Table II)**. Additional amino acid substitutions were found: V87I (B/Lisboa/4/2010 and B/Lisboa/10/2010), G185E (B/Lisboa/3/2010, B/Lisboa/11/2010, B/Lisboa/12/2010, B/Lisboa/39/2010, B/Lisboa/40/2010, B/Lisboa/48/2010, B/Lisboa/4/2011 and EE98), V283T (EE98), P341A and L345S (B/Lisboa/40/2010) **(Figure 1 and Table II)**. From all the observed substitutions only I146V occurred at an antigenic site of the hemagglutinin molecule **(Table II)**.

One *influenza* virus B/Yamagata was isolated and it was antigenically similar to B/Florida/4/2006 (vaccine strain for the 2008/2009 season). Genetically, this viral strain and one B/Yamagata virus detected in another clinical specimen grouped with reference strains B/Bangladesh/3333/2007 and B/Wisconsin/1/2010 **(Figure 2)**. The isolated strain (B/Lisboa/9/2011) presented 3 amino acid substitutions at antigenic sites whereas the clinical specimen (EE222) presented 4 **(Table III)**.

Table I – Antigenic characterisation of 30 isolated *influenza* B/Victoria strains (Hemagglutination Inhibition Test). Strains genetically characterised are shaded in yellow.

| Viruses | Laboratório Nacional de Referência para o Vírus da Gripe (INSA) | | | WHO Collaborating Centre (London) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Hemagglutination Inhibition Titre (ferret antisera) | | | Hemagglutination Inhibition Titre (ferret antisera) | | | | | | |
| | B/Brisbane/60/2008 | B/Florida/4/2006 | B/Wisconsin/1/2010 | B/Malaysia/2506/2004 | B/England/393/2008 | B/Brisbane/60/2008 | B/Paris/1762/2008 | B/HK/514/2009 | B/Odessa/3886/2010 |
| *Reference Strains* | | | | | | | | | |
| B/Malaysia/2506/2004 | | | | 640 | 80 | 160 | < | < | 10 |
| B/England/393/2008 | | | | 640 | 320 | 640 | 40 | 160 | 160 |
| B/Brisbane/60/2008 | 640 | 40 | 20 | 320 | 320 | 640 | 40 | 80 | 80 |
| B/Paris/1762/2008 | | | | 40 | 40 | 80 | 160 | 640 | 320 |
| B/Hong Kong/514/2009 | | | | 20 | 40 | 80 | 80 | 640 | 320 |
| B/Odessa/3886/2010 | | | | 20 | 80 | 80 | 160 | 640 | 640 |
| B/Florida/4/2006 | 20 | 320 | 40 | | | | | | |
| B/Wisconsin/1/2010 | 10 | 320 | 80 | | | | | | |
| *Test viruses* | | | | | | | | | |
| B/Lisboa/1/2010 | 160 | 40 | 40 | | | | | | |
| B/Lisboa/3/2010 | 320 | 20 | 20 | 20 | 40 | 40 | 80 | 320 | 320 |
| B/Lisboa/4/2010 | 1280 | 40 | 20 | | | | | | |
| B/Lisboa/5/2010 | 20 | 10 | 10 | | | | | | |
| B/Lisboa/8/2010 | 160 | 40 | 10 | 10 | 10 | 20 | 40 | 80 | 160 |
| B/Lisboa/9/2010 | 320 | 40 | 40 | 10 | 20 | 20 | 40 | 80 | 160 |
| B/Lisboa/10/2010 | 10 | 10 | 10 | | | | | | |
| B/Lisboa/11/2010 | 1280 | 40 | 40 | 20 | 40 | 40 | 80 | 320 | 320 |
| B/Lisboa/12/2010 | 160 | 40 | 10 | | | | | | |
| B/Lisboa/13/2010 | 160 | 20 | 20 | 10 | 10 | 40 | 40 | 80 | 160 |
| B/Lisboa/14/2010 | 80 | 10 | 10 | 20 | 40 | 40 | 80 | 320 | 160 |
| B/Lisboa/15/2010 | 320 | 20 | 10 | 20 | 40 | 40 | 80 | 320 | 160 |
| B/Lisboa/17/2010 | 40 | 10 | 10 | 20 | 40 | 40 | 80 | 80 | 160 |
| B/Lisboa/20/2010 | 80 | 20 | 10 | 20 | 40 | 40 | 80 | 320 | 160 |
| B/Lisboa/21/2010 | 80 | 20 | 20 | 20 | 40 | 40 | 80 | 320 | 160 |
| *Reference Strains* | | | | | | | | | |
| B/Malaysia/2506/2004 | | | | 640 | 160 | 160 | < | < | 10 |
| B/England/393/2008 | | | | 80 | 320 | 640 | 80 | 80 | 80 |
| B/Brisbane/60/2008 | 640 | 20 | 20 | 80 | 320 | 640 | 80 | 40 | 80 |
| B/Paris/1762/2008 | | | | < | < | 40 | 160 | 320 | 160 |
| B/Hong Kong/514/2009 | | | | < | < | 40 | 160 | 640 | 320 |
| B/Odessa/3886/2010 | | | | < | 40 | 160 | 320 | 640 | 320 |
| B/Florida/4/2006 | 80 | 320 | 40 | | | | | | |
| *Test viruses* | | | | | | | | | |
| B/Lisboa/39/2010 | 320 | 10 | 10 | | | | | | |
| B/Lisboa/40/2010 | 640 | 20 | 10 | < | 40 | 80 | 160 | 640 | 320 |
| B/Lisboa/42/2010 | 640 | 20 | 20 | | | | | | |
| B/Lisboa/44/2010 | 640 | 10 | 20 | < | 40 | 80 | 160 | 640 | 320 |
| B/Lisboa/47/2010 | 320 | 10 | 20 | < | 40 | 80 | 160 | 640 | 320 |
| B/Lisboa/48/2010 | 640 | 10 | 10 | | | | | | |
| B/Lisboa/50/2010 | 320 | 10 | 10 | < | 40 | 80 | 160 | 640 | 320 |
| B/Lisboa/53/2010 | 320 | 10 | 10 | < | < | 40 | 160 | 320 | 160 |
| B/Lisboa/54/2010 | 320 | 10 | 10 | < | 80 | 160 | 320 | 640 | 320 |
| B/Lisboa/57/2010 | 40 | 20 | | | | | | | |
| B/Lisboa/61/2010 | 1280 | 40 | | | | | | | |
| B/Lisboa/2/2010 | 20 | 10 | 10 | < | < | 80 | 160 | 640 | 320 |
| B/Lisboa/2/2011 | 20 | 10 | 10 | < | < | 80 | 160 | 640 | 320 |
| B/Lisboa/3/2011 | 640 | 20 | 10 | < | < | 80 | 160 | 640 | 320 |
| B/Lisboa/4/2011 | 320 | 10 | 10 | < | < | 80 | 160 | 640 | 320 |

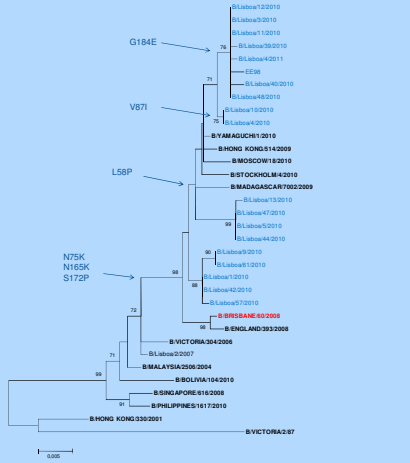


Figure 1 – Phylogenetic tree of HA1 nucleotide sequences from *influenza* B/Victoria viruses. Strains analysed are represented in blue, vaccine strain in red and reference strains in bold. Bootstrapp values above 70 are shown (500 replicates).

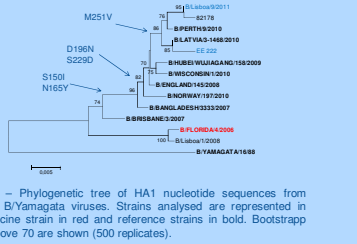


Figure 2 – Phylogenetic tree of HA1 nucleotide sequences from *influenza* B/Yamagata viruses. Strains analysed are represented in blue, vaccine strain in red and reference strains in bold. Bootstrapp values above 70 are shown (500 replicates).

## Conclusions:

The majority of *influenza* B viruses characterised in Portugal during the *influenza* season 2010/2011 were found similar, both antigenic and genetically, to the vaccine strain B/Brisbane/60/2008. Nucleotide sequence analysis revealed 2 different genetic subgroups within the B/Brisbane/60/2008 genetic clade. Isolated *influenza* B/Victoria viruses revealed only one amino acid substitution in antigenic sites of the HA molecule comparing with vaccine strain (Wang *et al.*, 2008). The *influenza* B/Yamagata viruses characterised grouped with the reference strains B/Wisconsin/1/2010 as did the majority of B/Yamagata viruses detected in circulation worldwide this season.

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Table III – Amino acid substitutions observed in the HA1 subunit of *influenza* B/Yamagata viruses comparing to the former vaccine strain B/Florida/4/2006. Antigenic sites of the hemagglutinin molecule are represented in red.

| Strains | Yamagata amino acid numbering | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 29 | 48 | 88 | 108 | 110 | 121 | 150 | 165 | 172 | 183 | 196 | 198 | 202 | 229 | 251 | 255 |
| *B/FLORIDA/4/2006* | V | R | K | P | L | T | S | N | L | G | D | T | N | S | M | G |
| *B/BRISBANE/3/2007* | . | K | R | A | . | . | . | . | . | . | N | . | . | G | . | . |
| *B/BANGLADESH/3333/2007* | . | . | R | . | . | . | I | Y | . | . | . | . | . | D | . | . |
| *B/ENGLAND/145/2008* | . | . | R | . | . | . | I | Y | . | . | N | A | . | D | . | . |
| *B/HUBEI/WUJIAGANG/158/2009* | . | . | R | . | . | . | I | Y | . | . | N | N | S | D | . | . |
| *B/WISCONSIN/1/2010* | . | . | R | . | . | . | I | Y | . | . | N | . | S | D | . | . |
| *B/PERTH/9/2010* | . | . | R | . | . | . | I | Y | . | . | N | . | . | D | V | . |
| *B/LATVIA/3-1468/2010* | . | . | R | . | . | A | I | Y | . | R | N | . | . | D | V | . |
| *B/NORWAY/197/2010* | . | . | R | . | I | . | I | Y | . | . | N | . | . | D | . | . |
| *B/Lisboa/1/2008* | . | . | R | . | . | . | . | . | . | . | N | . | . | . | . | . |
| B/Lisboa/9/2011 | A | . | R | . | . | . | I | Y | Q | . | N | . | . | D | V | . |
| EE222 | . | K | R | . | . | A | I | Y | . | R | N | . | . | D | V | . |

Table II – Amino acid substitutions observed in the HA1 subunit of *influenza* B/Victoria viruses comparing to the vaccine strain B/Brisbane/60/2008. Antigenic sites of the hemagglutinin molecule are represented in red.

| Strains | Victoria amino acid numbering | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 37 | 58 | 75 | 80 | 87 | 126 | 134 | 136 | 146 | 165 | 167 | 172 | 183 | 185 | 198 | 200 | 203 | 231 | 280 | 283 | 341 | 345 |
| *B/BRISBANE/60/2008* | T | L | K | R | V | N | P | K | I | K | K | P | T | G | N | T | A | G | R | V | P | L |
| *B/MALAYSIA/2506/2004* | . | . | N | . | . | . | S | . | V | N | . | S | . | . | . | A | . | . | . | . | . | . |
| *B/VICTORIA/304/2006* | . | . | N | . | . | . | . | . | V | N | . | S | . | . | T | . | . | D | . | . | . | . |
| *B/SINGAPORE/616/2008* | I | . | N | . | . | . | . | . | V | N | . | S | . | . | . | A | . | . | . | . | . | . |
| *B/ENGLAND/393/2008* | . | . | . | . | . | . | . | . | . | . | . | . | . | . | S | . | . | . | . | . | . | . |
| *B/HONG KONG/514/2009* | . | P | . | . | . | . | . | . | V | . | . | . | . | . | . | . | . | . | K | . | . | . |
| *B/MADAGASCAR/7002/2009* | . | P | . | . | . | . | . | . | V | . | . | . | . | . | . | . | V | . | . | . | . | . |
| *B/BOLIVIA/104/2010* | . | . | N | I | . | . | . | . | V | . | R | S | K | . | . | ? | . | . | . | . | . | . |
| *B/PHILIPPINES/1617/2010* | I | P | N | . | . | . | . | . | V | N | . | S | . | . | . | . | . | . | . | . | . | . |
| *B/MOSCOW/18/2010* | . | P | . | . | . | I | . | . | V | . | . | . | . | . | . | ? | . | . | . | . | . | . |
| *B/STOCKHOLM/4/2010* | . | P | . | . | . | . | . | . | V | . | . | . | . | . | . | . | . | . | . | . | . | . |
| *B/YAMAGUCHI/1/2010* | . | P | . | . | . | . | R | V | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| *B/Lisboa/2/2007* | . | . | N | . | . | . | . | . | V | N | . | S | . | . | . | . | . | . | . | . | . | . |
| B/Lisboa/1/2010 | . | . | . | . | . | . | . | . | V | . | . | . | . | . | . | . | . | . | . | . | . | . |
| B/Lisboa/42/2010 | . | . | . | . | . | . | . | . | V | . | . | . | . | . | . | . | . | . | . | . | . | . |
| B/Lisboa/57/2010 | . | . | . | . | . | . | . | . | V | . | . | . | . | . | . | . | . | . | . | . | . | . |
| B/Lisboa/9/2010 | . | . | . | . | . | . | . | . | V | . | . | . | . | . | . | . | . | . | . | . | . | . |
| B/Lisboa/61/2010 | . | . | . | . | . | . | . | . | V | . | . | . | . | . | . | . | . | . | . | . | . | . |
| B/Lisboa/5/2010 | . | P | . | . | . | . | . | . | V | . | . | . | . | . | . | . | . | . | . | . | . | . |
| B/Lisboa/13/2010 | . | P | . | . | . | . | . | . | V | . | . | . | . | . | . | . | . | . | . | . | . | . |
| B/Lisboa/44/2010 | . | P | . | . | . | . | . | . | V | . | . | . | . | . | . | . | . | . | . | . | . | . |
| B/Lisboa/47/2010 | . | P | . | . | . | . | . | . | V | . | . | . | . | . | . | . | . | . | . | . | . | . |
| B/Lisboa/4/2010 | . | P | . | I | . | . | . | . | V | . | . | . | . | . | . | . | . | . | . | . | . | . |
| B/Lisboa/10/2010 | . | P | . | I | . | . | . | . | V | . | . | . | . | . | . | . | . | . | . | . | . | . |
| B/Lisboa/11/2010 | . | P | . | . | . | . | . | . | V | . | . | . | . | E | . | . | . | . | . | . | . | . |
| B/Lisboa/12/2010 | . | P | . | . | . | . | . | . | V | . | . | . | . | E | . | . | . | . | . | . | . | . |
| B/Lisboa/39/2010 | . | P | . | . | . | . | . | . | V | . | . | . | . | E | . | . | . | . | . | . | . | . |
| B/Lisboa/40/2010 | . | P | . | . | . | . | . | . | V | . | . | . | . | E | . | . | . | . | . | . | A | S |
| B/Lisboa/48/2010 | . | P | . | . | . | . | . | . | V | . | . | . | . | E | . | . | . | . | . | . | . | . |
| B/Lisboa/3/2010 | . | P | . | . | . | . | . | . | V | . | . | . | . | E | . | . | . | . | . | . | . | . |
| B/Lisboa/4/2011 | . | P | . | . | . | . | . | . | . | . | . | . | . | E | . | . | . | . | . | . | . | . |
| EE98 | . | P | . | . | . | . | . | . | V | . | . | . | . | E | . | . | . | . | . | T | . | . |

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