

# **Antigenic and genetic characteristics of H5N1 viruses and candidate vaccine viruses developed for potential use in human vaccines**

**February 2009**

Since their reemergence in 2003, H5N1 influenza viruses have become endemic in some countries and continue to cause outbreaks in poultry and sporadic human infections. It is not known if the next influenza pandemic will be caused by H5N1 viruses or, should one occur, which of the clades of H5N1 viruses would be responsible. However, because an H5N1 pandemic is a possibility, and one with potential for an unusually severe outcome, the development of representative H5N1 candidate vaccine viruses from those available for characterization, coordinated by the World Health Organization (WHO), is an essential component of the overall global strategy for pandemic preparedness. This summary provides an update on the characterization of available H5N1 viruses circulating in birds, including those that have caused human infections, and the current status of the development of candidate H5N1 vaccine viruses. This information may be used to guide national decisions on procurement of H5N1 vaccines.

H5N1 vaccines continue to be developed by manufacturers using clade 1 and clade 2 viruses. Clinical trials have been conducted or are under way in several countries and stockpiles of clade 1 and clade 2 vaccines are being acquired by a number of countries ([http://www.who.int/vaccine\\_research/diseases/influenza/flu\\_trials\\_tables/en/index.html](http://www.who.int/vaccine_research/diseases/influenza/flu_trials_tables/en/index.html)). Clinical trials using different viruses should continue as an essential element in pandemic preparedness. Such trials should provide data on priming vaccination schedules, and induction of cross-reactive immunity by vaccines containing viruses from different clades.

Companies are recommended to consult individual national authorities on the specific H5N1 viruses to be used for preparing experimental pilot lots and stockpiles of H5N1 vaccines. Decisions should be based on several considerations including the epidemiology and geographical distribution of the circulating H5N1 viruses.

Comparisons of the candidate H5N1 vaccine viruses with respect to immunogenicity and cross-reactivity and their relationship to newly emerging H5N1 viruses are ongoing, and will be updated periodically by WHO.

## **Molecular epidemiology of H5N1 viruses**

A nomenclature for phylogenetic relationships among the haemagglutinin (HA) genes of H5N1 viruses was devised in consultation with representatives of FAO, OIE, and WHO ([http://www.who.int/csr/disease/avian\\_influenza/guidelines/nomenclature/en/index.html](http://www.who.int/csr/disease/avian_influenza/guidelines/nomenclature/en/index.html)) (Figure 1). The nomenclature has been recently updated and a manuscript describing this update will be published (<http://www3.interscience.wiley.com/cgi-bin/fulltext/122211969/HTMLSTART>). The HA sequences of the majority of H5N1 viruses circulating in avian species are separated into a number of distinct clades. Clade 1 viruses have continued to circulate and have been recently isolated in poultry in Cambodia, Thailand

and Viet Nam. Although recent human clade 1 infections have been limited to Cambodia, clade 1 viruses have also been previously isolated from humans in China Hong Kong Special Administrative Region, Thailand and Viet Nam. Clade 2.1 viruses have continued to circulate in poultry and have caused human infections in Indonesia. Clade 2.2 viruses have the most geographically diverse distribution and have caused outbreaks in birds in over 60 countries in Africa, Asia and Europe with human infections in Azerbaijan, Bangladesh, China, Djibouti, Egypt, Iraq, Nigeria, Pakistan and Turkey. Clade 2.3.2 and 2.3.4 viruses continue to circulate in birds in Asia; clade 2.3.4 viruses have been responsible for human infections in China, Lao People's Democratic Republic, Myanmar and Viet Nam. Viruses from other clades, including clade 7, have been detected in birds in Asia. Since September 2008, human infections have been caused by clade 2.3.2 viruses in China, clade 2.3.4 viruses in China and Viet Nam, clade 1 viruses in Cambodia, clade 2.2 viruses in Egypt and by clade 2.1 viruses in Indonesia.

### **Antigenic characteristics of H5N1 viruses**

Haemagglutination inhibition (HI) tests of available H5N1 viruses demonstrate that current vaccine candidates continue to provide good antigenic coverage of most available isolates within corresponding clades. In addition, recent data show that the clade 2.1 vaccine virus A/duck/Hunan/795/2002 is antigenically similar to the vaccine virus A/Indonesia/5/2005. However, some viruses within clades 1, 2.1, 2.2, 2.3.2, 2.3.4, and 7 show evidence of antigenic and/or genetic heterogeneity (Table 1 and Figure 1). Within clade 2.3.4, A/chicken/Hong Kong/AP156/2008-like viruses are antigenically distinct from reference strains (Table 2). Antigenic diversity is also present within clade 7 and viruses such as A/chicken/Vietnam/NCDV-03/2008 show reduced reactivity with antisera to the clade 7 reference strain A/chicken/Vietnam/NCDV-016/2008. Of the most recent human isolates from China, A/Guangxi/1/2009 belongs to clade 2.3.2, the first human isolate from this clade. A/Hunan/2/2009, belonging to clade 2.3.4, shows genetic divergence from other viruses of this clade. Both viruses should be considered as potential vaccine strains pending further characterization.

### **Potential H5N1 vaccine viruses**

Potential H5N1 vaccine viruses are listed in Table 3. On the basis of the geographical spread, epidemiology, and antigenic and genetic properties of the H5N1 viruses, national authorities may recommend the use of one or more of these for pilot lot vaccine production, clinical trial, and subsequent stockpiling of vaccines, should such national policies exist.

- Additional H5N1 candidate vaccine viruses are being developed as the viruses continue to evolve, and will be announced as they become available. Institutions, companies and others interested in pandemic vaccine development, who wish to receive these prototype viruses, should contact the WHO Global Influenza Program at [GISN@who.int](mailto:GISN@who.int) or the institutions listed in announcements published at WHO web site [http://www.who.int/csr/disease/avian\\_influenza/guidelinstopics/en/index5.html](http://www.who.int/csr/disease/avian_influenza/guidelinstopics/en/index5.html)

**Table 1. Antigenic properties of H5N1 viruses**

REFERENCE ANTIGENS	CLADE	REFERENCE FERRET ANTISERA														
		1	2.1	2.1	2.2	2.2	2.2	2.2	2.2	2.3.2	2.3.4	2.3.4	2.3.4	2.3.4	4	7
		VN/1203	Indo/5	dk/Hun	ws/MG	tk/65	bg/QI-R	ck/India	EG/321	ck/KO	Anh/1	Anh/1-R	ck/MY	jwe/HK	gs/Gui	ck/VN/16
A/VIETNAM/1203/2004	1	640	10	160	10	40	40	40	80	5	5	80	40	5	80	5
A/INDONESIA/5/2005	2.1	5	1280	320	160	160	40	160	320	5	160	80	20	20	20	80
A/DUCK/HUNAN/795/2002	2.1	5	640	320	320	160	80	320	640	5	5	20	5	5	5	5
A/WHOOPER SWAN/MG/244/2005	2.2	5	160	320	640	320	160	640	320	5	5	40	10	40	5	5
A/TURKEY/65-596/2006	2.2	5	1280	640	1280	2560	320	1280	1280	320	160	160	5	5	40	80
A/BARHEADED GOOSE/QINGHAI/1A/2005xPR8	2.2	5	320	320	640	320	80	320	160	5	5	80	10	40	5	20
A/CHICKEN/INDIA/NIV-33487/2006	2.2	5	80	160	320	320	80	320	80	5	5	20	5	20	5	10
A/EGYPT/321-NAMRU3/2007	2.2	5	320	320	320	320	160	320	640	5	5	10	20	80	5	20
A/CHICKEN/KOREA/GIMJE/2008	2.3.2	5	80	160	80	40	20	40	10	160	5	10	5	5	10	5
A/ANHUI/1/2005	2.3.4	5	80	80	10	80	10	20	40	5	1280	320	320	160	80	20
A/ANHUI/1/2005xPR8 IBCDC RG-6	2.3.4	40	80	80	20	80	20	20	80	5	1280	640	640	160	160	40
A/CHICKEN/MALAYSIA/935/2006	2.3.4	5	5	5	5	10	5	5	10	5	320	160	80	80	40	5
A/JAPANESE/WHITE EYE/HK/1038/2006	2.3.4	5	80	80	20	40	640	640	160	5	1280	640	640	640	80	40
A/GOOSE/GUIYANG/337/2006	4	40	5	5	5	10	5	10	40	5	5	40	5	5	320	5
A/CHICKEN/VIETNAM/NCVD-016/2008	7	5	5	5	5	5	5	10	5	5	5	5	5	5	40	320
<b>TEST ANTIGENS</b>																
A/VIETNAM/1203/2007xPR8	1	80	5	5	5	10	10	10	20	5	5	20	5	5	10	5
A/THAILAND/676/05	1	320	10	40	20	20	20	40	40	5	40	80	20	40	40	5
A/DUCK/VIETNAM/NCVD-16/07	1	80	5	5	5	10	5	5	20	5	5	10	5	5	10	5
A/CAMBODIA/R0405050/2007	1	40	5	20	10	10	10	10	10	5	5	5	5	5	5	5
A/INDONESIA/5/05 RG-2	2.1	5	640	160	80	80	40	80	80	5	80	40	10	5	5	20
A/INDONESIA/CDC1031/07	2.1	5	1280	160	160	80	40	160	80	5	80	5	5	5	5	40
A/INDONESIA/CDC625L/06	2.1	5	80	80	40	40	40	40	20	5	5	10	5	10	5	5
A/TURKEY/TURKEY/1/05 X PR8	2.2	5	640	640	1280	1280	160	1280	320	80	5	80	10	80	5	40
A/TURKEY/TURKEY/1/05	2.2	5	640	640	1280	1280	320	1280	320	5	40	80	20	80	5	40
A/EGRET/EGYPT/NAMRU-3-1162/06	2.2	5	640	640	1280	1280	160	1280	640	5	5	80	10	40	5	20
A/CHICKEN/EGYPT/9403-NAMRU3/07	2.2	5	160	80	10	40	20	80	20	5	5	40	20	5	5	20
A/EGYPT/3300-NAMRU3/2008	2.2	5	160	160	10	80	10	20	5	5	5	10	10	5	10	20
A/BANGLADESH/207095/2008	2.2	5	160	160	640	640	160	2560	160	5	5	80	5	20	5	20
A/DUCK/LAOS/3295/06	2.3.4	5	5	5	5	10	5	20	10	5	160	160	80	40	40	5
A/LAOS/JP085/2007	2.3.4	5	5	5	5	5	5	5	5	5	80	80	40	20	10	5
A/CHICKEN/VIETNAM/NCVD-74/07	2.3.4	5	5	5	5	10	5	10	10	5	80	80	40	10	10	5
A/DUCK/VIETNAM/NCVD-81/2007	2.3.4	5	5	5	5	10	5	5	10	5	160	160	160	20	20	10
A/CHICKEN/VIETNAM/NCVD-138/2008	2.3.4	5	5	5	5	5	5	5	5	5	40	20	80	80	5	5
A/CHICKEN/VIETNAM/NCVD-142/2008	2.3.4	5	5	5	5	20	5	5	5	5	320	80	80	80	20	5
A/MUSCOVY DUCK/VIETNAM/NCVD-154/2008	2.3.4	5	5	5	5	10	5	5	5	5	160	80	40	40	5	5
A/DUCK/VIETNAM/NCVD-187/2008	2.3.4	5	5	5	5	20	5	5	5	5	320	80	40	40	5	5
A/CHICKEN/VIETNAM/NCVD-03/2008	7	5	5	5	5	5	5	5	5	5	5	5	5	5	10	40
A/GOOSE/GUANGDONG/1/96	0	10	10	5	10	160	160	160	20	5	5	20	20	5	10	5

Table 2. Antigenic properties of clade 2.3.4 H5N1 viruses								
REFERENCE ANTIGENS	CLADE	1	2.1	2.2	2.3.4	2.3.4	2.3.4	2.3.4
		VN/1203	Indo/5	ws/MG	Anh/1	cm/HK	jwe/HK	ck/HK
A/VIETNAM/1203/2004	1	<b>320</b>	40	<40	160	160	80	<40
A/INDONESIA/5/2005	2.1	40	<b>1280</b>	160	160	160	<40	<40
A/COMMON MAGPIE/HK/645/06	2.3.4	80	40	<40	160	<b>320</b>	80	<40
A/JAPANESE/WHITE EYE/HK/1038/2006	2.3.4	80	320	80	2560	2560	<b>5120</b>	<40
TEST ANTIGENS								
A/FALCON/HK/2142/2008	2.3.4	160	40	<40	40	80	<40	80
A/CHICKEN/HK/AP156/2008	2.3.4	<40	40	<40	<40	<40	<40	<b>80</b>
A/CHICKEN/HK/8825.2/2008	2.3.4	<40	<40	<40	<40	<40	<40	10

**Table 3. Status of H5N1 vaccine virus development as of February 2009**

<b>Reassortants with completed regulatory approval</b>			
<b>Virus</b>	<b>Clade</b>	<b>Institution*</b>	<b>Availability</b>
A/Cambodia/R0405050/2007	1	NIBSC	Yes
A/Viet Nam/1194/2004	1	NIBSC	Yes
A/Viet Nam/1203/2004	1	CDC and SJ/HKU/NIAID	Yes
A/duck/Hunan/795/2002	2.1	SJ/HKU/NIAID	Yes
A/Indonesia/5/2005	2.1	CDC	Requires Indonesian Government permission
A/bar-headed goose/Qinghai/1A/2005	2.2	SJ/HKU/NIAID	Yes
A/turkey/Turkey/1/2005	2.2	NIBSC	Yes
A/whooper swan/Mongolia/244/2005	2.2	SJ/NIAID	Yes
A/Anhui/1/2005	2.3.4	CDC	Yes
A/duck/Laos/3295/2006	2.3.4	FDA	Yes
A/Japanese white-eye/Hong Kong/1038/2006	2.3.4	SJ/HKU/NIAID	Yes
A/goose/Guiyang/337/2006	4	SJ/HKU/NIAID	Yes
<b>Reassortants prepared and awaiting regulatory approval</b>			
<b>Virus</b>	<b>Clade</b>	<b>Institution*</b>	<b>Availability</b>
A/chicken/India/NIV33487/2006	2.2	CDC/NIV	Pending
A/Egypt/2321/2007-like	2.2	CDC	Pending
A/Egypt/3300-NAMRU3/2008	2.2	CDC	Pending
A/common magpie/Hong Kong/5052/2007	2.3.2	SJ/HKU/NIAID	Pending
A/chicken/Viet Nam/NCVD-016/2008-like	7	CDC	Pending
<b>Viruses proposed by WHO for candidate vaccine preparation</b>			
<b>Virus</b>	<b>Clade</b>	<b>Institution*</b>	
A/chicken/Hong Kong/AP156/2008-like	2.3.4	SJ/HKU/NIAID	
A/chicken/Vietnam/NCDV-03/2008	7	CDC	

\* CDC- Centers for Disease Control and Prevention, USA  
 FDA- Food and Drug Administration, USA  
 NIAID- National Institute of Allergy and Infectious Disease, NIH, USA  
 NIBSC- National Institute for Biological Standards and Control, UK  
 NIV- National Institute of Virology, India  
 SJ- St Jude Children's Research Hospital, USA  
 HKU-University of Hong Kong, Hong Kong SAR China

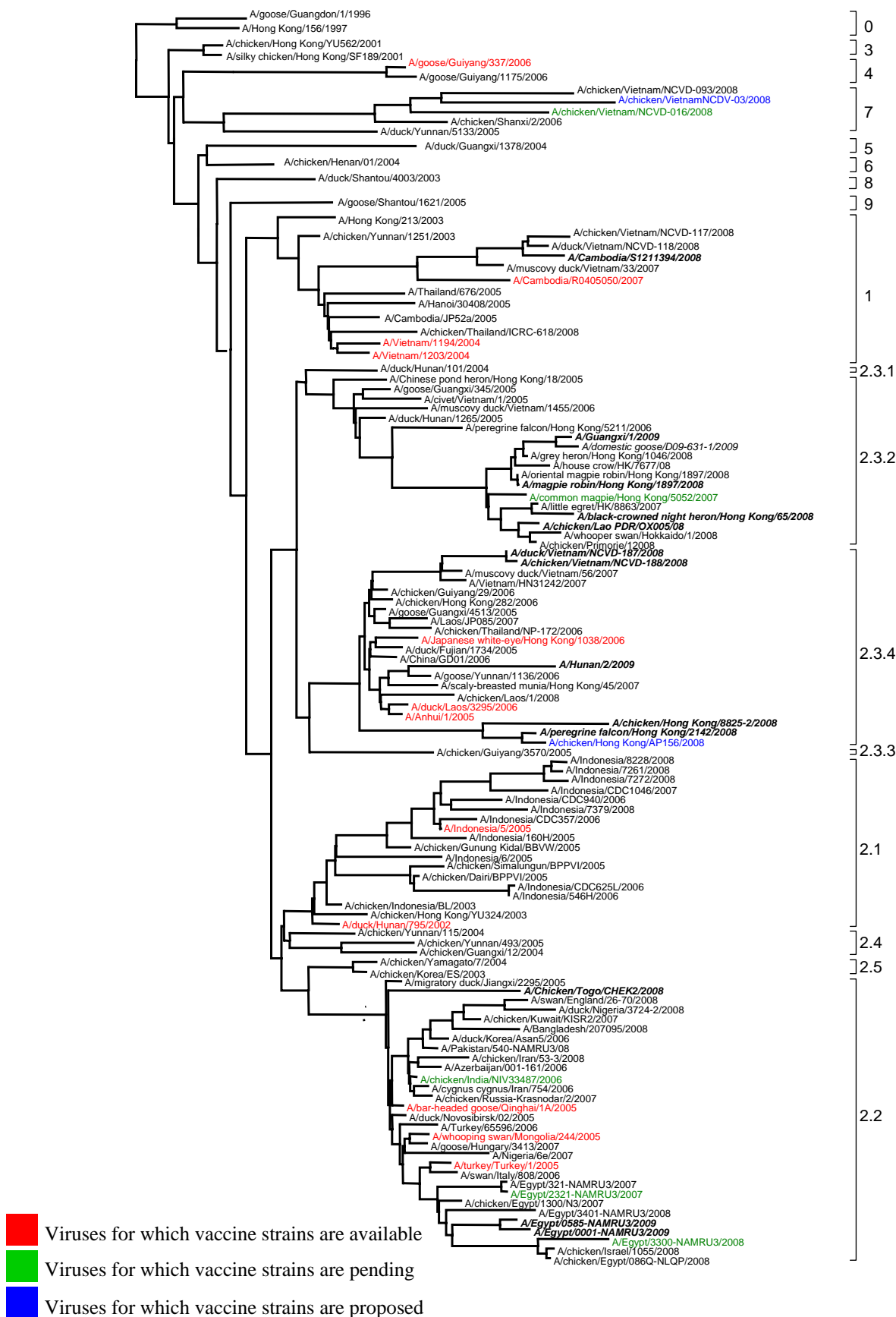


Figure 1. Phylogenetic relationships of H5N1 viruses showing availability of vaccine strains. We gratefully acknowledge the contributions of the originating laboratories and countries that have provided samples and/or submitted sequence data to DDBJ, EMBL-Bank, GenBank, GISAID and other public databases. Recent viruses (where date of isolation known) are shown in italics.