

# ANTIGENIC AND GENETIC ANALYSIS OF PANDEMIC INFLUENZA A(H1N1)2009 VIRUSES FROM PORTUGAL

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## Background:

In April 2009, a new pandemic strain of influenza infected thousands of persons in Mexico and the United States and spread rapidly worldwide. This virus had origin in a reassortment between a North American swine lineage (already a triple reassortant, circulating in pigs since the late 1990's) and a Eurasian swine lineage (Garten *et al.*, 2009). The influenza pandemic virus (A(H1N1)2009) was first detected in Portugal in 4th May 2009. The early diversification of the A(H1N1)2009 viruses (based on concatenated whole genomes) resulted into seven lineages, clade 1–7 (Nelson *et al.*, 2009). However, none of the amino acid changes that define clades are located in HA antigenic sites or is associated in the NA with resistance to antiviral drugs (Nelson *et al.*, 2009).

In contrast with recent seasonal human H1N1 viruses, the A(H1N1)2009 hemagglutinin (North American swine lineage) contains almost the same amino acid composition in the antigenic sites that pandemic 1918 AH1N1 viruses (Igarashi *et al.*, 2010). As A(H1N1)2009 viruses continue to circulate in the human population, HA antigenic sites will continue to be targeted by antibody-mediated selection pressure and certainly acquire amino acid substitutions. Therefore it is important, also from a public health perspective, continue to characterize the HA and to monitor the antigenic and genetic properties of the H1N1 pandemic viruses in order to detect any changes and thus any necessity for selecting further vaccine candidates or changes in antiviral recommendations.

In this study, is presented a genetic and antigenic characterization of influenza A(H1N1)2009 viruses, isolated in Portugal over the 2009 influenza pandemic.

## Material and Methods:

In Portugal, during the 2009 influenza pandemic, about 16500 clinical samples were tested by the National Influenza Reference Laboratory for the presence of influenza A(H1N1)2009 virus. From near 8000 A(H1N1)2009-positive (real-time RT-PCR) samples, 147 were isolated in MDCK-SIAT1 cell cultures and characterised antigenically by hemagglutination-inhibition assays (HI) performed by the WHOcc - London. Of these, 56 isolates were taken for sequence analysis of the HA1 gene segment. Nucleotide sequences were aligned (ClustalW) and the phylogenetic trees constructed (Neighbour-joining, Kimura 2-parameter) using MEGA 4.0 software.

Table I – Antigenic analysis (HI titres) of pandemic influenza A(H1N1)2009 strains isolated in Portugal.

Virus	Collection date	Passage history	Post infection ferret sera					
			A/California/4/09	A/California/7/09	A/Bangkok/1/05	A/Bangkok/3/05	A/Bayern/69/09	A/Lviv/6/09
<b>REFERENCE VIRUSES</b>								
A/California/4/09	01/02	MDCK	2560	2560	5120	5120	2560	5120
A/California/7/09	01/02	MDCK	2560	2560	5120	5120	1280	2560
A/Bangkok/1/05	01/02	MDCK	2560	2560	2560	5120	1280	2560
A/Bangkok/3/05	01/02	MDCK	2560	2560	2560	5120	1280	2560
A/Bayern/69/09	01/02	MDCK	2560	2560	2560	5120	1280	2560
A/Lviv/6/09	01/02	MDCK	320	1280	320	160	1280	2560
<b>TEST VIRUSES</b>								
A/Lisboa/7/09/2009	18/08/2009	MDCK SA1	160	640	160	160	640	640
A/Lisboa/10/09/2009	18/08/2009	MDCK SA1	2560	2560	1280	1280	1280	1280
A/Lisboa/15/09/2009	15/09/2009	MDCK SA1	2560	2560	2560	5120	2560	640
A/Lisboa/16/09/2009	14/09/2009	MDCK SA1	1280	2560	1280	1280	2560	2560
A/Lisboa/17/09/2009	14/09/2009	MDCK SA1	1280	2560	2560	5120	1280	5120
A/Lisboa/18/09/2009	14/09/2009	MDCK SA1	2560	2560	2560	5120	1280	640
A/Lisboa/19/09/2009	14/09/2009	MDCK SA1	5120	5120	5120	5120	5120	640
A/Lisboa/20/09/2009	14/09/2009	MDCK SA1	2560	5120	5120	5120	2560	2560
A/Lisboa/21/09/2009	15/09/2009	MDCK SA1	1280	1280	1280	1280	640	1280
A/Lisboa/22/09/2009	15/09/2009	MDCK SA1	2560	2560	2560	5120	1280	1280
A/Lisboa/23/09/2009	15/09/2009	MDCK SA1	2560	2560	2560	5120	1280	1280
A/Lisboa/24/09/2009	15/09/2009	MDCK SA1	2560	2560	2560	5120	1280	1280
A/Lisboa/25/09/2009	15/09/2009	MDCK SA1	2560	2560	2560	5120	1280	1280
A/Lisboa/26/09/2009	15/09/2009	MDCK SA1	2560	2560	2560	5120	1280	1280
A/Lisboa/27/09/2009	15/09/2009	MDCK SA1	320	1280	320	320	1280	1280
A/Lisboa/28/09/2009	15/09/2009	MDCK SA1	640	640	640	640	1280	1280
A/Lisboa/29/09/2009	15/09/2009	MDCK SA1	2560	2560	1280	5120	1280	1280
A/Lisboa/30/09/2009	15/09/2009	MDCK SA1	2560	2560	5120	5120	1280	1280
A/Lisboa/31/09/2009	15/09/2009	MDCK SA1	2560	2560	1280	5120	640	1280
A/Lisboa/01/10/2009	14/10/2009	MDCK SA1	2560	2560	1280	2560	640	640
A/Lisboa/02/10/2009	14/10/2009	MDCK SA1	2560	2560	1280	5120	1280	1280
A/Lisboa/03/10/2009	14/10/2009	MDCK SA1	2560	2560	1280	5120	1280	1280
A/Lisboa/04/10/2009	14/10/2009	MDCK SA1	2560	2560	2560	5120	1280	2560
A/Lisboa/05/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/06/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/07/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/08/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/09/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/10/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/11/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/12/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/13/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/14/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/15/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/16/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/17/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/18/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/19/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/20/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/21/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/22/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/23/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/24/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/25/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/26/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/27/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/28/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/29/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/30/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560
A/Lisboa/31/10/2009	14/10/2009	MDCK SA1	2560	2560	5120	5120	1280	2560

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<b>REFERENCE VIRUSES</b>								
A/California/4/09	01/02	MDCK	2560	2560	2560	5120	2560	2560
A/California/7/09	01/02	MDCK	2560	2560	2560	5120	1280	2560
A/Bangkok/1/05	01/02	MDCK	2560	2560	2560	5120	1280	2560
A/Bangkok/3/05	01/02	MDCK	2560	2560	2560	5120	1280	2560
A/Bayern/69/09	01/02	MDCK	2560	2560	2560	5120	1280	2560
A/Lviv/6/09	01/02	MDCK	80	320	160	80	640	320
<b>TEST VIRUSES</b>								
A/Lisboa/7/09/2009	18/08/2009	MDCK SA1	2560	2560	2560	5120	1280	2560
A/Lisboa/10/09/2009	18/08/2009	MDCK SA1	1280	1280	1280	1280	640	1280
A/Lisboa/15/09/2009	15/09/2009	MDCK SA1	2560	2560	2560	5120	1280	1280
A/Lisboa/16/09/2009	14/09/2009	MDCK SA1	640	1280	1280	1280	640	1280
A/Lisboa/17/09/2009	14/09/2009	MDCK SA1	640	640	640	1280	640	1280
A/Lisboa/18/09/2009	14/09/2009	MDCK SA1	2560	2560	2560	5120	1280	2560
A/Lisboa/19/09/2009	14/09/2009	MDCK SA1	2560	2560	2560	5120	1280	2560
A/Lisboa/20/09/2009	14/09/2009	MDCK SA1	1280	1280	1280	2560	1280	2560
A/Lisboa/21/09/2009	14/09/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/22/09/2009	14/09/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/23/09/2009	14/09/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/24/09/2009	14/09/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/25/09/2009	14/09/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/26/09/2009	14/09/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/27/09/2009	14/09/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/28/09/2009	14/09/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/29/09/2009	14/09/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/30/09/2009	14/09/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/01/10/2009	14/10/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/02/10/2009	14/10/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/03/10/2009	14/10/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/04/10/2009	14/10/2009	MDCK SA1	1280	1280	1280	2560	640	1280
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A/Lisboa/08/10/2009	14/10/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/09/10/2009	14/10/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/10/10/2009	14/10/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/11/10/2009	14/10/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/12/10/2009	14/10/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/13/10/2009	14/10/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/14/10/2009	14/10/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/15/10/2009	14/10/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/16/10/2009	14/10/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/17/10/2009	14/10/2009	MDCK SA1	1280	1280	1280	2560	640	1280
A/Lisboa/18/10/2009								