Molecular epidemiology of influenza A(H1N1)PDM09 hemagglutinin gene circulating in São *Paulo State*, Brazil: 2016 anticipated influenza season

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ABSTRACT

Compared to previous years, seasonal influenza activity commenced early in São Paulo State, Brazil, Southern hemisphere during the 2016 year. In order to investigate the genetic pattern of influenza A(H1N1)pdm09 in the State of Sao Paulo a total of 479 respiratory samples, collected in January by Sentinel Surveillance Units, were screened by real-time RT-PCR. A total of 6 Influenza viruses A(H1N1)pdm09 presenting ct values ≤ 30 were sequenced following phylogenetic analysis. The present study identified the circulation of the new 6B.1 subgroup (A/Sao Paulo/10-118/2016 and A/Sao Paulo/3032/2016). In addition, influenza A(H1N1)pdm09 group 6B has also been identified during January in the State of Sao Paulo. Despite amino acid changes and changes in potential glycosylation motifs, 6B.1 viruses were well inhibited by the reference ferret antiserum against A/California/07/2009 virus, the A(H1N1)pdm09 component of the vaccine for the 2016 influenza season.

KEYWORDS: Influenza. National Influenza Surveillance Network. Molecular epidemiology. Vaccination strategy. Public health.

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Compared to previous years, seasonal influenza activity commenced early in *São Paulo State*, Brazil, Southern hemisphere. Influenza A(H1N1)pdm09 virus detection started in January 2016 during the summer season with hot temperatures, and it was the predominating strain in autumn[1,2]. In contrast, influenza seasonal activity commenced late in some countries in Western Europe, North America and Eastern Asia. Based on the WHO global influenza surveillance, in countries with influenza A(H1N1)pdm09 virus predominance, the hospitalization and intensive care unit (ICU) admission patterns seem to be similar to previous seasons when this virus predominated and young/middle-aged adults experienced severe disease[3]. The aim of this study was to investigate the genetic pattern of influenza A(H1N1)pdm09 in the *São Paulo State*.

A total of 479 respiratory samples, collected in January by Sentinel Surveillance Units, were screened by real-time RT-PCR (qRT – PCR)[4]. Among them, 30 Influenza virus A(H1N1)pdm09 presenting ct values ≤ 30 were identified. A total of 6 viruses were sequenced by using an Applied Biosystems BigDye® Terminator v3.1 Cycle Sequencing Kit with reaction products resolved on an Applied Biosystems Sequencer 3730 DNA Analyzer. Nucleotide sequences were aligned using MUSCLE[5]. Sequences alignment results were further analyzed using the BioEdit program[6].

Our Institution is one of the National Influenza Centers accredited by the World Health Organization. The present follow up study of influenza surveillance has been

approved by the *Conselho Nacional de* Ética *em Pesquisa (CONEP)* –135/2002.

Phylogenetic analyses

The TREESUB phylogenetic program (available from https://github.com/tamuri/treesub) was used to estimate the maximum likelihood phylogenetic trees using RAxML and PAML, followed by branch annotation of amino acid substitutions. The general time reversible+Γ (GTR+GAMMA) nucleotide substitution model was selected in RAxML v.7.3.0 for tree inference[7]. Ancestral codon substitutions for each gene were estimated using baseml, as implemented in PAML[8] using the ML trees inferred. Nonsynonymous substitutions were then transcribed onto the consensus gene phylogenies and visualized in Figure 1 Tree v1.4.2 (available from http://tree.bio.ed.ac.uk/software/figtree/).
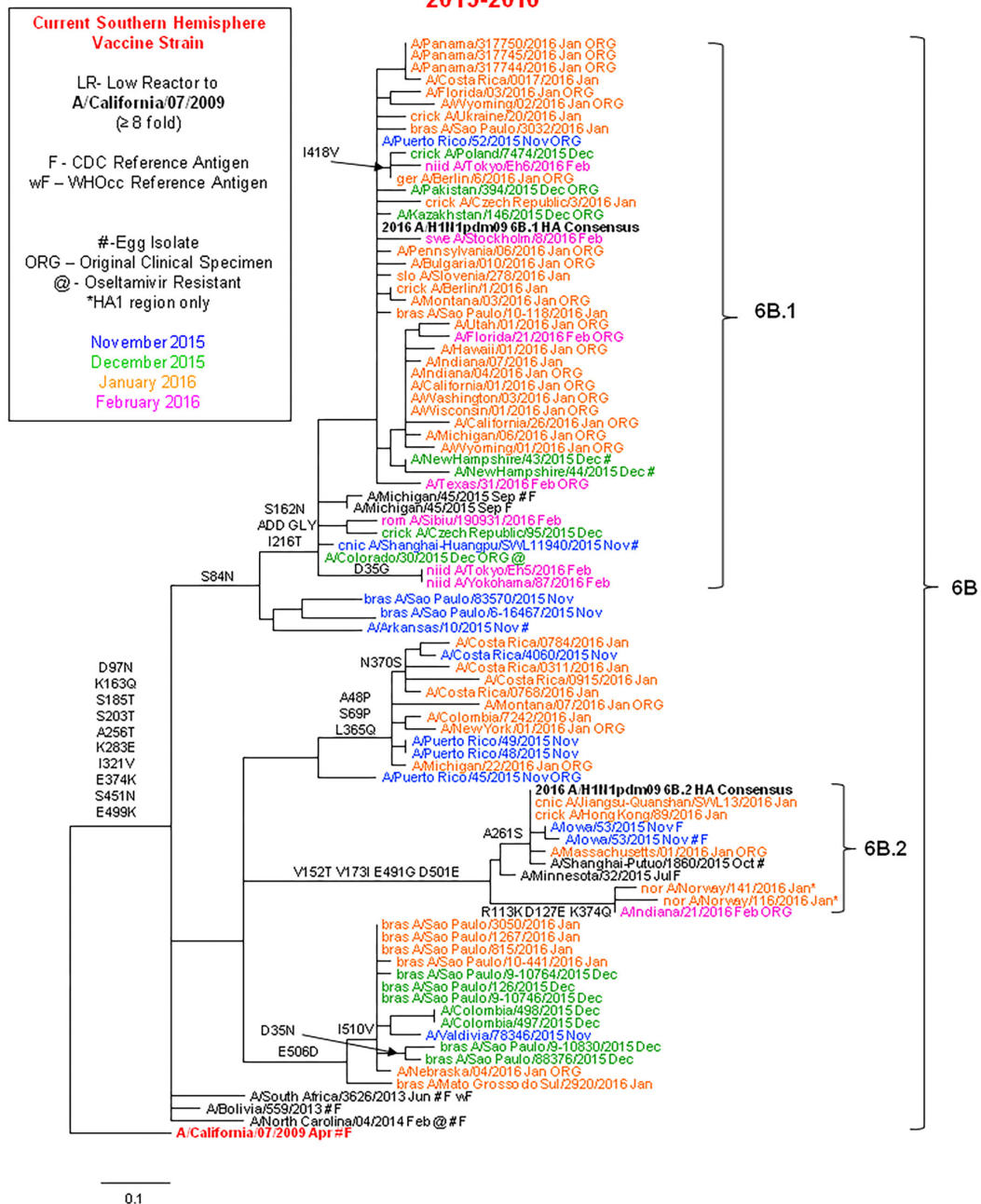

Figure 1 - Molecular epidemiology of influenza A(H1N1)pdm09 hemagglutinin gene circulating in São Paulo, state, Brazil: 2016 anticipated influenza season.

Influenza A(H1N1)pdm09 viruses identified in *São Paulo State* during November 2015 belong to the 6B genetic group, presenting AA changes in the HA at residue E179D and I183V in the HA2 region. Figure 1 shows two viruses from Brazil collected in January that belong to the new 6B.1 subgroup (A/Sao Paulo/10-118/2016 and A/Sao Paulo/3032/2016). The HA sequences from *São Paulo State* obtained in this study, which were also used in the phylogenetic analysis, were deposited in the EpiFlu database of the Global Initiative on sharing Avian Influenza Data (GISAID) under the following accession numbers: EPI725841, EPI725838.

Influenza A(H1N1)pdm09 group 6B has also been identified during January in *São Paulo State* as shown in Figure 1, which were also used in the phylogenetic analysis; A/Sao Paulo/3050/2016; A/Sao Paulo/1267/2016; A/Sao Paulo/815/2016; A/Sao Paulo/10-441/2016 deposited in the GISAID under the follow accession numbers EPI725839, EPI725844, EPI704027, EPI725842, respectively.

The phylogenetic analysis of the A(H1N1)pdm09 HA gene demonstrated that HA genes of the recent viruses diverged into genetic groups 6A, 6B or 6C, with all viruses collected since September 1st, 2015 belonging to the genetic subgroup 6B. Subgroups 6B and 6C share AA changes in the HA at residues K283E and E499K (mature A(H1N1)pdm09 numbering after the signal peptide). Subgroup 6B viruses also possess AA changes in the HA at residues K163Q and A256T. Within subgroup 6B, additional subgroups with shared amino acid changes have emerged. The majority of viruses share an AA change at residue S84N; among these more than half share two additional changes at residues S162N (adds a glycosylation motif) and I216T (new subgroup 6B.1). A smaller group of 6B viruses (new subgroup 6B.2) shares AA changes at residues V152T, V173I, E491G and D501E[9].

Despite amino acid changes and changes in glycosylation, 6B.1 viruses were well inhibited by the reference ferret antiserum against A/California/07/2009 virus the A(H1N1)pdm09 component of the vaccine for 2016 influenza season[9].

In addition, a pool of human post-vaccination sera collected from healthy adults in the United States of America who received influenza vaccine in the 2015-2016 seasons well inhibited all recent viruses tested in the WHO CC at the Centers for Disease Control and Prevention (CDC) in Atlanta[9].

According to the literature data, the United Kingdom (UK), as others countries in Europe, has experienced a season dominated by circulation of influenza A(H1N1)pdm09 with reports of increases in hospitalizations and ICU admissions mainly in younger adults. The epidemiological observations are consistent with earlier seasons in the UK dominated by circulation of A(H1N1)pdm09, in particular in 2010/11, the first post-pandemic season[10]. In *São Paulo State* this influenza virus A(H1N1)pdm09 pattern of seasonality occurred in 2013 (personnel communication), and in the currently anticipated influenza virus season 2016, influenza A(H1N1)pdm09 predominated.

Influenza virological surveillance follow-up will provide the antigenic and phylogenetic patterns of influenza A(H1N1)pdm09 virus circulation during the coming winter and early spring period in *São Paulo State*. Taking into account that Brazilian vacation period corresponds to the North America and Western Europe influenza season (January/February), and also considering the late influenza virus seasonality in these regions, this pattern of seasonality may be considered to interpret the earlier influenza seasonality in *São Paulo State* in 2016.

ACKNOWLEDGEMENTS

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Supplemental Table - Origin of the haemaglutinin sequences of influenza A(H1N1)pdm09 isolates for the phylogenetic analysis . We acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu™ Database on which this research is based. The list is detailed below

Segment ID	Segment	Country	Collection date	Isolate name	Originating Lab	Submitting Lab	Authors
EPI691137	HA	United States	2015-Nov-05	A/Arkansas/10/2015	Arkansas Children's Hospital	Centers for Disease Control and Prevention	
EPI706882	HA	Germany	2016-Jan-11	A/Berlin/1/2016	Robert-Koch-Institute	Crick Worldwide Influenza Centre	
EPI733770	HA	Germany	2016-Jan-19	A/Berlin/6/2016	Robert-Koch-Institute	Crick Worldwide Influenza Centre	
EPI465091	HA	Bolivia, Plurinational State of	2013-Jun-08	A/Bolivia/559/2013	Instituto Nacional de Laboratoriosde Salud (INLASA)	Centers for Disease Control and Prevention	
EPI700599	HA	Bulgaria	2016-Jan-08	A/Bulgaria/010/2016	National Influenza Laboratory, Natl. Center of Inf. & Parasitic Dis	Centers for Disease Control and Prevention	
EPI700615	HA	United States	2016-Jan-04	A/California/01/2016	California Department of Health Services	Centers for Disease Control and Prevention	
EPI176620	HA	United States	2009-Apr-09	A/California/07/2009	Naval Health Research Center	Centers for Disease Control and Prevention	
EPI717170	HA	United States	2016-Jan-08	A/California/26/2016	California Department of Health Services	Centers for Disease Control and Prevention	
EPI725887	HA	Colombia	2015-Dec-10	A/Colombia/497/2015	Instituto Nacional de Salud de Columbia	Centers for Disease Control and Prevention	
EPI725895	HA	Colombia	2015-Dec-10	A/Colombia/498/2015	Instituto Nacional de Salud de Columbia	Centers for Disease Control and Prevention	
EPI725911	HA	Colombia	2016-Jan-01	A/Colombia/7242/2016	Instituto Nacional de Salud de Columbia	Centers for Disease Control and Prevention	
EPI700671	HA	United States	2015-Dec-14	A/Colorado/30/2015	Colorado Department of Health Lab	Centers for Disease Control and Prevention	
EPI706013	HA	Costa Rica	2016-Jan-04	A/Costa Rica/0017/2016	Laboratorio Nacional de Influenza	Centers for Disease Control and Prevention	
EPI716691	HA	Costa Rica	2016-Jan-08	A/Costa Rica/0311/2016	Laboratorio Nacional de Influenza	Centers for Disease Control and Prevention	
EPI706021	HA	Costa Rica	2016-Jan-08	A/Costa Rica/0768/2016	Laboratorio Nacional de Influenza	Centers for Disease Control and Prevention	

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EPI706029	HA	Costa Rica	2016-Jan-08	A/Costa Rica/0784/2016	Laboratorio Nacional de Influenza	Centers for Disease Control and Prevention	
EPI716699	HA	Costa Rica	2016-Jan-09	A/Costa Rica/0915/2016	Laboratorio Nacional de Influenza	Centers for Disease Control and Prevention	
EPI690877	HA	Costa Rica	2015-Nov-20	A/Costa Rica/4060/2015	Laboratorio Nacional de Influenza	Centers for Disease Control and Prevention	
EPI706894	HA	Czech Republic	2016-Jan-01	A/Czech Republic/3/2016	National Institute of Public Health	Crick Worldwide Influenza Centre	
EPI706896	HA	Czech Republic	2015-Dec-01	A/Czech Republic/95/2015	National Institute of Public Health	Crick Worldwide Influenza Centre	
EPI709468	HA	United States	2016-Jan-13	A/Florida/03/2016	Florida Department of Health-Jacksonville	Centers for Disease Control and Prevention	
EPI726014	HA	United States	2016-Feb-19	A/Florida/21/2016	Florida Department of Health-Tampa	Centers for Disease Control and Prevention	
EPI706061	HA	United States	2016-Jan-12	A/Hawaii/01/2016	State of Hawaii Department of Health	Centers for Disease Control and Prevention	
EPI697711	HA	Hong Kong (SAR)	2016-Jan-04	A/Hong Kong/89/2016	Government Virus Unit	Crick Worldwide Influenza Centre	
EPI732918	HA	United States	2016-Jan-25	A/Indiana/04/2016	Indiana State Department of Health Laboratories	Centers for Disease Control and Prevention	
EPI715972	HA	United States	2016-Jan-24	A/Indiana/07/2016	Indiana State Department of Health Laboratories	Centers for Disease Control and Prevention	
EPI717210	HA	United States	2016-Feb-04	A/Indiana/21/2016	Indiana State Department of Health Laboratories	Centers for Disease Control and Prevention	
EPI709638	HA	United States	2015-Nov-04	A/Iowa/53/2015	Iowa State Hygienic Laboratory	Centers for Disease Control and Prevention	
EPI709630	HA	United States	2015-Nov-04	A/Iowa/53/2015	Iowa State Hygienic Laboratory	Centers for Disease Control and Prevention	
EPI697117	HA	China	2016-Jan-05	A/Jiangsu-Quanshan/SWL13/2016	WHO Chinese National Influenza Center	WHO Chinese National Influenza Center	Yu Lan,Weijuan Huang, Xiyan Li, Xiang Zhao, Yanhui Cheng, Minju Tan, Dayan Wang, Yuelong Shu
EPI691262	HA	Kazakhstan	2015-Dec-02	A/Kazakhstan/146/2015	CSEE	Centers for Disease Control and Prevention	
EPI700788	HA	United States	2016-Jan-11	A/Massachusetts/01/2016	Massachusetts Department of Public Health	Centers for Disease Control and Prevention	
EPI725840	HA	Brazil	2016-Jan-18	A/Mato Grosso do Sul/2920/2016	Instituto Adolfo Lutz	Instituto Adolfo Lutz	Santos, Katia; Silva, Daniela; Benega, Margarete; Santos, Cecilia; Paiva, Terezinha
EPI732896	HA	United States	2016-Jan-19	A/Michigan/06/2016	University of Michigan SPH EPID	Centers for Disease Control and Prevention	
EPI717234	HA	United States	2016-Jan-04	A/Michigan/22/2016	Michigan Department of Community Health	Centers for Disease Control and Prevention	
EPI699812	HA	United States	2015-Sep-07	A/Michigan/45/2015	Michigan Department of Community Health	Centers for Disease Control and Prevention	

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EPI685579	HA	United States	2015-Sep-07	A/Michigan/45/2015	Michigan Department of Community Health	Centers for Disease Control and Prevention	
EPI699804	HA	United States	2015-Jul-09	A/Minnesota/32/2015	Minnesota Department of Health	Centers for Disease Control and Prevention	
EPI706181	HA	United States	2016-Jan-09	A/Montana/03/2016	Montana Laboratory Services Bureau	Centers for Disease Control and Prevention	
EPI709516	HA	United States	2016-Jan-10	A/Montana/07/2016	Montana Laboratory Services Bureau	Centers for Disease Control and Prevention	
EPI716444	HA	United States	2016-Jan-21	A/Nebraska/04/2016	Nebraska Public Health Lab	Centers for Disease Control and Prevention	
EPI695421	HA	United States	2015-Dec-03	A/New Hampshire/43/2015	New Hampshire Public Health Laboratories	Centers for Disease Control and Prevention	
EPI695469	HA	United States	2015-Dec-04	A/New Hampshire/44/2015	New Hampshire Public Health Laboratories	Centers for Disease Control and Prevention	
EPI700817	HA	United States	2016-Jan-05	A/New York/01/2016	New York City Department of Health	Centers for Disease Control and Prevention	
EPI541854	HA	United States	2014-Feb-16	A/North Carolina/04/2014	Mission Hospital Microbiology Lab	Centers for Disease Control and Prevention	
EPI695331	HA	Norway	2016-Jan-04	A/Norway/116/2016	Drammen Hospital / Vestreviken HF, Depto for Medical Microbiology section Drammen	Norwegian Institute of Public Health	Bragstad, K; Dudman, SG; Waalen, K; Hungnes, O
EPI695336	HA	Norway	2016-Jan-04	A/Norway/141/2016	Unilabs Telelab, Laboratory for Medical Microbiology	Norwegian Institute of Public Health	Bragstad, K; Dudman, SG; Waalen, K; Hungnes, O
EPI706259	HA	Pakistan	2015-Dec-01	A/Pakistan/394/2015	National Institute of Health	Centers for Disease Control and Prevention	
EPI716460	HA	Panama	2016-Jan-09	A/Panama/317744/2016	Instituto Conmemorativo Gorgas de Estudios de la Salud	Centers for Disease Control and Prevention	
EPI716468	HA	Panama	2016-Jan-09	A/Panama/317745/2016	Instituto Conmemorativo Gorgas de Estudios de la Salud	Centers for Disease Control and Prevention	
EPI716476	HA	Panama	2016-Jan-12	A/Panama/317750/2016	Instituto Conmemorativo Gorgas de Estudios de la Salud	Centers for Disease Control and Prevention	
EPI709524	HA	United States	2016-Jan-05	A/Pennsylvania/06/2016	Pennsylvania Department of Health	Centers for Disease Control and Prevention	
EPI697742	HA	Poland	2015-Dec-31	A/Poland/7474/2015	National Institute of Public Health - National Institute of Hygiene	Crick Worldwide Influenza Centre	
EPI716012	HA	Puerto Rico	2015-Nov-15	A/Puerto Rico/45/2015	Puerto Rico Department of Health	Centers for Disease Control and Prevention	
EPI706290	HA	Puerto Rico	2015-Nov-18	A/Puerto Rico/48/2015	Puerto Rico Department of Health	Centers for Disease Control and Prevention	

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EPI710546	HA	Puerto Rico	2015-Nov-20	A/Puerto Rico/49/2015	Puerto Rico Department of Health	Centers for Disease Control and Prevention	
EPI716020	HA	Puerto Rico	2015-Nov-27	A/Puerto Rico/52/2015	Puerto Rico Department of Health	Centers for Disease Control and Prevention	
EPI725841	HA	Brazil	2016-Jan-04	A/Sao Paulo/10-118/2016	Instituto Adolfo Lutz	Instituto Adolfo Lutz	Santos, Katia; Silva, Daniela; Benega, Margarete; Santos, Cecilia; Paiva, Terezinha
EPI725842	HA	Brazil	2016-Jan-11	A/Sao Paulo/10-441/2016	Instituto Adolfo Lutz	Instituto Adolfo Lutz	Santos, Katia; Silva, Daniela; Benega, Margarete; Santos, Cecilia; Paiva, Terezinha
EPI704025	HA	Brazil	2015-Dec-29	A/Sao Paulo/126/2016	Instituto Adolfo Lutz	Instituto Adolfo Lutz	Santos, Katia; Silva, Daniela; Benega, Margarete; Santos, Cecilia; Paiva, Terezinha
EPI704028	HA	Brazil	2016-Jan-11	A/Sao Paulo/1267/2016	Instituto Adolfo Lutz	Instituto Adolfo Lutz	Santos, Katia; Silva, Daniela; Benega, Margarete; Santos, Cecilia; Paiva, Terezinha
EPI725838	HA	Brazil	2016-Jan-24	A/Sao Paulo/3032/2016	Instituto Adolfo Lutz	Instituto Adolfo Lutz	Santos, Katia; Silva, Daniela; Benega, Margarete; Santos, Cecilia; Paiva, Terezinha
EPI725839	HA	Brazil	2016-Jan-25	A/Sao Paulo/3050/2016	Instituto Adolfo Lutz	Instituto Adolfo Lutz	Santos, Katia; Silva, Daniela; Benega, Margarete; Santos, Cecilia; Paiva, Terezinha
EPI687384	HA	Brazil	2015-Nov-25	A/Sao Paulo/6-16467/2015	Instituto Adolfo Lutz	Instituto Adolfo Lutz	Santos, Katia; Silva, Daniela; Benega, Margarete; Santos, Cecilia; Paiva, Terezinha
EPI704027	HA	Brazil	2016-Jan-08	A/Sao Paulo/815/2016	Instituto Adolfo Lutz	Instituto Adolfo Lutz	Santos, Katia; Silva, Daniela; Benega, Margarete; Santos, Cecilia; Paiva, Terezinha
EPI685425	HA	Brazil	2015-Nov-20	A/Sao Paulo/83570/2015	Instituto Adolfo Lutz	Instituto Adolfo Lutz	Santos, Katia; Silva, Daniela; Benega, Margarete; Santos, Cecilia; Paiva, Terezinha
EPI687387	HA	Brazil	2015-Dec-15	A/Sao Paulo/88376/2015	Instituto Adolfo Lutz	Instituto Adolfo Lutz	Santos, Katia; Silva, Daniela; Benega, Margarete; Santos, Cecilia; Paiva, Terezinha
EPI687386	HA	Brazil	2015-Dec-07	A/Sao Paulo/9-10746/2015	Instituto Adolfo Lutz	Instituto Adolfo Lutz	Santos, Katia; Silva, Daniela; Benega, Margarete; Santos, Cecilia; Paiva, Terezinha
EPI687385	HA	Brazil	2015-Dec-08	A/Sao Paulo/9-10764/2015	Instituto Adolfo Lutz	Instituto Adolfo Lutz	Santos, Katia; Silva, Daniela; Benega, Margarete; Santos, Cecilia; Paiva, Terezinha
EPI704003	HA	Brazil	2015-Dec-17	A/Sao Paulo/9-10830/2015	Instituto Adolfo Lutz	Instituto Adolfo Lutz	Santos, Katia; Silva, Daniela; Benega, Margarete; Santos, Cecilia; Paiva, Terezinha
EPI697225	HA	China	2015-Nov-20	A/Shanghai-Huangpu/ SWL11940/2015	WHO Chinese National Influenza Center	WHO Chinese National Influenza Center	Yu Lan, Weijuan Huang, Xiyan Li, Xiang Zhao, Yanhui Cheng, Minju Tan, Dayan Wang, Yuelong Shu
EPI709614	HA	China	2015-Oct-29	A/Shanghai-Putuo/1860/2015	WHO Chinese National Influenza Center	Centers for Disease Control and Prevention	

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EPI711230	HA	Romania	2016-Feb-02	A/Sibiu/190931/2016	Cantacuzino Institute	Cantacuzino Institute	Lazar, M.; Dinu, S.; Oprea, M.; Necula, G.; Ivanciuc, A.; Lupulescu, E
EPI709640	HA	Slovenia	2016-Jan-20	A/Slovenia/278/2016		National Laboratory for Health Environment and Food	
EPI577031	HA	South Africa	2013-Jun-06	A/South Africa/3626/2013	National Institute for Medical Research	Centers for Disease Control and Prevention	
EPI712265	HA	Sweden	2016-Feb-02	A/Stockholm/8/2016		Swedish Institute for Infectious Disease Control	
EPI725683	HA	United States	2016-Feb-09	A/Texas/31/2016	Texas Department of State Health Services-Laboratory Services	Centers for Disease Control and Prevention	
EPI710931	HA	Japan	2016-Feb-01	A/TOKYO/EH5/2016	National Institute of Infectious Diseases (NIID)	National Institute of Infectious Diseases (NIID)	Takashita, Emi; Fujisaki, Seiichiro; Shirakura, Masayuki; Watanabe, Shinji; Odagiri, Takato
EPI710939	HA	Japan	2016-Feb-02	A/TOKYO/EH6/2016	National Institute of Infectious Diseases (NIID)	National Institute of Infectious Diseases (NIID)	Takashita, Emi; Fujisaki, Seiichiro; Shirakura, Masayuki; Watanabe, Shinji; Odagiri, Takato
EPI706970	HA	Ukraine	2016-Jan-03	A/Ukraine/20/2016	Institute of Epidemiology and Infectious Diseases AMS of Ukraine	Crick Worldwide Influenza Centre	
EPI706298	HA	United States	2016-Jan-05	A/Utah/01/2016	Utah Department of Health	Centers for Disease Control and Prevention	
EPI715948	HA	Chile	2015-Nov-02	A/Valdivia/78346/2015	Instituto de Salud Publica de Chile	Centers for Disease Control and Prevention	
EPI732837	HA	United States	2016-Jan-07	A/Washington/03/2016	Marshfield Clinic Research Foundation	Centers for Disease Control and Prevention	
EPI698049	HA	United States	2016-Jan-08	A/Wisconsin/01/2016	Wisconsin State Laboratory of Hygiene	Centers for Disease Control and Prevention	
EPI710586	HA	United States	2016-Jan-14	A/Wyoming/01/2016	Wyoming Public Health Laboratory	Centers for Disease Control and Prevention	
EPI710594	HA	United States	2016-Jan-16	A/Wyoming/02/2016	Wyoming Public Health Laboratory	Centers for Disease Control and Prevention	
EPI715789	HA	Japan	2016-Feb-16	A/YOKOHAMA/87/2016	Yokohama City Institute of Public Health.	National Institute of Infectious Diseases (NIID)	Takashita,Emi; Fujisaki,Seiichiro; Shirakura,Masayuki; Watanabe,Shinji; Odagiri,Takato