Antigenic and genetic characteristics of zoonotic influenza viruses and development of candidate vaccine viruses for pandemic preparedness

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The development of representative candidate influenza vaccine viruses, coordinated by the World Health Organization (WHO), remains an essential component of the overall global strategy for pandemic preparedness.

Zoonotic influenza viruses continue to be identified and evolve both genetically and antigenically, leading to the need for update of candidate vaccine viruses for pandemic preparedness purposes. Evaluation of the genetic and antigenic characteristics of these viruses, their relationship to existing candidate vaccine viruses, and their potential risks to public health, justify the need to select and develop new candidate vaccine viruses.

Selection and development of a candidate vaccine virus represents a first step only towards timely vaccine production and does not imply a recommendation for initiating manufacture. National authorities may consider the use of one or more of these candidate vaccine viruses for pilot lot vaccine production, clinical trials and other pandemic preparedness purposes based on their assessment of public health risk and need.

This document summarizes the genetic and antigenic characteristics of recent zoonotic influenza viruses from humans and related viruses circulating in animals and updates the availability of candidate vaccine viruses. Institutions that wish to receive these candidate vaccine viruses should contact WHO at gisrs-whohq@who.int or the institutions listed in announcements published on the WHO website[1].

Influenza A(H5N1)

Since their re-emergence in 2003, highly pathogenic avian influenza A(H5N1) viruses have become enzootic in some countries and continue to cause outbreaks in poultry as well as sporadic human infections. The A(H5N1) viruses have diversified both genetically and antigenically leading to the need for multiple candidate vaccine viruses. This summary provides updates on the characterization of A(H5N1) viruses and the current status of the development of influenza A(H5N1) candidate vaccine viruses.

Influenza A(H5N1) activity from 24 September 2013 to 17 February 2014

A(H5N1) viruses have been detected in birds in Africa and Asia. Human infections have been reported to the WHO by Cambodia, China, Indonesia, and Viet Nam, countries in which infections have been detected in birds (Table 1). An A(H5N1) virus was also isolated from an individual in Canada who had recently travelled to China.

[1] http://www.who.int/influenza/vaccines/virus/en/

Table 1. Recent influenza A(H5N1) activity reported to international agencies

Reporting country, area or territory	Host/source	Genetic clade*
Bangladesh	Poultry	2.3.2.1a
Cambodia	Poultry	1.1.2
	Human (9)#	1.1.2
Canada	Human (1)	2.3.2.1c
China	Poultry/environmental	2.3.2.1b, 2.3.2.1c, 2.3.4, 7.2
	Human (1)	2.3.4
Egypt	Poultry	2.2.1
Indonesia	Poultry	2.1.3.2a, 2.3.2.1c
	Human (2)	2.1.3.2a
Viet Nam	Poultry	1.1.2, 2.3.2.1c
	Human (2)	1.1.2, 2.3.2.1c

* based on available sequences
# denotes number of human cases with illness onset dates falling within reporting period

Antigenic and genetic characteristics of influenza A(H5N1) viruses

The nomenclature for phylogenetic relationships among the haemagglutinin (HA) genes of A(H5N1) viruses is defined in consultation with representatives of the WHO, the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE) and academic institutions. An updated nomenclature report has been published[2].

Viruses circulating and characterized from 24 September 2013 to 17 February 2014 belonged to the following clades.

Clade 1.1.2 viruses were detected in poultry and humans in Cambodia and Viet Nam. Genetic characterization of the HA genes showed that these viruses were closely related to viruses detected previously in these countries. While some of the recent avian viruses from Viet Nam had reduced reactivity to post-infection ferret antisera raised against A/Cambodia/W0526301/2012, for which a candidate vaccine virus is in development, the majority of recent clade 1.1.2 viruses reacted well.

Clade 2.1.3.2a viruses continue to circulate in Indonesia. The HA gene sequence of a recent 2013 human virus was similar to that of A/Indonesia/NIHRD11771/2011 for which a candidate vaccine virus has been derived. No antigenic information for this virus is available.

Clade 2.2.1 viruses were detected in poultry in Egypt although no human infections were identified during this period. As compared to the candidate vaccine viruses produced from A/Egypt/N03072/2010 and A/Egypt/2321-NAMRU3/2007, the HA proteins of recent clade 2.2.1 viruses have accumulated a number of amino acid substitutions. These viruses showed reduced reactivity to post-infection ferret antisera raised against the candidate vaccine viruses. Further virus characterization is underway.

Clade 2.3.2.1a viruses were detected in birds in Bangladesh. The HA genes of these viruses were similar to those of viruses detected previously. The viruses reacted well with post-infection ferret antiserum raised against A/duck/Bangladesh/19097/2013 for which a candidate vaccine virus is in development.

Clade 2.3.2.1b viruses were detected in environmental samples from China. Genetically and antigenically these viruses were similar to the viruses previously detected and reacted well to post-infection ferret antisera raised against available candidate vaccine viruses.

[2] WHO/OIE/FAO H5N1 Evolution Working Group. Revised and updated nomenclature for highly pathogenic avian influenza A(H5N1) viruses. John Wiley & Sons Ltd. 2014 (http://onlinelibrary.wiley.com/doi/10.1111/irv.12230/full#irv12230)

Clade 2.3.2.1c viruses were detected in birds and/or environmental samples in China, Indonesia and Viet Nam and in humans in Canada and Viet Nam. The HA genes of these viruses were similar to those of viruses previously detected. Antigenic analysis showed that many of these viruses, including the human virus from Canada, reacted well with a post-infection ferret antiserum raised against A/duck/Viet Nam/NCVD-1584/2012 for which a candidate vaccine virus has been proposed.

Clade 2.3.4 viruses were detected in environmental samples and a human in China. The HA genes of these viruses were similar to A/Anhui/1/2005. Further antigenic characterization of viruses from this clade is pending and will determine if additional candidate vaccine viruses are required.

Clade 7.2 viruses were detected in environmental samples collected in China. Genetically and antigencially these viruses were distinct from the available candidate vaccine viruses (Figure 1, Table 2). As clade 7.2 viruses have continued to be detected, the development of a new candidate vaccine virus derived from an A/environment/Hubei/950/2013-like virus is proposed.

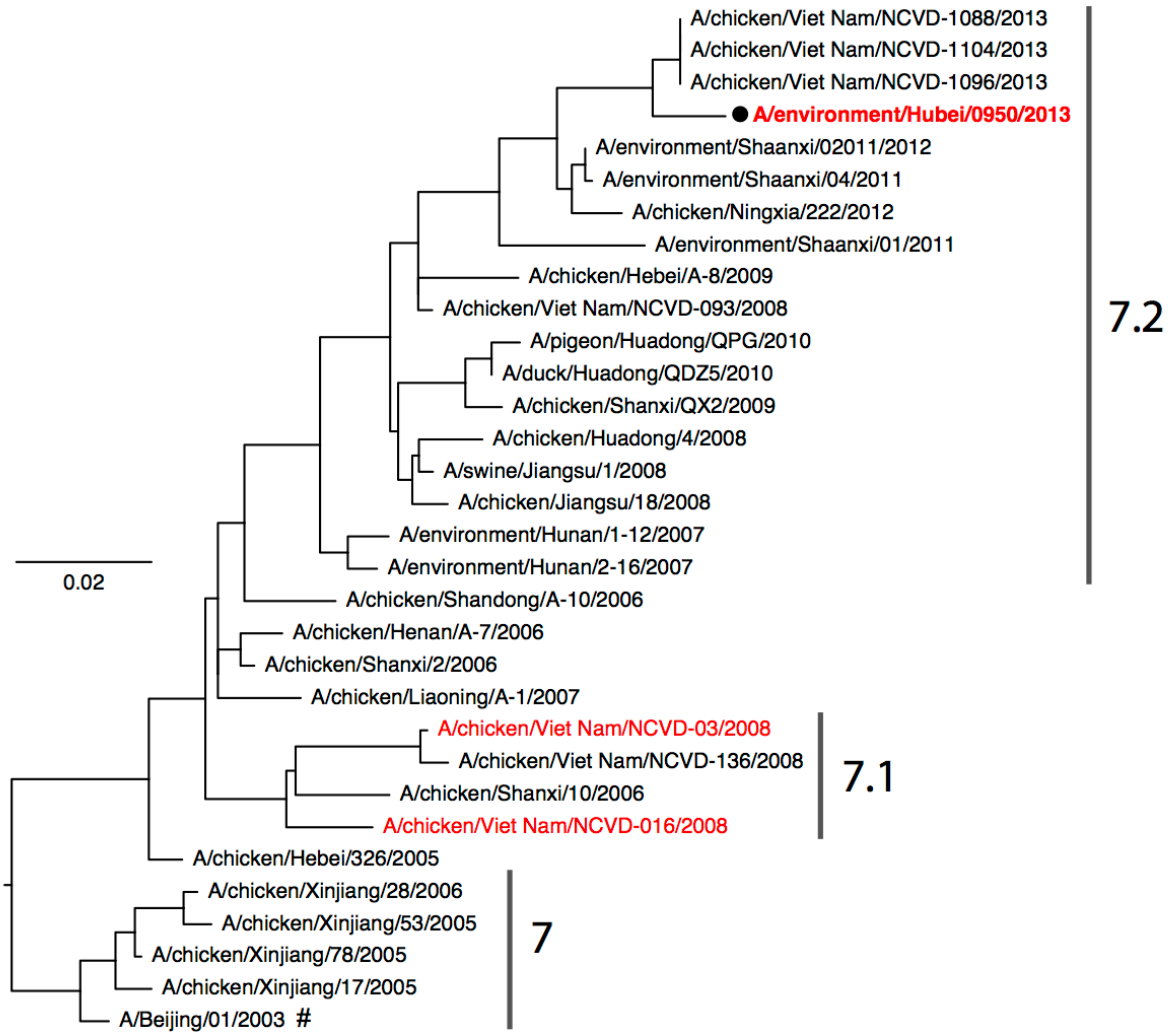

Figure 1. Phylogenetic relationships of A(H5N1) clade 7 HA genes. The available candidate vaccine viruses are in red. The proposed vaccine candidate is indicated by a circle. Human viruses are indicated (#). The scale bar represents the number of substitutions per site.

Table 2. Haemagglutination inhibition reactions of influenza A(H5N1) Clade 7.2 viruses

	clade	Post-infection ferret antiserum NCVD-016
REFERENCE ANTIGEN		
A/chicken/Viet Nam/NCVD-016/2008 (RG12)	7.1	**160**
TEST ANTIGEN		
A/environment/Hubei/950/2013	7.2	<20
A/environment/Guizhou/00320/2013	7.2	<20
A/environment/Shaanxi/02011/2012	7.2	<20
A/environment/Shaanxi/01/2011	7.2	<20
A/environment/Shaanxi/04/2011	7.2	<20

Influenza A(H5N1) candidate vaccine viruses

Based on the available antigenic, genetic and epidemiologic data, an A/environment/Hubei/950/2013-like (clade 7.2) candidate vaccine virus is proposed. The available and proposed candidate A(H5N1) vaccine viruses are listed in Table 3. National authorities may consider the use of one or more of these candidate A(H5N1) vaccine viruses for pilot lot vaccine production, clinical trials and other pandemic preparedness purposes based on their assessment of public health risk and need.

As the viruses continue to evolve, new A(H5N1) candidate vaccine viruses may be developed.

Table 3. Status of influenza A(H5N1) candidate vaccine virus development

Candidate vaccine viruses	**Clade**	**Institution***	**Available**
A/Viet Nam/1203/2004 (CDC-RG; SJRG-161052)	1	CDC and SJCRH	Yes
A/Viet Nam/1194/2004 (NIBRG-14)	1	NIBSC	Yes
A/Cambodia/R0405050/2007 (NIBRG-88)	1.1	NIBSC	Yes
A/duck/Hunan/795/2002 (SJRG-166614)	2.1.1	SJCRH	Yes
A/Indonesia/5/2005 (CDC-RG2)	2.1.3.2	CDC	Yes
A/Indonesia/NIHRD11771/2011 (NIIDRG-9)	2.1.3.2a	NIID	Yes
A/bar-headed goose/Qinghai/1A/2005 (SJRG-163222)	2.2	SJCRH	Yes
A/chicken/India/NIV33487/2006 (IBCDC-RG7)	2.2	CDC/NIV	Yes
A/whooper swan/Mongolia/244/2005 (SJRG-163243)	2.2	SJCRH	Yes
A/Egypt/2321-NAMRU3/2007 (IDCDC-RG11)	2.2.1	CDC	Yes
A/turkey/Turkey/1/2005 (NIBRG-23)	2.2.1	NIBSC	Yes
A/Egypt/N03072/2010 (IDCDC-RG29)	2.2.1	CDC	Yes
A/Egypt/3300-NAMRU3/2008 (IDCDC-RG13)	2.2.1.1	CDC	Yes
A/common magpie/Hong Kong/5052/2007 (SJRG-166615)	2.3.2.1	SJCRH	Yes
A/Hubei/1/2010 (IDCDC-RG30)	2.3.2.1a	CDC	Yes
A/barn swallow/Hong Kong/D10-1161/2010 (SJ-003)	2.3.2.1b	SJCRH	Yes
A/chicken/Hong Kong/AP156/2008 (SJ-002)	2.3.4	SJCRH	Yes
A/Anhui/1/2005 (IBCDC-RG6)	2.3.4	CDC	Yes
A/duck/Laos/3295/2006 (CBER-RG1)	2.3.4	FDA	Yes
A/Japanese white eye/Hong Kong/1038/2006 (SJRG-164281)	2.3.4	SJCRH	Yes
A/goose/Guiyang/337/2006 (SJRG-165396)	4	SJCRH	Yes
A/chicken/Viet Nam/NCVD-016/2008 (IDCDC-RG12)	7.1	CDC	Yes
A/chicken/Viet Nam/NCDV-03/2008 (IDCDC-RG25A)	7.1	CDC	Yes
Candidate vaccine viruses in preparation	**Clade**	**Institution**	**Availability**
A/chicken/Bangladesh/11RS1984-30/2011-like	2.3.4.2	CDC	Pending
A/Guizhou/1/2013-like	2.3.4.2	CDC/CCDC	Pending
A/duck/Bangladesh/19097/2013-like	2.3.2.1a	SJCRH	Pending
A/duck/Viet Nam/NCVD-1584/2012-like	2.3.2.1c	NIBSC	Pending
A/Cambodia/W0526301/2012-like	1.1.2	CDC	Pending
A/environment/Hubei/950/2013-like	7.2	CDC/CCDC	Pending

* **Institutions distributing the candidate vaccine viruses:**

CDC - Centers for Disease Control and Prevention, United States of America

CDC/NIV - Centers for Disease Control and Prevention, United States of America/National Institute of Virology, India

CDC/CCDC - Centers for Disease Control and Prevention, United States of America/China Center for Disease Control and Prevention

FDA - Food and Drug Administration, United States of America

NIBSC - National Institute for Biological Standards and Control, a centre of the Medicines and Healthcare products Regulatory Agency (MHRA), United Kingdom of Great Britain and Northern Ireland

NIID - National Institute of Infectious Diseases, Japan

SJCRH - St Jude Children's Research Hospital, United States of America

Influenza A(H7N9)

Influenza A(H7) viruses have been detected in poultry populations worldwide with the associated disease ranging from mild to severe. Human infections with avian influenza A(H7N9) viruses were first reported to WHO on 31 March 2013.

Influenza A(H7N9) activity from 24 September 2013 to 17 February 2014

During this period, 220 human cases of avian influenza A(H7N9) virus infection were reported to WHO, bringing the total number of cases to 355 including 112[3] deaths. Human cases (and genetically related avian and/or environmental viruses) have been restricted to China except for a single case in Malaysia detected in a traveler from Guangdong Province, China. Comparison of avian influenza A(H7N9) viruses isolated from humans, poultry and environmental samples using haemagglutination inhibition assays shows that limited antigenic diversity exists among this group of viruses and they remain antigenically similar to the candidate vaccine viruses derived from A/Anhui/1/2013-like viruses (Figure 2, Table 4). All recent avian influenza A(H7N9) viruses that have been tested remain susceptible to the neuraminidase inhibitor class of antiviral drugs.

Table 4. Haemagglutination inhibition reactions of influenza A(H7N9) viruses

	Post-infection ferret antiserum	
REFERENCE ANTIGENS	Anhui 1	Shanghai 2
A/Anhui/1/2013	**160**	640
A/Shanghai/2/2013	320	**2560**
TEST ANTIGENS		
A/Hong Kong/5942/2013	80	1280
A/Shanghai/02619/2014	320	640
A/Guangdong/02620/2014	320	1280
A/Guizhou/01502/2014	320	1280
A/Guangdong/02125/2014	160	640
A/Fujian/1/2014	640	2560
A/Zhejiang/07807/2014	320	2560
A/ Zhejiang /07802/2014	640	2560
A/Hunan/07833/2014	160	1280
A/Hunan/08963/2014	320	1280
A/Guangxi/08970/2014	320	2560
A/Guangxi/08971/2014	320	2560
A/environment/Guangdong/25003/2013	160	640
A/environment/Zhejiang/07818/2014	320	1280
A/environment/Hunan/07836/2014	160	640

[3] Communication from Chinese Centers for Disease Control and Prevention (CDC)

Figure 2. Phylogenetic relationships of A(H7N9) HA genes. The available candidate vaccine viruses are in red. Human viruses are indicated (#). The viruses of 2nd wave outbreak are in magenta. The scale bar represents the number of substitutions per site.

Influenza A(H7N9) candidate vaccine viruses

Based on the current epidemiologic and virologic data, no new A(H7N9) candidate vaccine viruses have been proposed. Available A(H7N9) candidate vaccine viruses are shown in Table 5. National authorities may consider the use of one or more of these candidate A(H7N9) vaccine viruses for pilot lot vaccine production, clinical trials and other pandemic preparedness purposes based on their assessment of public health risk and need.

As the viruses continue to evolve, new A(H7N9) candidate vaccine viruses may be developed.

Table 5. Status of influenza A(H7N9) candidate vaccine virus development

Candidate vaccine virus	Type	Institution*	Available
A/Anhui/1/2013 (H7N9) IDCDC-RG33A	Reverse Genetics	CDC	Yes
A/Anhui/1/2013 (H7N9) NIBRG-268	Reverse Genetics	NIBSC	Yes
A/Anhui/1/2013 (H7N9) NIIDRG-10.1	Reverse Genetics	NIID	Yes
A/Anhui/1/2013 (H7N9) SJ005	Reverse Genetics	SJCRH	Yes
A/Shanghai/2/2013 (H7N9) NIBRG-267	Reverse Genetics	NIBSC	Yes
A/Shanghai/2/2013 (H7N9) CBER-RG4A	Reverse Genetics	FDA	Yes
A/Shanghai/2/2013 (H7N9) IDCDC-RG32A	Reverse Genetics	CDC	Yes
A/Shanghai/2/2013 (H7N9) IDCDC-RG32A.3	Reverse Genetics	CDC	Yes

* **Institutions distributing the candidate vaccine viruses:**
CDC - Centers for Disease Control and Prevention, United States of America
FDA - Food and Drug Administration, United States of America
NIBSC - National Institute for Biological Standards and Control, a centre of the Medicines and Healthcare products Regulatory Agency (MHRA), United Kingdom of Great Britain and Northern Ireland
NIID - National Institute of Infectious Diseases, Japan
SJCRH - St Jude Children's Research Hospital, United States of America

Influenza A(H9N2)

Influenza A(H9N2) viruses are enzootic in poultry populations in parts of Africa, Asia and the Middle East. The majority of viruses that have been sequenced belong to the G1, chicken/Beijing (Y280/G9), or Eurasian clades. Since 1998, when the first human infection was detected, the isolation of A(H9N2) viruses from humans and swine has been reported infrequently. In all human cases the associated disease symptoms have been mild and there has been no evidence of human-to-human transmission.

Influenza A(H9N2) activity from 24 September 2013 to 17 February 2014

Two human cases of A(H9N2) infection have been reported, one in China and the other in China Hong Kong Special Administrative Region (Hong Kong SAR) in this period. Both human viruses had HA genes belonging to the Y280/G9 genetic lineage. A(H9N2) viruses continue to be isolated from birds in many regions of the world. Recent Y280/G9 lineage viruses demonstrate increased genetic heterogeneity and some, including the human virus from Hong Kong SAR and closely related viruses, show reduced reactivity to a post-infection ferret antiserum to the A/chicken/Hong Kong/G9/1997 candidate vaccine virus (Figure 3, Table 6).

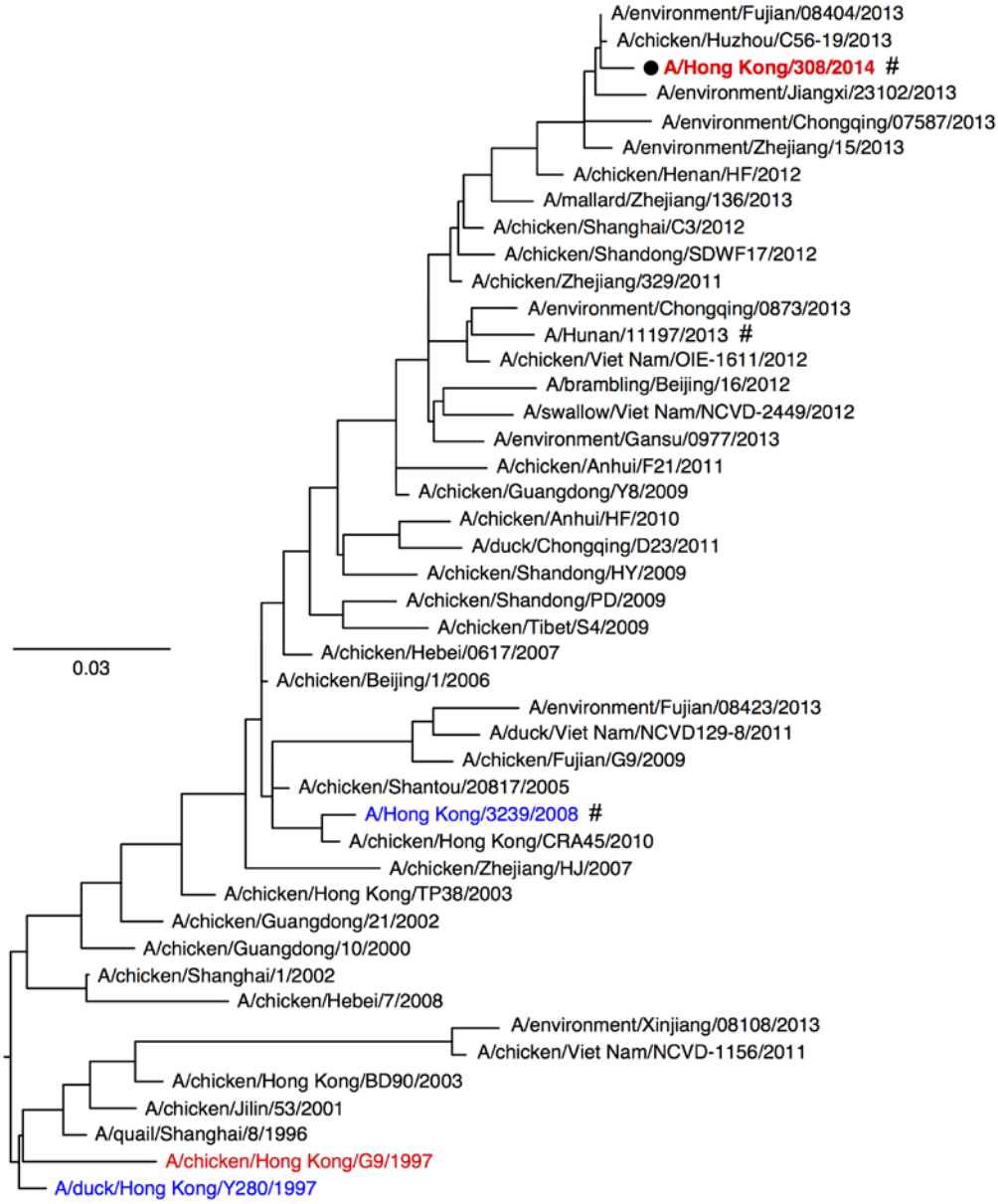

Figure 3. Phylogenetic relationships of A(H9N2) Y280-like HA genes. The available candidate vaccine viruses are in red and the HI reference viruses are in blue. The proposed vaccine candidate is indicated by a circle. Human viruses are indicated (#). The scale bar represents the number of substitutions per site.

Table 6. Haemagglutination inhibition reactions of influenza A(H9N2) viruses

		Post-infection ferret antiserum			
	Lineage	HK/1073	HK/33982	BA/994	IBCDC-2
REFERENCE ANTIGENS					
A/Hong Kong/1073/1999	G1	**320**	640	10	<10
A/Hong Kong/33982/2009	G1	320	**2560**	20	10
A/Bangladesh/994/2011	G1	80	160	**1280**	80
A/chicken/Hong Kong/G9/1997 IBCDC-2	G9/Y280	20	40	80	**320**
TEST ANTIGENS					
A/swallow/Vietnam/NCVD-2449/2012	G9/Y280	20	80	80	320
A/chicken/Vietnam/NCVD-1156/2011	G9/Y280	<10	20	20	20
A/Hong Kong/308/2014	G9/Y280	40	80	40	20

Influenza A(H9N2) candidate vaccine viruses

Based on the current antigenic, genetic and epidemiologic data, an A/Hong Kong/308/2014-like candidate vaccine virus is proposed. The available A(H9N2) candidate vaccine viruses are listed in Table 7. National authorities may consider the use of one or more of these candidate A(H9N2) vaccine viruses for pilot lot vaccine production, clinical trials and other pandemic preparedness purposes based on their assessment of public health risk and need.

As the viruses continue to evolve, new A(H9N2) candidate vaccine viruses may be developed.

Table 7. Status of influenza A(H9N2) candidate vaccine virus development

Candidate vaccine viruses	Type	Clade	Institution*	Available
A/Hong Kong/1073/1999	Wild type	G1	NIBSC	Yes
A/chicken/Hong Kong/G9/1997 (NIBRG-91)	Reverse genetics	Y280/G9	NIBSC	Yes
A/chicken/Hong Kong/G9/1997 (IBCDC-2)	Conventional	Y280/G9	CDC	Yes
A/Hong Kong/33982/2009 (IDCDC-RG26)	Reverse genetics	G1	CDC	Yes
A/Bangladesh/994/2011 (IDCDC-RG31)	Reverse genetics	G1	CDC	Yes
Candidate vaccine viruses in preparation				
A/Hong Kong/308/2014-like	Reverse genetics	Y280/G9	SJCRH	Pending

* **Institutions distributing the candidate vaccine viruses:**
CDC - Centers for Disease Control and Prevention, United States of America
NIBSC - National Institute for Biological Standards and Control, a centre of the Medicines and Healthcare products Regulatory Agency (MHRA), United Kingdom of Great Britain and Northern Ireland
SJCRH - St Jude Children's Research Hospital, United States of America

Influenza A(H10N8)

Three cases of human infection with avian influenza A(H10N8) viruses were reported from Jiangxi Province, China, with onset dates from December 2013 to February 2014. All of these individuals had severe disease and two have died. All had reported contact with poultry or contaminated environments. To date, genetic information from one virus isolate is available, which showed all genes to be of avian origin and the internal genes to be derived from A(H9N2) viruses currently circulating widely in poultry in China[4]. This virus is susceptible to the neuraminidase inhibitor class of antiviral drugs. Information on the prevalence and distribution of A(H10N8) viruses in poultry in the region is limited, thus the assessment of its impact on public health is difficult.

At this time, the virus is being evaluated for its growth and antigenic properties and diagnostic reagents are being prepared. WHO is monitoring the situation closely.

[4] Chen H et al. Clinical and epidemiological characteristics of a fatal case of avian influenza A H10N8 virus infection : a descriptive study. Lancet. 2014 (http://dx.doi.org/10.1016/S0140-6736(14)60111-2)