

## WHO Process for Monitoring Novel Influenza Viruses

FAO-OIE-WHO Joint Technical Consultation  
on Avian Influenza at the Human-Animal Interface  
Verona, 7-9 October 2008

### WHO Global Influenza Surveillance Network (GISN) (Established in 1952)

5 WHO Collaborating Centres (Atlanta, London, Melbourne, Tokyo, Memphis)  
~120 National Influenza Centres (>80 countries) (gaps!)

**Main Objectives:**

- **Monitor - epidemiology of influenza and burden of disease**
  - antigenic/genetic changes in circulating A and B viruses
  - spread of antigenic variants
  - emergence and persistence of drug resistance

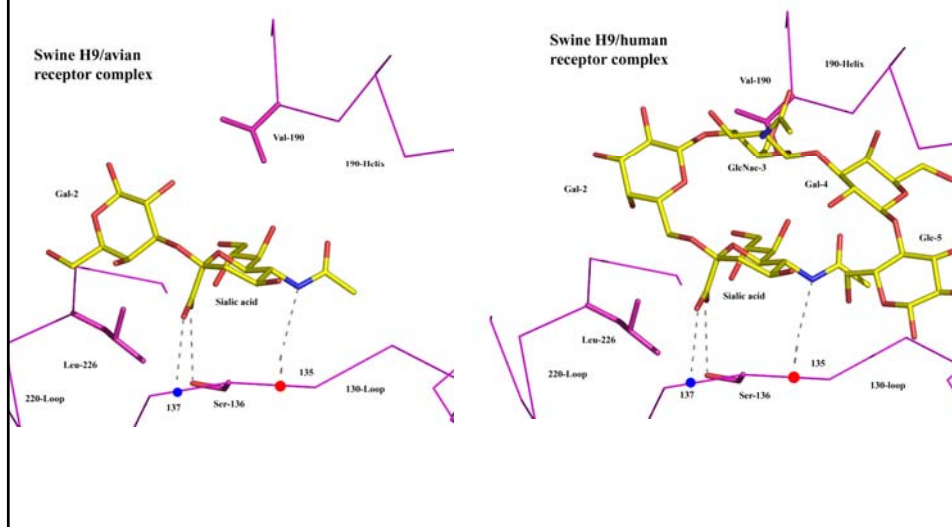
Make biannual recommendations on vaccine composition
- **Early detection of novel human viruses, assessment of pandemic risk**
  - identify the virus (genetic/antigenic) – new human subtype?
  - identify source of infection and extent
  - sporadic or local clusters of infection (serological evidence)
  - human-human transmission?
  - geographical spread
  - develop candidate vaccine strains

## Monitoring emergence of human virus with pandemic potential (characteristics)

Identity of the virus (genetic/antigenic) – new human subtype?

- Swine H1N1, H1N2, H3N2 - antigenically different from human subtypes (not considered likely pandemic threat)  
important to monitor changes among swine viruses
  - to readily identify source of infection (e.g. A/HK/1774/99, H3N2)
  - emergence of novel subtypes (e.g. H2N3) with increased pandemic risk
- Avian viruses:
  - H7N7, H7N3, H7N2 - mainly mild infections, conjunctivitis
    - little change (animal/human)
    - source of infections removed
  - H9N2 - mild infections; partial human receptor-binding characteristics
    - widespread
  - H5N1 - highly pathogenic, diverse genetically and antigenically
    - increasing spread

The HA of H9N2 virus, with leucine 226, has preference for the human-like ( $\alpha$ 2,6-linked sialic acid) receptor - intermediate in human adaptation? - does it pose a greater pandemic threat?



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## Monitoring changes in novel human influenza viruses

### Genetic changes/increased diversity (all genes):

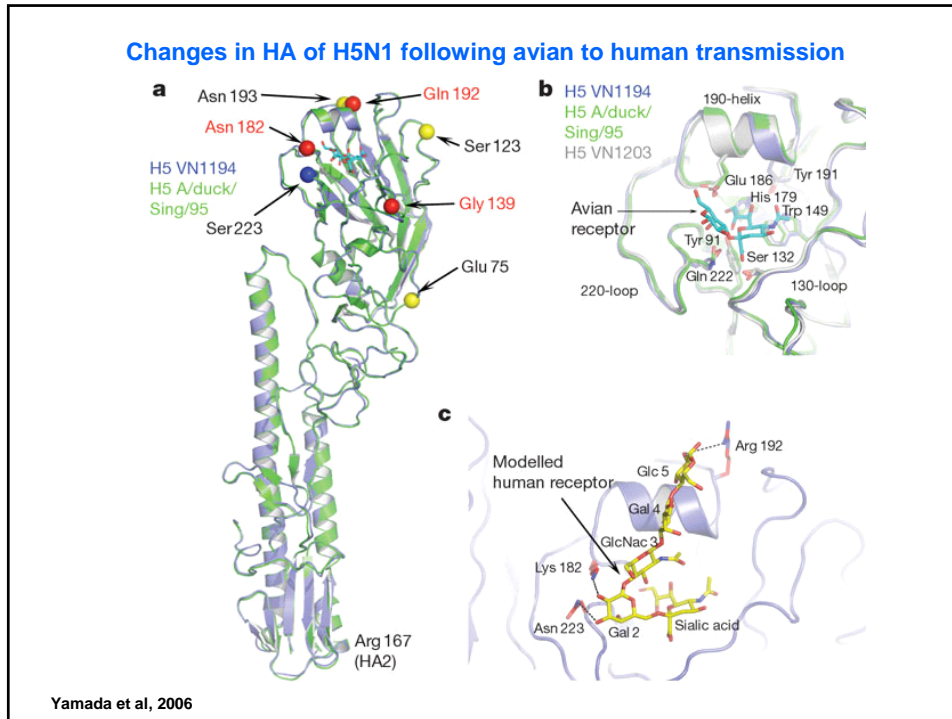
- In source viruses - genetic reassortment (avian/human)?
  - diagnosis: update primers/probes? [H5N1 ]( H7N7)  
(WHO Working Group on PCR Protocols)
  - increase human infection (pandemic risk) [PB2 E627K of clade 2.2]
  - change in clinical outcome?
  - drug resistance (established mutations)?
- Following animal to human transmission - adaptive changes? (increase human transmission)
  - HA receptor binding (increased preference for human receptors)
  - Polymerase activity, e.g. PB2: E627K, D701N
  - Altered virulence?, e.g. NS1, PB1- F2

### Antigenic changes/increased diversity (using ferret post-infection antisera):

- Diagnostics
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- Cross-reactivity of antibody responses to natural infection

### Resistance to anti-M2, anti-NA drugs :

- Effectiveness of antivirals (stockpiles)



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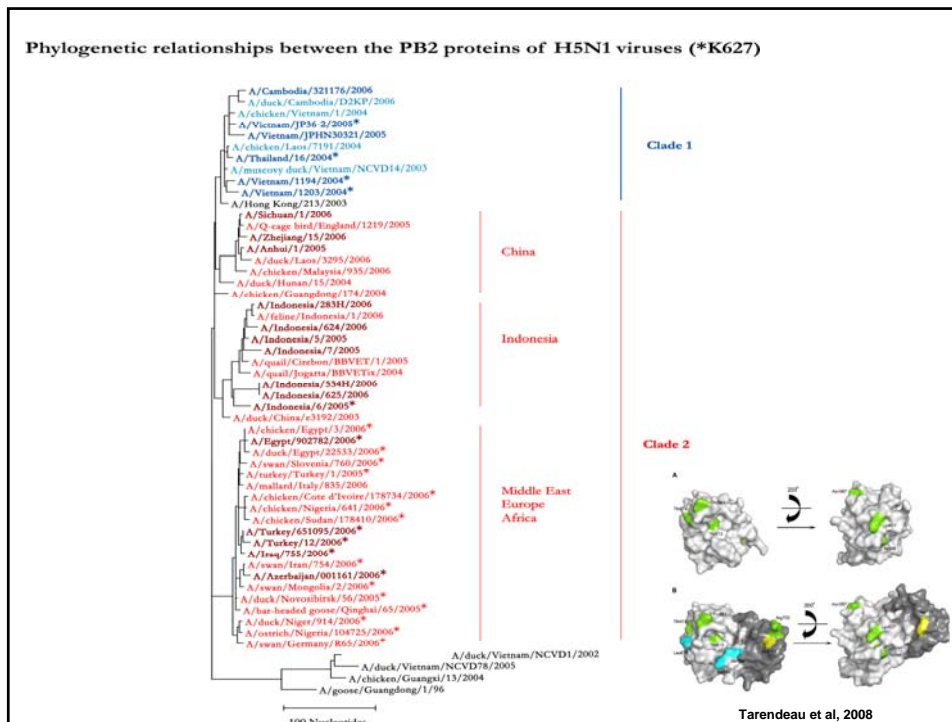
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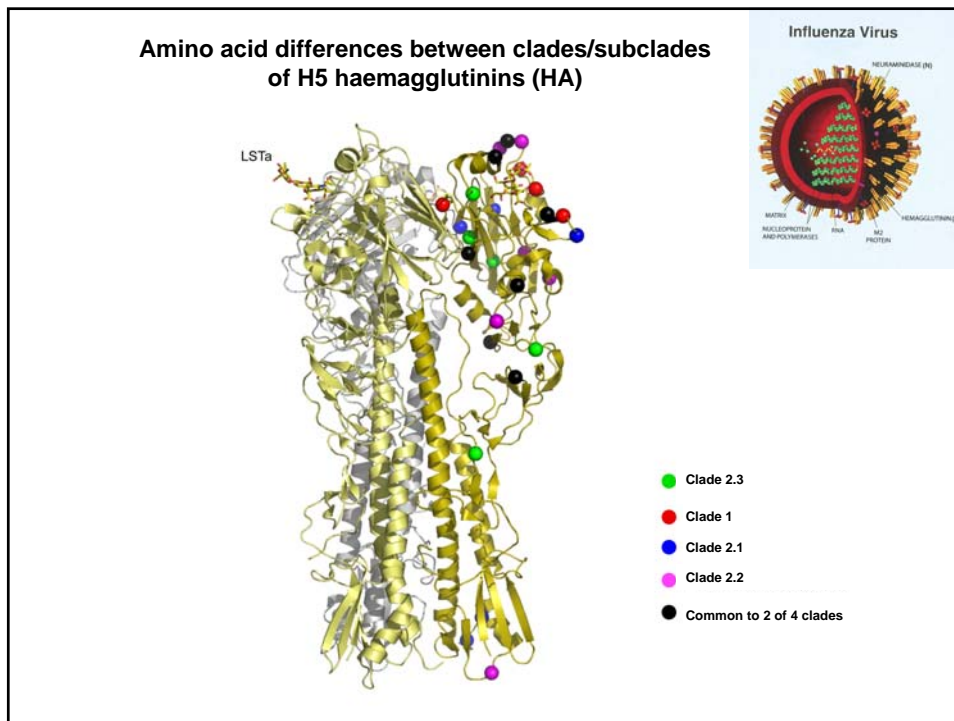
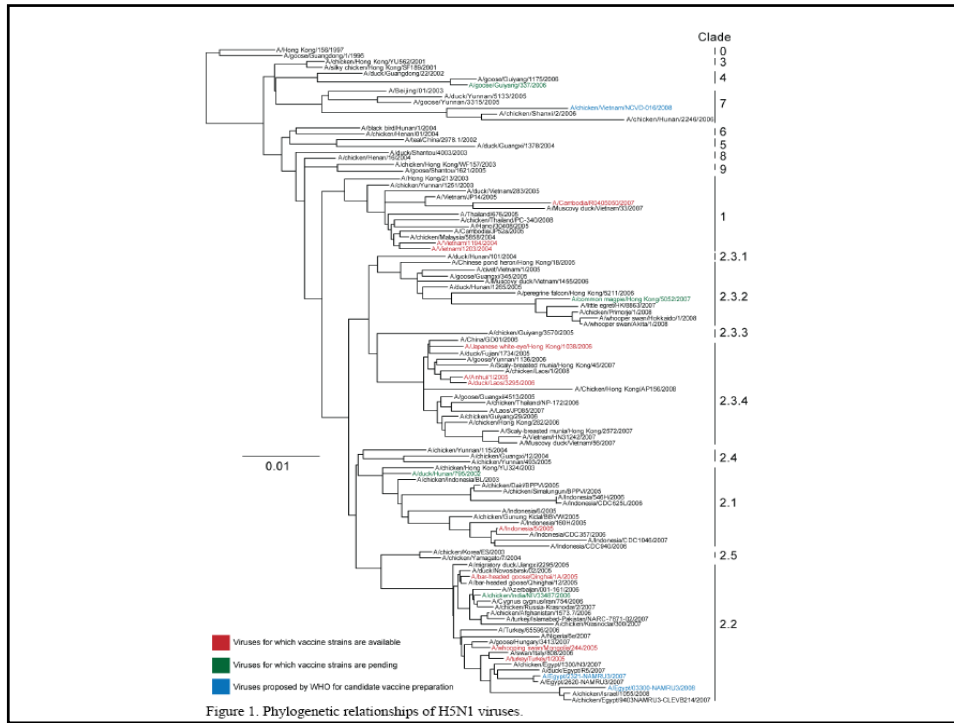
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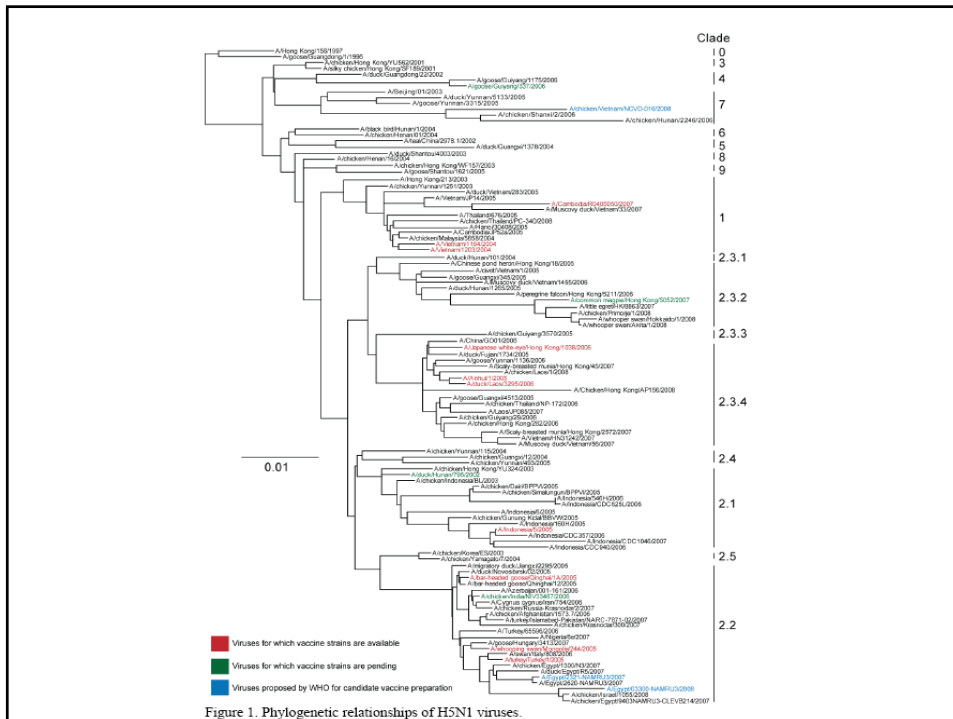


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Table 1. Antigenic properties of H5N1 viruses

REFERENCE ANTIGENS	CLADE	REFERENCE FERRET ANTISERA						
		1	2.1	2.1	2.2	2.3.2	2.3.4	7
		VN-1203	IND-5	DK/HU	MG-244	MD/VN	ANB-1	CK/VN
A/Viet Nam/1203/2004 (VN-1203)	1	320	20	40	-10	40	-	-10
A/Indonesia-5/2005 (IND-5)	2.1	10	640	80	80	160	-	80
A/duck/Human/795/2002 (DK/HU)	2.1	80	1280	160	-	-	40	-
A/wisconsin/osca/Mongolia/744/2005 (MG-244)	2.2	20	160	160	320	80	-	-
A/moscow/duck/Viet Nam/1455/2006 (MD/VN)	2.3.2	40	160	-	160	320	-10	-10
A/Andhra/1/2005 (ANB-1)	2.3.4	40	320	-	-10	-	640	-10
A/chicken/Viet Nam/NCVD-016/2008 (CK/VN)	7	-10	-10	-	-	-	-10	640
<b>TEST ANTIGENS</b>								
A/Thailand/6/76/2003	1	160	20	-	-10	-	40	-
A/duck/Viet Nam/NCVD16/2007	1	40	<10	-	-10	-	-10	-
A/Indonesia CDC1031/2007	2.1	-10	640	-	160	-	160	-
A/Indonesia CDC623L/2006	2.1	40	80	40	20	-	-10	-
A/Turkey/65-596/2006	2.2	160	1280	-	5120	-	320	-
A/great Egypt/1162- NAMRU3/2006	2.2	-10	320	-	320	-	-10	-
A/Egypt/3300- NAMRU3/2008	2.2	-10	160	-	80	-	20	-
A/Bangladesh/20/7095/2008	2.2	-10	320	-	320	-	-10	-
A/vietnam/mainghe/Hong Kong/3052/2007	2.3.2	80	320	40	-	320	-10	-
A/hong kong/719/2007	2.3.4	-10	80	-	-10	-	320	-
A/chicken/Viet Nam/NCVD74/2007	2.3.4	20	<10	-	-10	-	40	-
A/duck/Viet Nam/NCVD81/2007	2.3.4	-10	<10	-	-10	-	80	-
A/chicken/Hong Kong/AP156/2008	2.3.4	-10	<10	40	-10	40	-10	-
A/chicken/Viet Nam/NCVD-03/2008	7	-10	-10	-	-	-	-10	40



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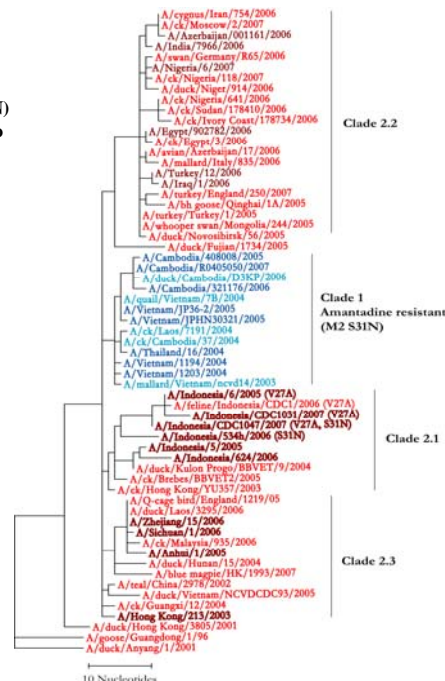
### Resistance to anti-M2, anti-NA drugs :

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## Phylogenetic comparison of the M genes of H5N1 viruses

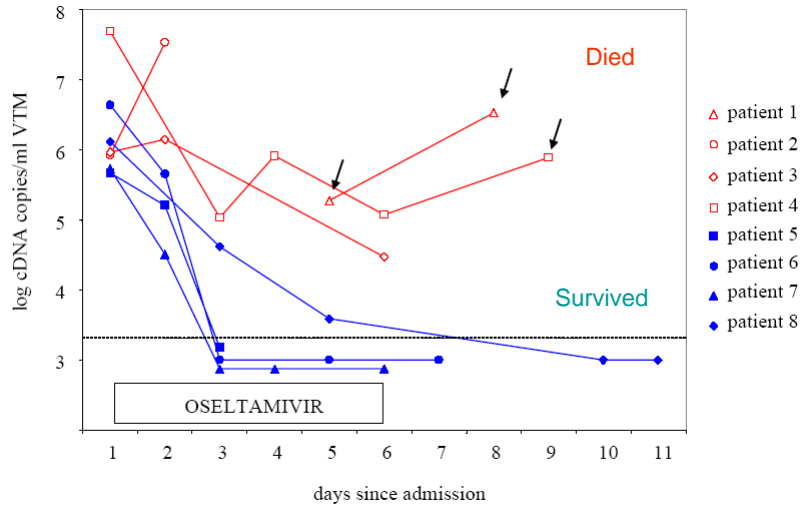
• Presence of mutations (V27A, S31N) in the M2 protein confer resistance to amantadine and rimantadine

- Clade 1 resistant
- Clade 2.1 ~80% resistant
- Clade 2.2, 2.3 sensitive



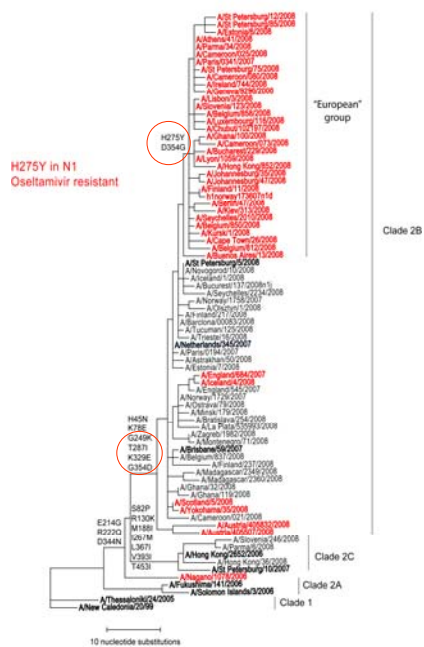


## Effect of Oseltamivir Treatment on Virus Load in H5N1 Patients

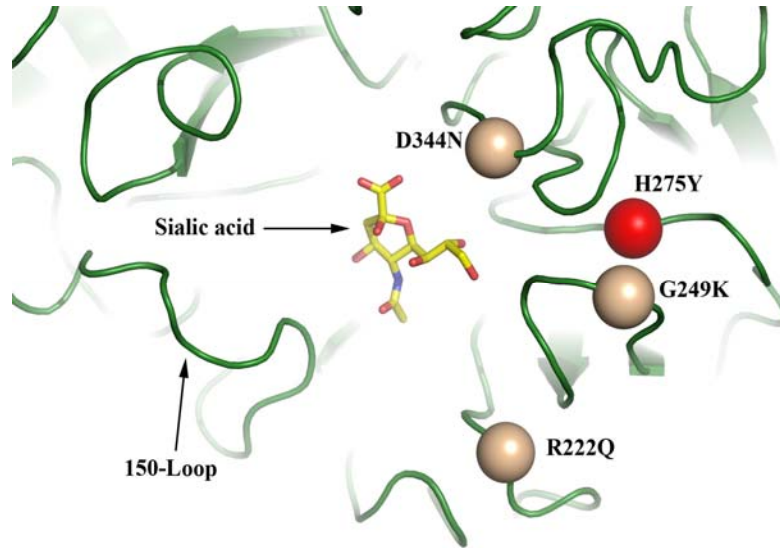


M. de Jong et al. N. Eng. J. Med. 2005

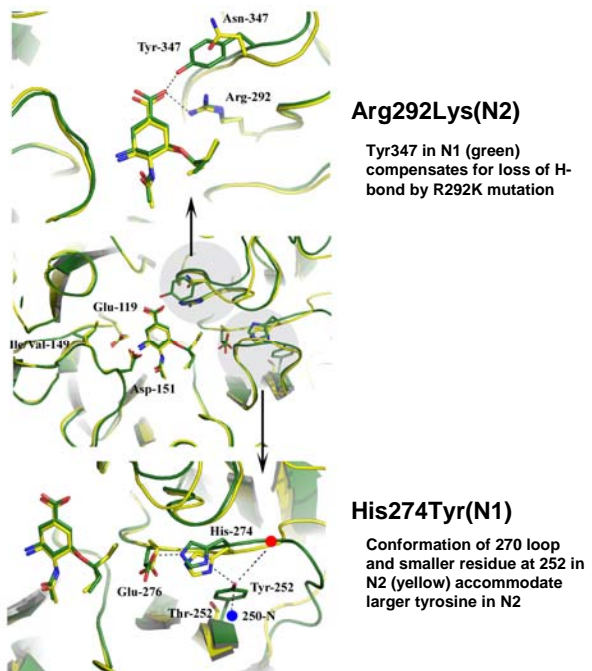
## Phylogenetic comparison of N1 neuraminidase genes



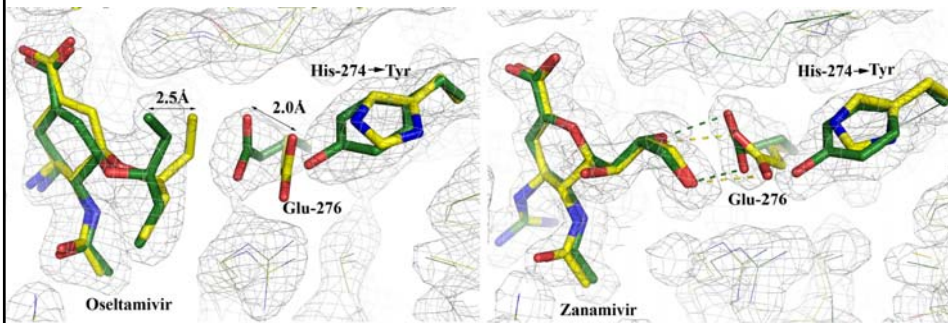
**Proximity of recent amino acid changes in NA of H1N1 viruses to the catalytic site**



**Structural basis for difference in Oseltamivir-resistance determinants**



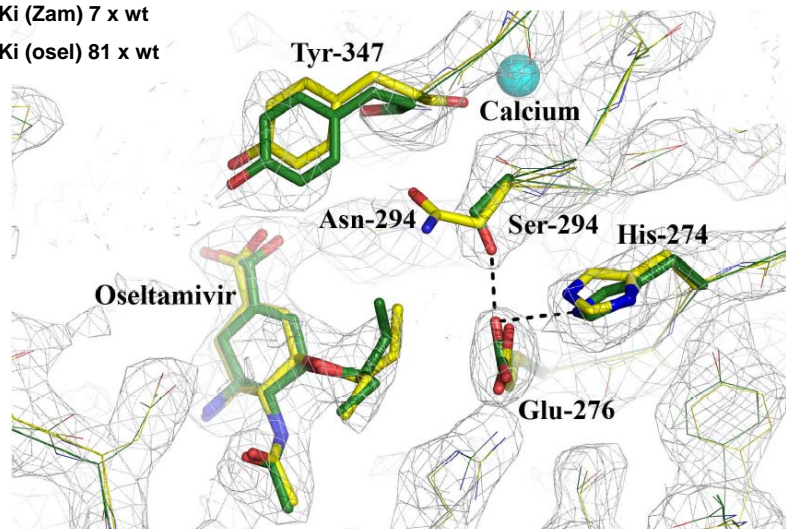
Effects of the H274Y mutation on the location of Glu 276 of N1 of A/Vietnam/1203/04(H5N1) in complex with with oseltamivir or zanamivir



Wild type (yellow); H274Y mutant (green)

Effect of the Asn294Ser mutation on oseltamivir binding to N1 of H5N1

Km 8 x wt  
Ki (Zam) 7 x wt  
Ki (osel) 81 x wt



## Acknowledgements

### **NIMR (Mill Hill)**

#### **WHO Flu Centre**

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John Skehel  
Steve Gamblin

Ben Blackburne  
Richard Goldstein

#### **WHO Influenza Network**

**Collaborating Centres**  
**National Centres**  
**H5 Reference Labs**

#### **HPA, Colindale**

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#### **VLA, Weybridge**

Ian Brown

