

Control human influencias

Influenza: key facts

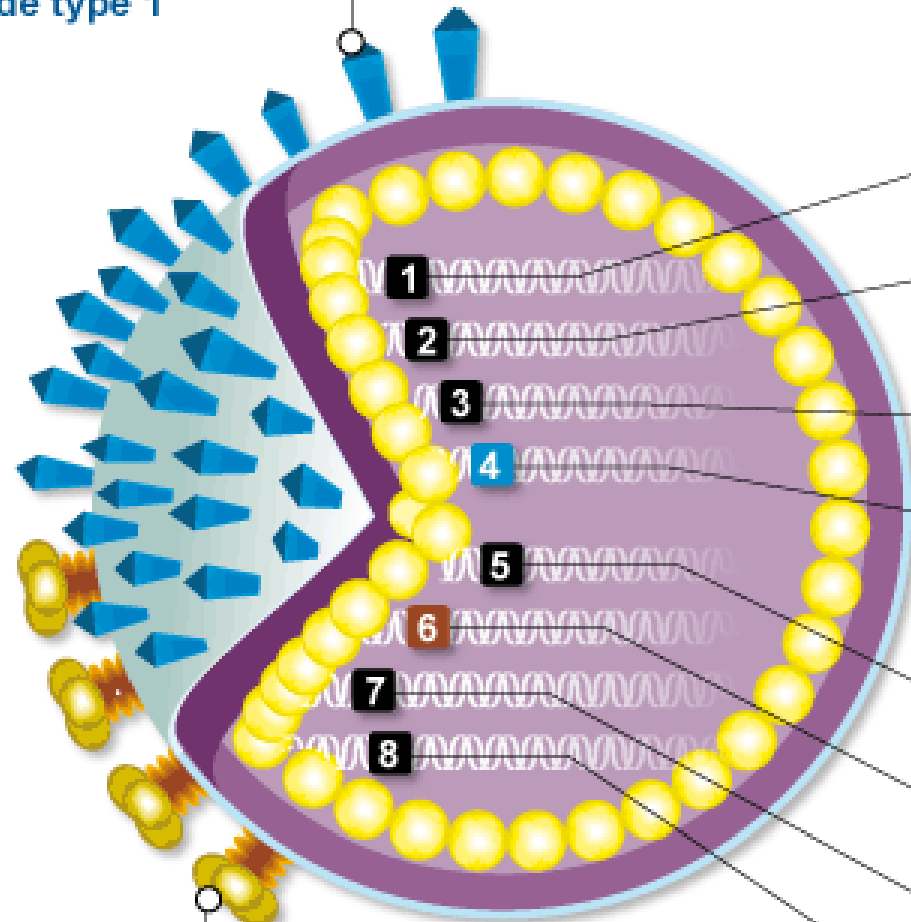
- Acute viral infection that spreads easily from person to person.
- Circulates worldwide and can affect anybody in any age group.
- Causes annual epidemics that peak during winter in temperate regions and pandemics.
- A serious public health problem that causes severe illnesses and deaths for higher risk populations.

Ses **huit gènes** proviennent de virus apparus à des époques différentes, dans des zones géographiques variées, qui s'épanouissent chez le porc, les oiseaux et l'homme.

HA :

Hemagglutinine de type 1

(se lie aux récepteurs
des cellules hôtes)



Patrimoine génétique

(fragments d'acide ribonucléique)

ORIGINE

PB2 :

Aviaire nord-américaine

PB1 :

Humaine (déjà présent
dans un virus H3-N2 de 1993)

PA :

Aviaire nord-américaine

HA :

Porcine (présent dans la souche
du virus de la « grippe espagnole »
de 1918)

NP :

Porcine nord-américaine

NA :

Porcine eurasienne

M :

Porcine eurasienne

NS :

Porcine nord-américaine

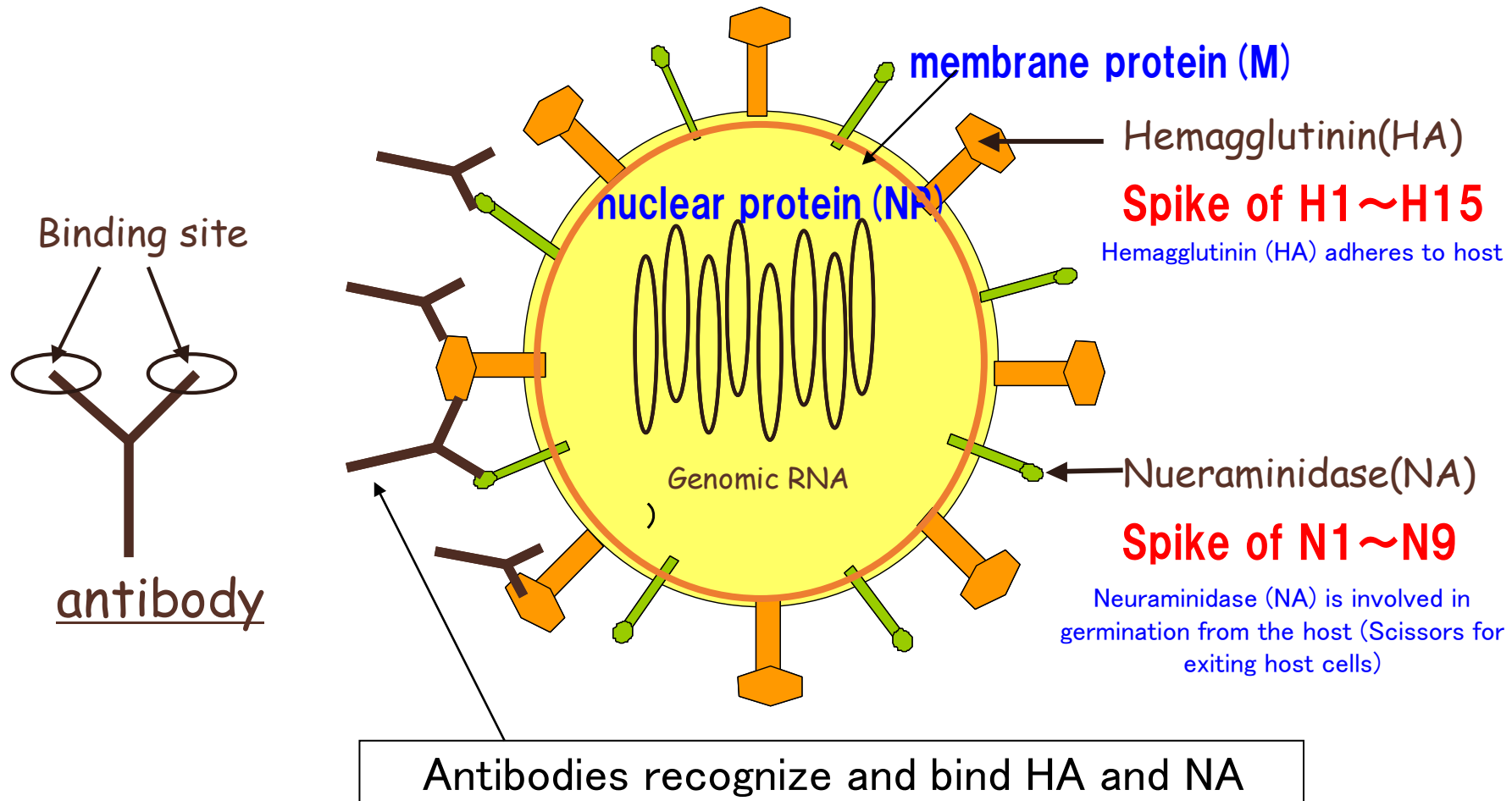
NA :

Neuraminidase de type 1

(aide à enclencher l'infection)

What is influenza virus?

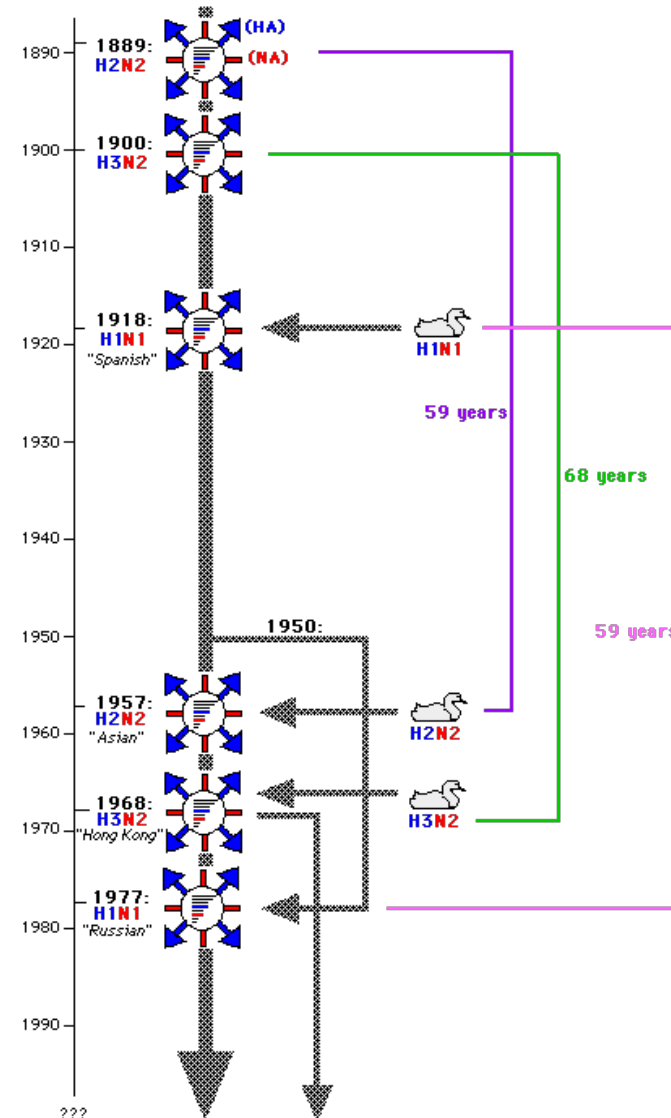
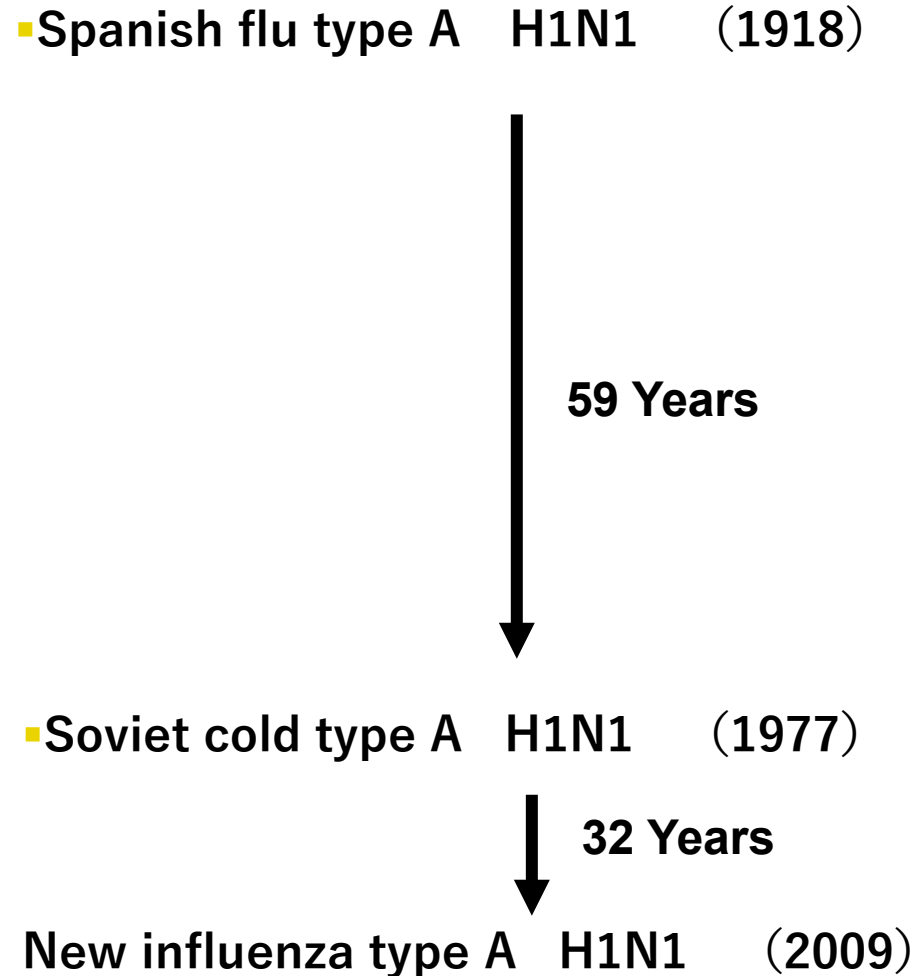
- Spherical with a diameter of 90–110 nm.
- The surface is covered with two types (H and N) of spikes.
- There are eight RNA genes inside that determine the nature of the virus.



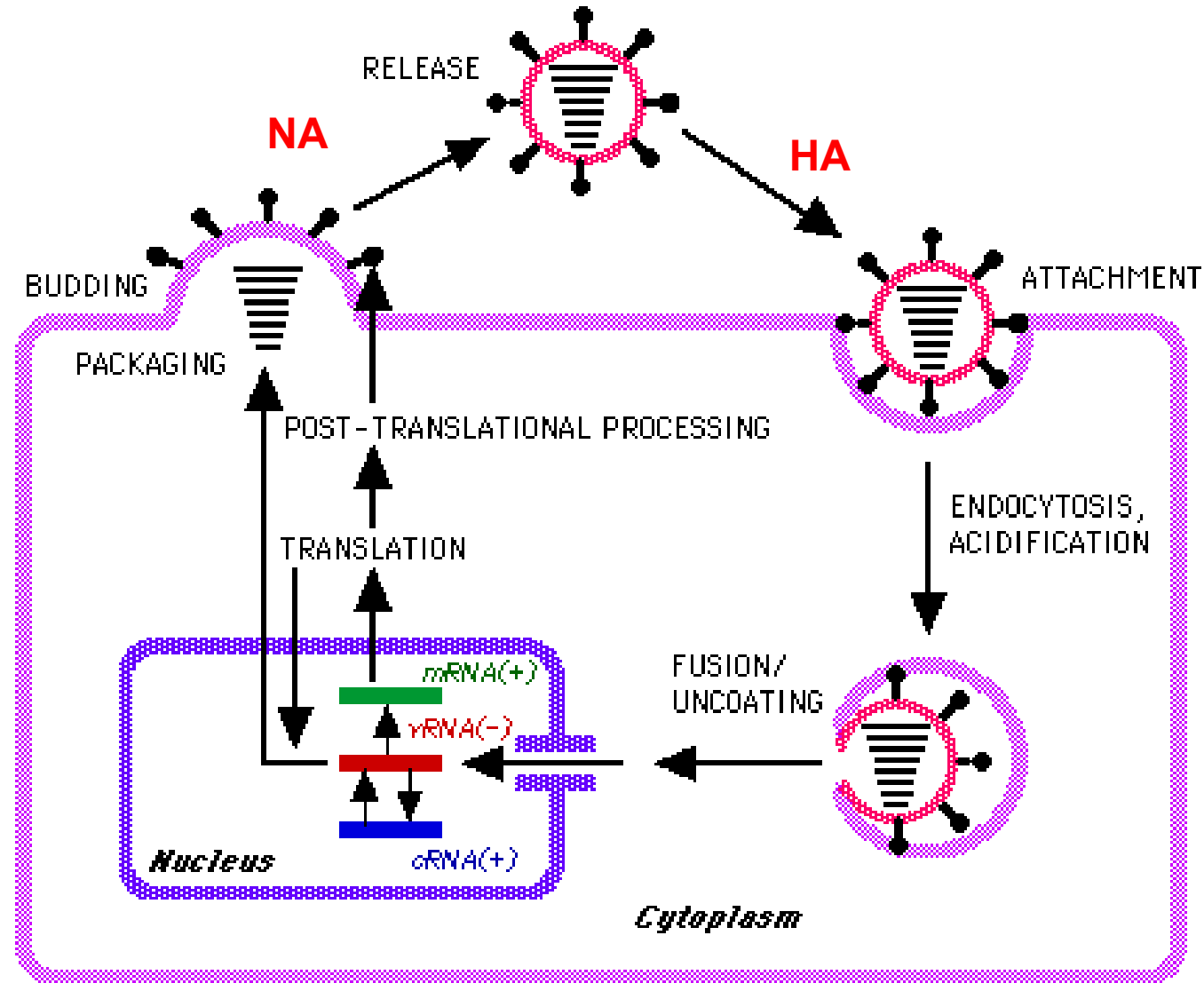
Influenza epidemiology: change in serotypes

| | |
|----------------|---|
| 1889-1892 | ? A/H2N2 |
| 1900 | ? A/H3N8 mild pandemic |
| 1918 | A/H1N1, Spanish Influenza |
| 1957 | A/H2N2, Asian Influenza |
| 1968 | A/H3N2, Hong Kong |
| 1976 | A/H1N1 swine-like virus, Fort Dix, NJ, USA |
| 1977* | A/H1N1, Russian, global re-introduction (re-emergence) |
| 1997 | A/H5N1 avian influenza, Hong Kong |
| 1999 | A/H9N2 human cases, Hong Kong |
| 2003/04 | A/H7N7 human cases, Netherlands & British Columbia |
| 2003/04 | A/H5N1 further human cases (Thailand, Korea, Vietnam) |
| 2009* ~ | A/H1N1 |

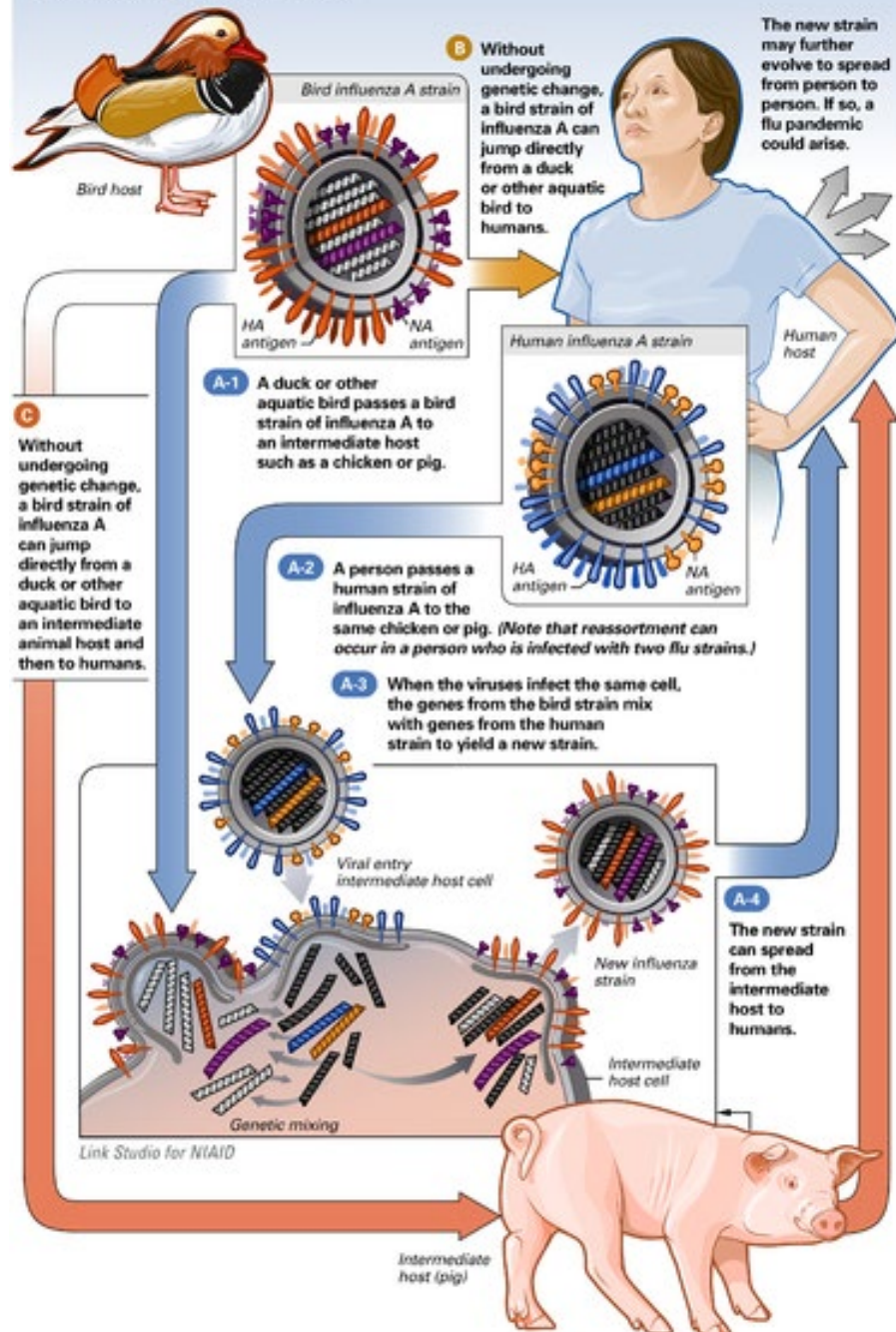
Influenza virus epidemic serotype



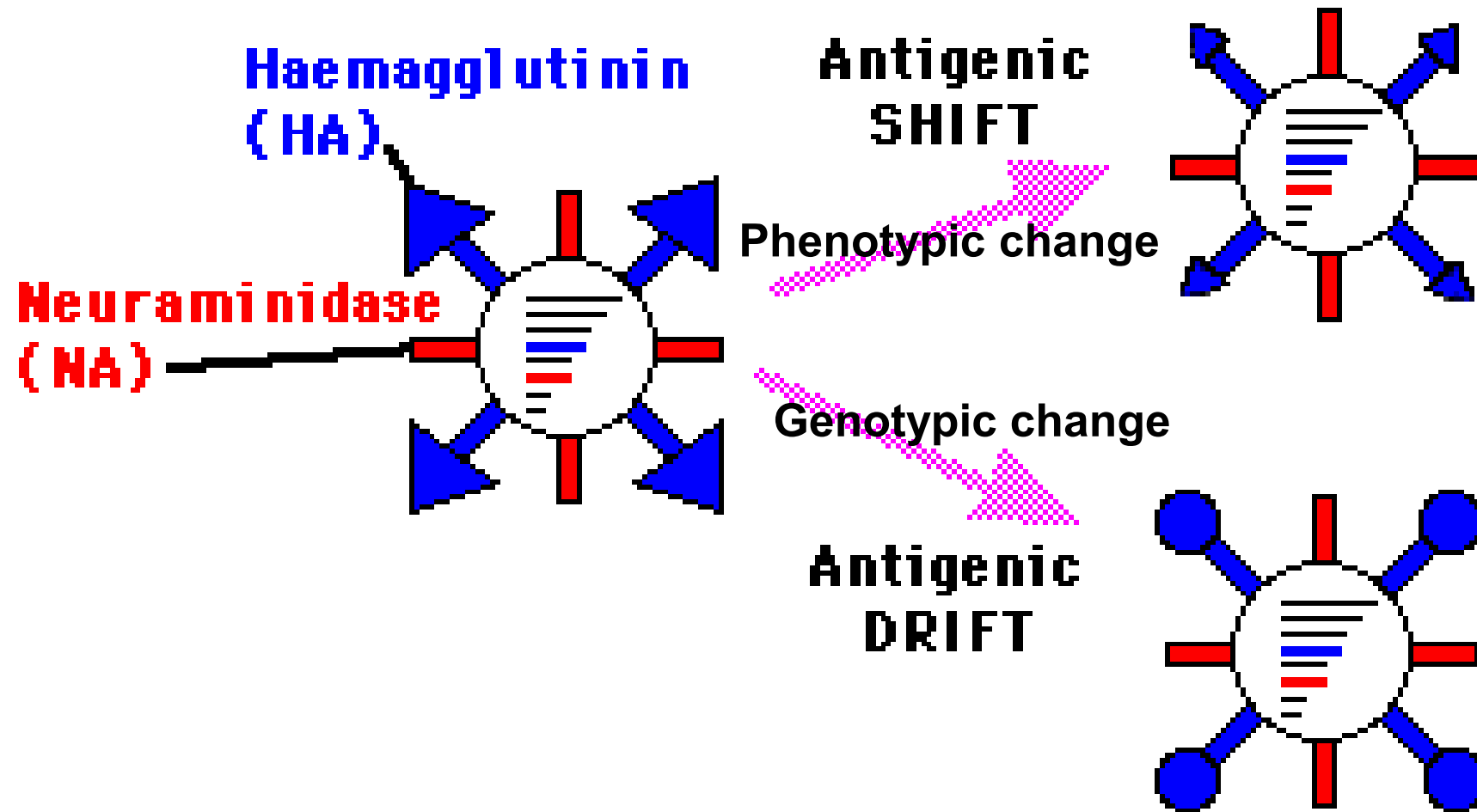
Influenza virus Replication



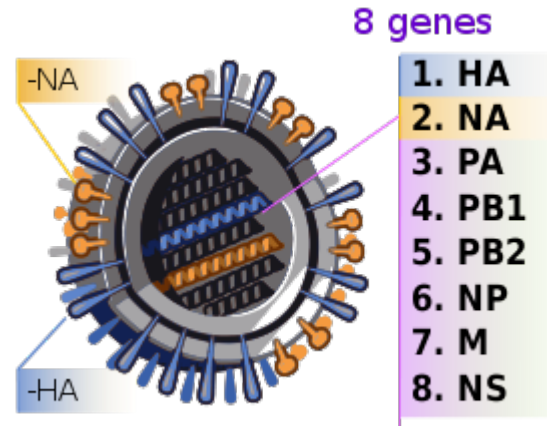
The genetic change that enables a flu strain to jump from one animal species to another, including humans, is called "ANTIGENIC SHIFT."
Antigenic shift can happen in three ways:



Mechanism for Influenza virus Serotype Change



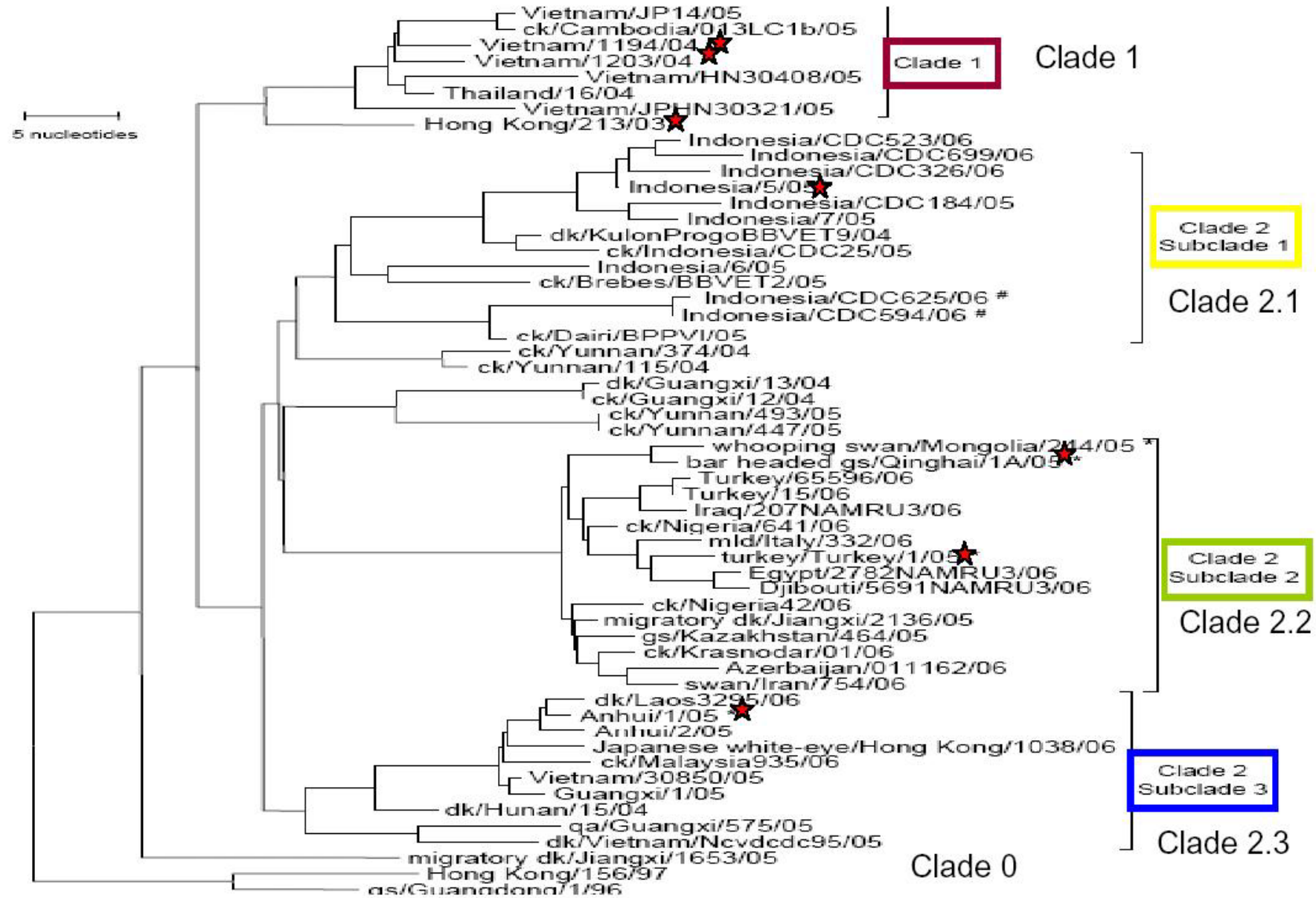
The genetics of the Pandemic novel H1N1/09 virus



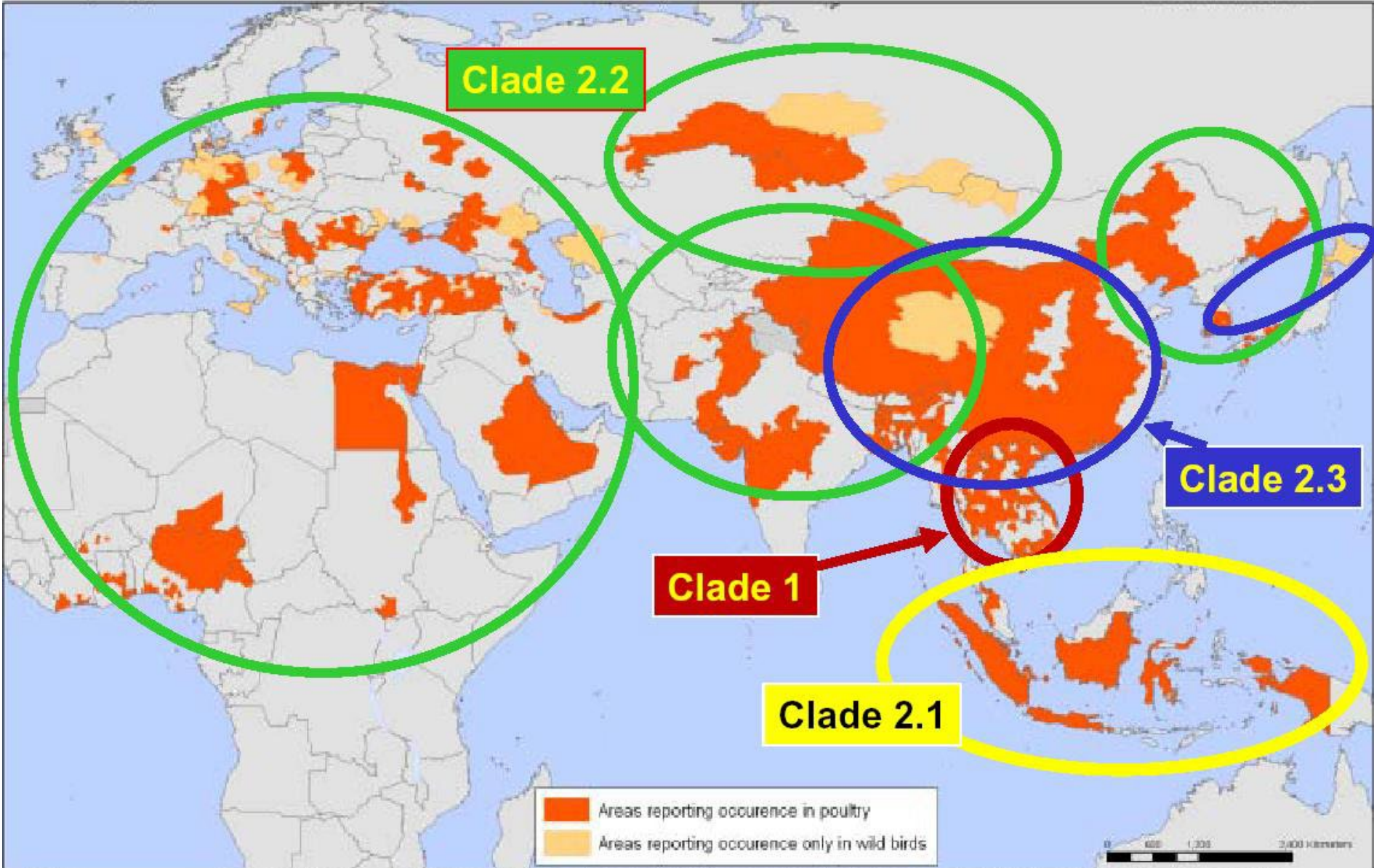
| | | | |
|---------------|--|-------------------|-------------------------|
| HA | <u>Hemagglutinin</u> | Swine (H1) | North America |
| NA | <u>Neuraminidase</u> | Swine (N1) | Europe |
| PA | <u>RNA polymerase subunit PA</u> | Avian | North America |
| PB1 | <u>RNA polymerase subunit PB1</u> | Human | 1993 H3N2 strain |
| PB2 | <u>RNA polymerase subunit PB2</u> | Avian | North America |
| NP | <u>Nucleoprotein</u> | Swine | North America |
| M | <u>Matrix protein M1, M2</u> | Swine | Eurasia |
| NS/NEP | <u>Non-structural proteins NS1, NEP</u> | Swine | North America |

source: "[The identity card of a composite virus](http://www.lemonde.fr/planete/infographie/2009/04/30/la-fiche-d-identite-d-un-virus-inedit_1187597_3244.html#ens_id=1185166)" (in French). [Le Monde](http://www.lemonde.fr). 2009/04/29. http://www.lemonde.fr/planete/infographie/2009/04/30/la-fiche-d-identite-d-un-virus-inedit_1187597_3244.html#ens_id=1185166.

avian influenza virus strains



Endemic areas of avian influenza virus strains



H5N1 pandemic threat

- 2003 up to date – **602** H5N1 cases of human infection in 15 countries with a death rate **>50%** (**355**).
- 2012: **24** human H5N1 cases, **15** of which have died.
- Countries with high incidence are Cambodia, China, Egypt, Indonesia, Thailand, and Vietnam.

Current approaches

Active immunization:

- Intramuscular (IM) injection of inactivated whole or split viruses.
- Intranasal (IN) spray of adjuvanted influenza virosomes or live attenuated cold-adapted influenza virus (CAIV) vaccines.

Not all vaccinees respond to vaccines.

Antiviral drugs: Tamiflu[®] (oseltamivir) and Relenza[®] (zanamivir).

Problems with emerging of drug-resistant strains.

Passive immunization: (transfer of specific antibodies):

- Human mAbs using Epstein-Barr virus (EBV) immortalization of B cells isolated from patients infected with H5N1.
- Phage display.
- Humanized mAbs.
- Human recombinant Abs.

Costly and under development.

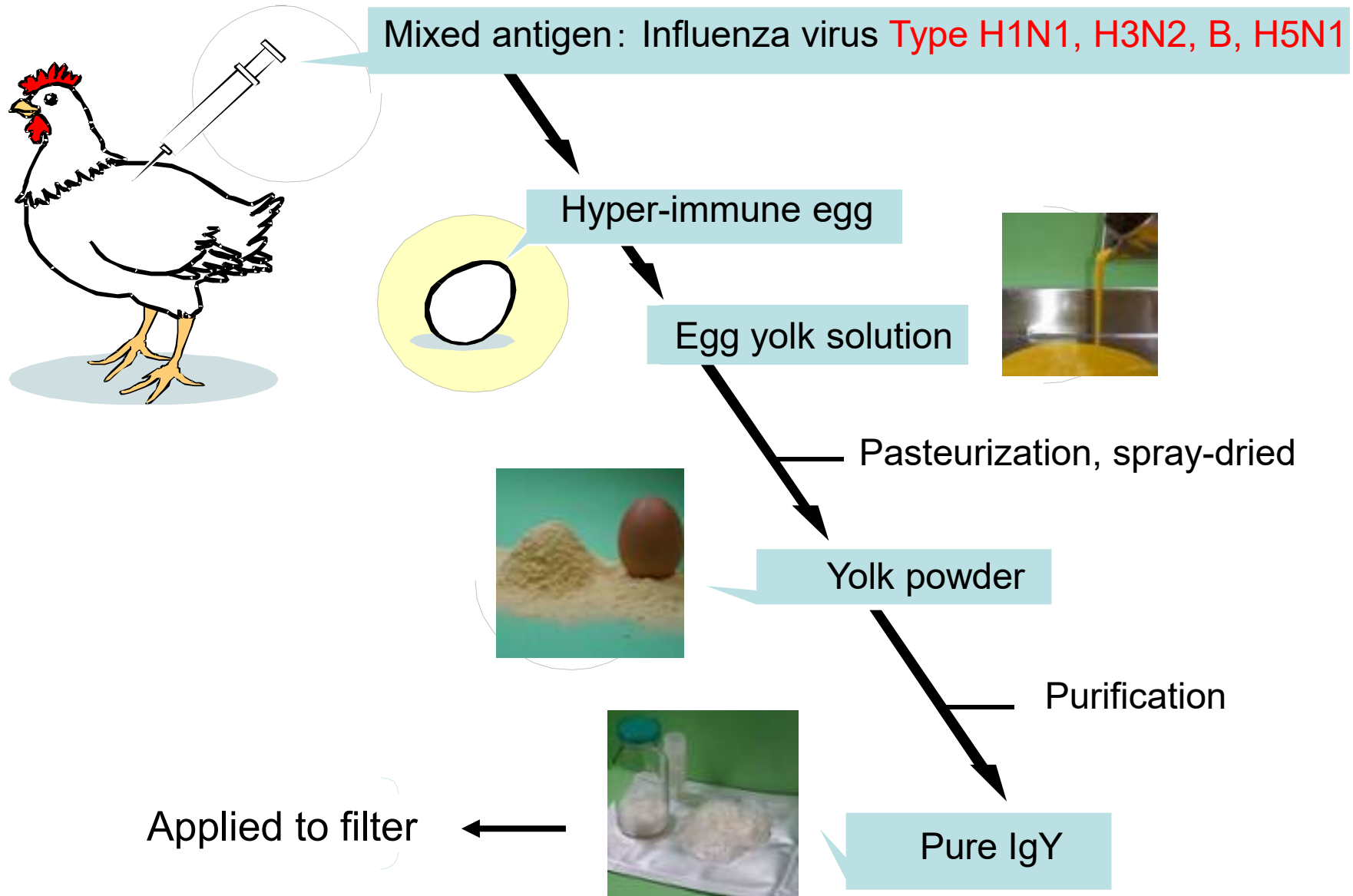
Influenza virus strains used as antigen to produce

- Human A type H1N1
- Human A type H3N2
- Human B type
- Pig A type H1N1
- Pig A type H3N2
- Bird A type H5N1 Clade 1
- Bird A type H5N1 Clade 2.1

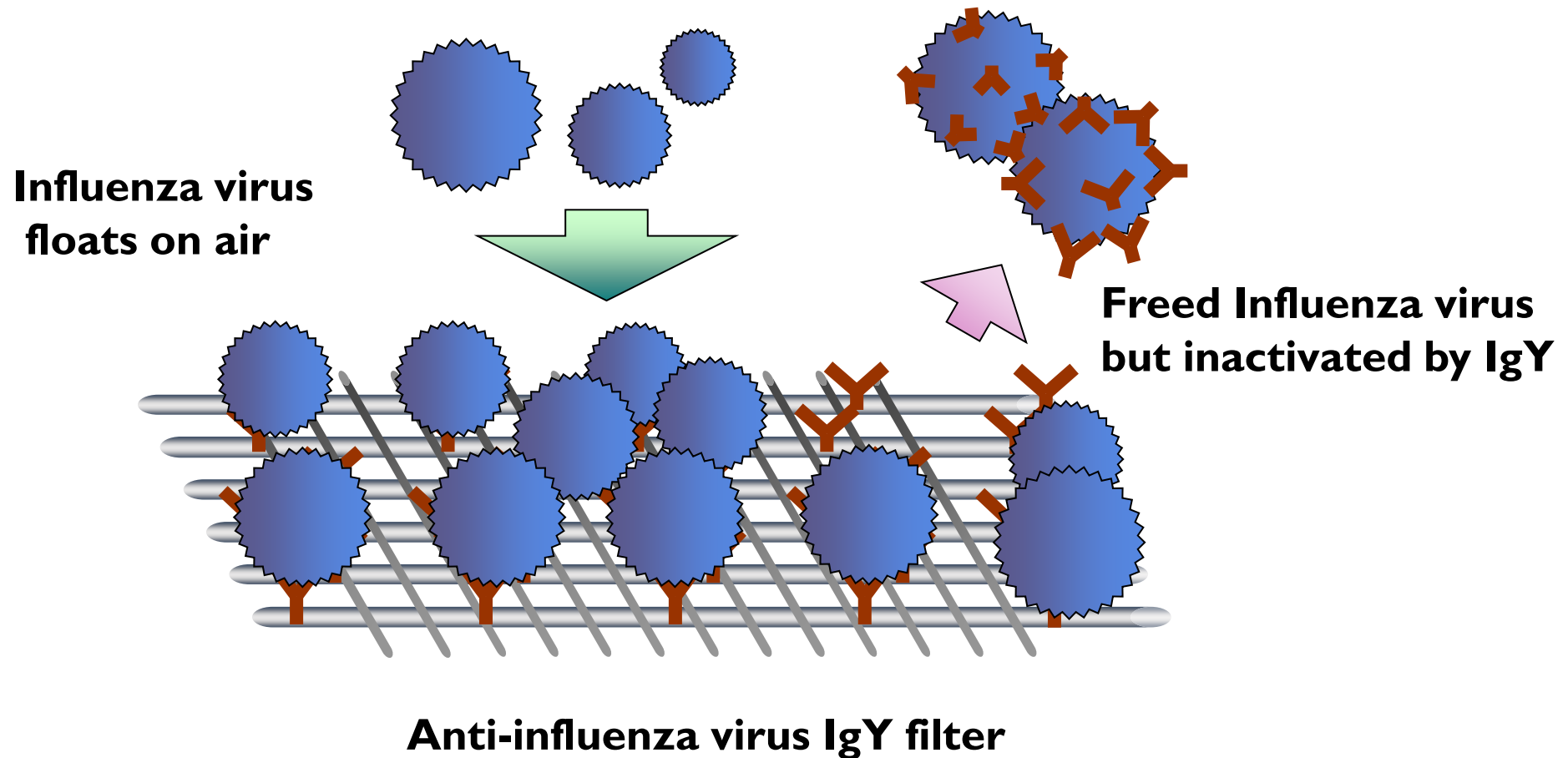
In-vitro study for the evaluation of Anti-influenza virus IgY incorporated air-filter

**Sponsored by : Waseda University
Daikin Industries, *Ltd.*
EW Nutrition Japan**

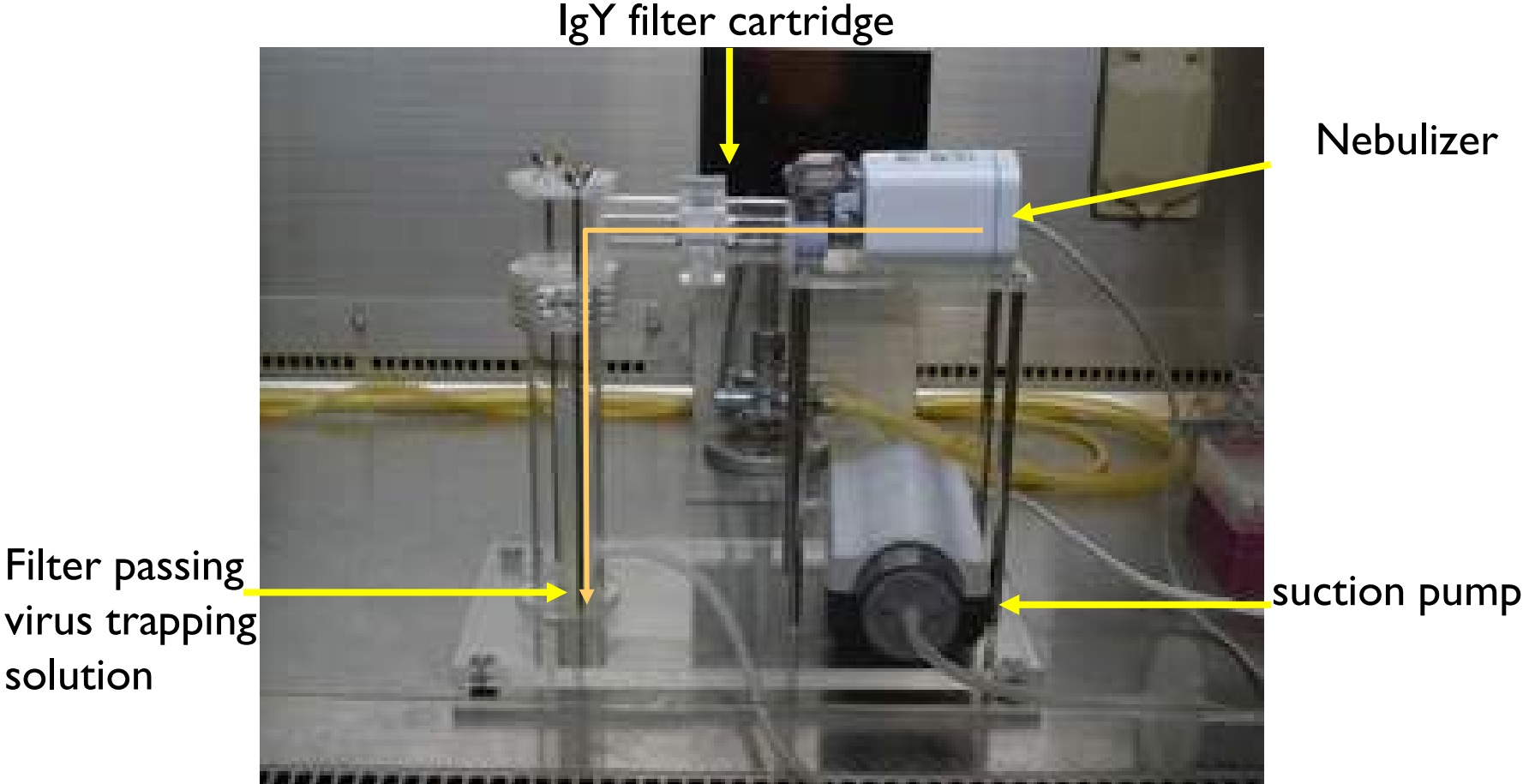
Anti-influenza IgY Preparation



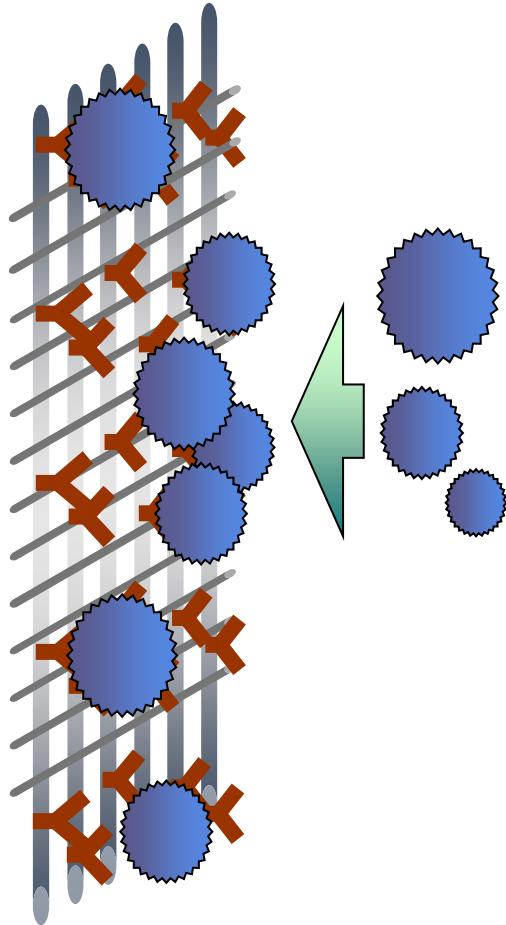
Characteristic of Anti-influenza virus IgY incorporated filter



Anti-influenza IgY filter evaluation by wind tunnel device



Influenza virus trapping in Anti-influenza IgY coated filter



Anti-influenza virus IgY filter evaluation experiment

Method

- - **Types of IgY-coated filter**

Anti-influenza virus IgY and Control IgY added filter

- - **Influenza virus incorporation into filter**

Spray influenza virus solution (1.2×10^9 EID₅₀/50 μ L) for three minutes to each filter (1cm²) by using nebulizer and incubated at the room temperature for ten minutes.

Anti-influenza virus IgY filter evaluation experiment

- **Virus washout**

The adhering virus is washed out from each filter by PBS (-) of 500 μ L at room temperature for ten minutes.

- **Inoculating to egg for growth**

100 μ L from each dilution (10^{-1} ~ 10^{-8} dilutions of washed-out virus) is vaccinated to the four eggs and cultured for 48 hours at 37°C.

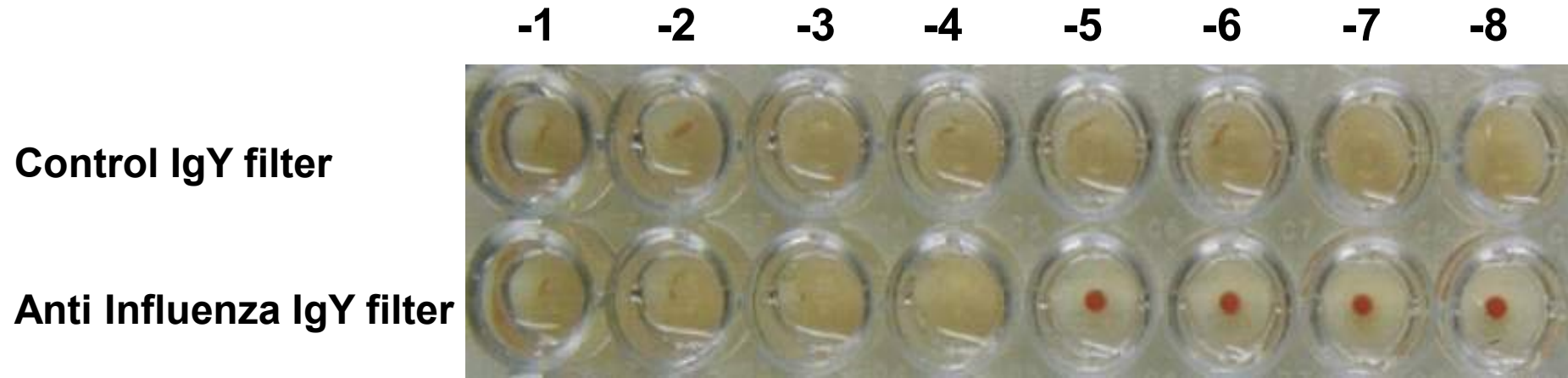
- **Passage of inoculated egg**

100 μ L from the inoculated egg is re-inoculated to the egg after diluting 1000 times and incubated for 48 hours at 37°C.

- **Virus titration**

HA is tested by using the chicken red blood corpuscle, and the virus titer is calculated by the Karber method.

Anti-influenza virus IgY filter evaluation results



Influenza virus titer

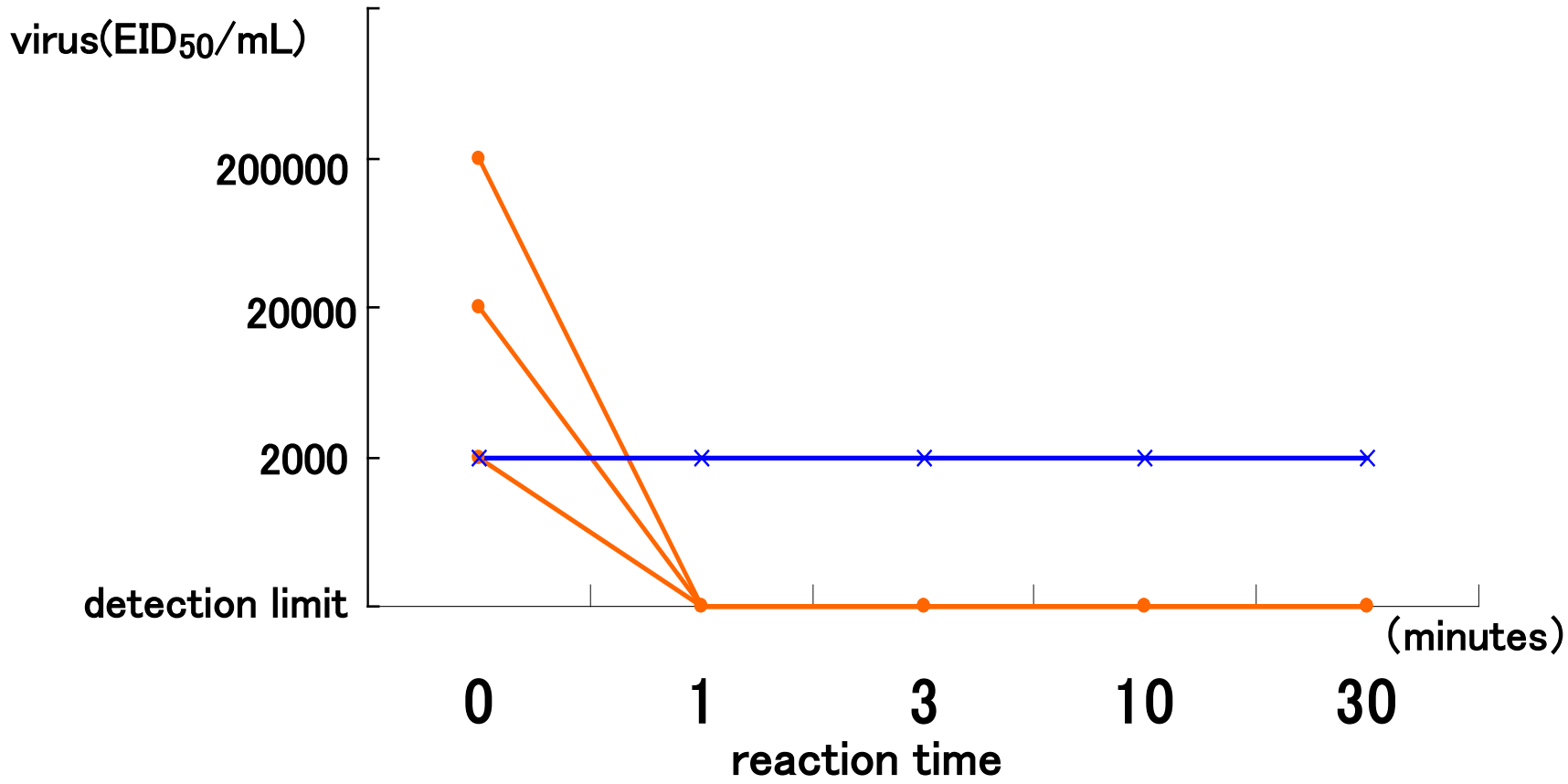
Control IgY filter $>0.5 \times 10^{8.5} \text{EID}_{50}/\text{cm}^2$

Anti Influenza IgY filter $0.5 \times 10^{4.75} \text{EID}_{50}/\text{cm}^2$

99.99% virus inactivated (neutralized)

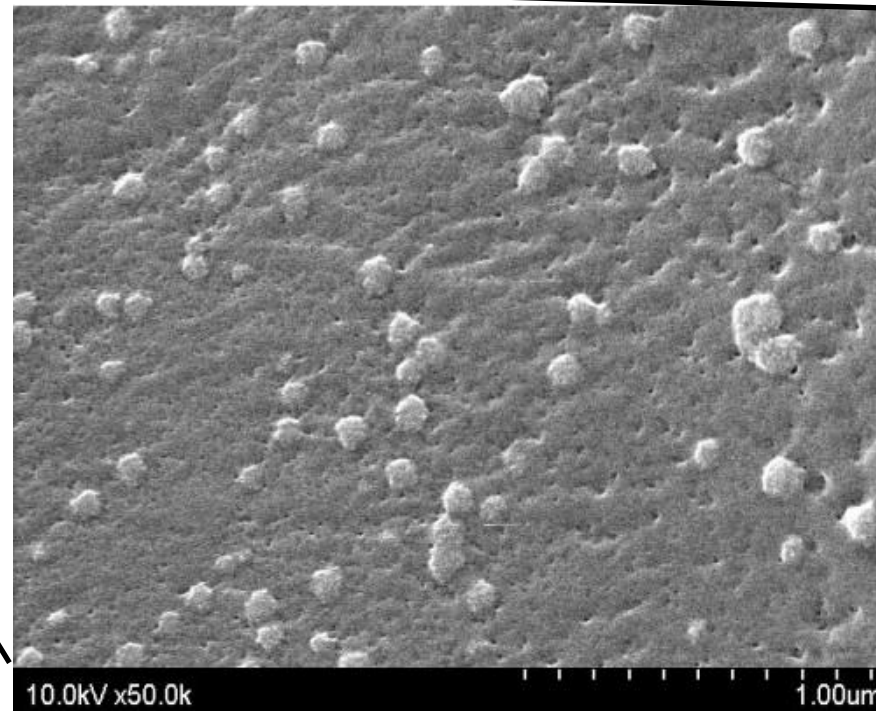
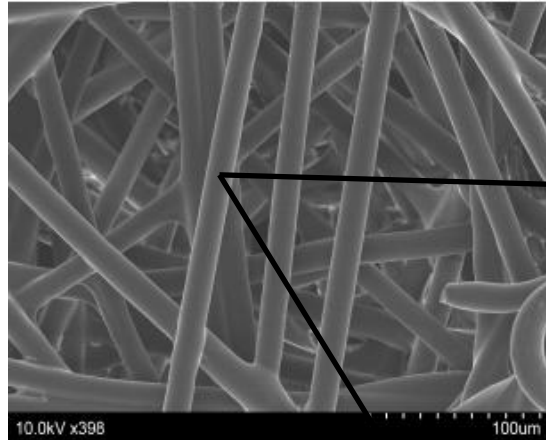
Rapid inactivation effect of bio-antibody filter

n=10 — : anti-influenza antibody — : control antibody



Influenza virus adsorbed on bioantibody filter

Taken with the cooperation of Associate
Professor Shirasawa, Yamagata University School
of Medicine



Evaluation of Ecologen IgY against avian-influenza A/H5N1 virus by serological tests

Supervised by: Dr. Le thi Quynh Mai

Tested in : Laboratory of Special Viral Pathogen
 Department of Virology
 National Institute of Hygiene and Epidemiology Vietnam

National Institute of Hygiene and Epidemiology, Vietnam



Viral Special Pathogen Laboratory, Department of Virology Supervised by : Dr. Le thi Quynh Mai



National Influenza center



Laboratory BSL1 Area



Safety Cabin



BSL3 Area



BSL3 Area Monitoring

Clade of the H5N1 virus

- **10 Clades are available, according to the HA antigen.**
- **Clade which causes human diseases are: 0, 1, 2.1, 2.2, and 2.3.**
- **Clade 1 is most common in Southeast Asian (chicken and human).**
- **Clade 2 (2.1, 2.2, 2.3, and 2.3.2, etc.) is isolated from the chicken in Japan, South Korea, and China.**

Material and method

- **IgY antibody**

1. Anti-influenza samples (4 samples)
2. Mock-infected IgY

- **Virus neutralization test**

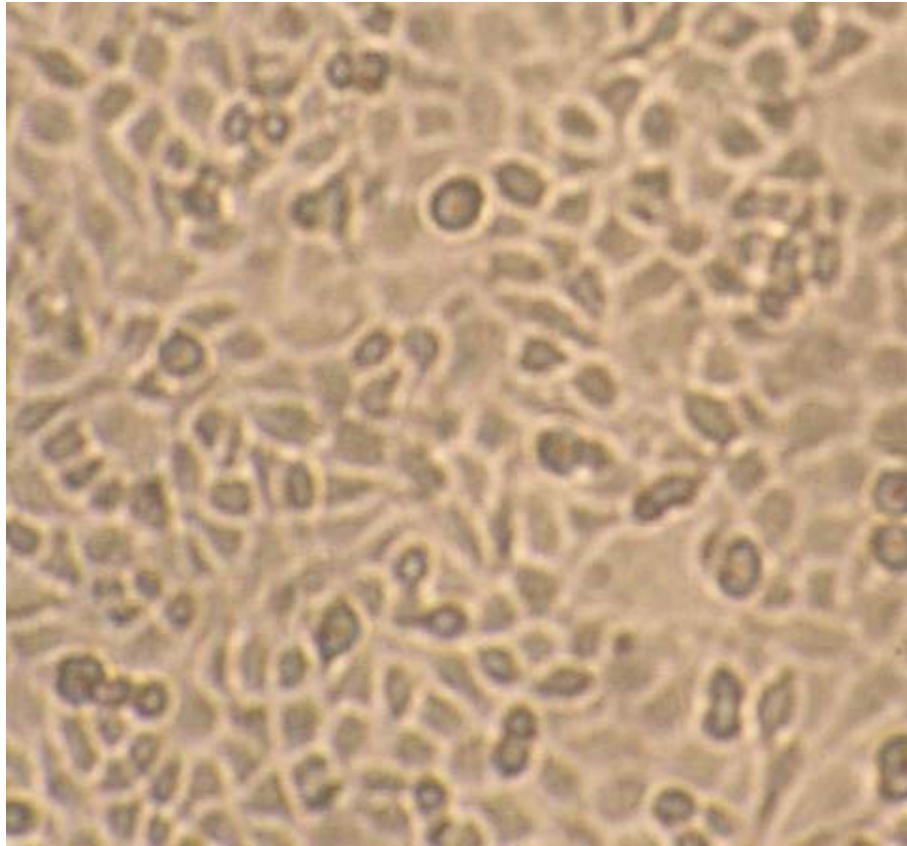
Method

1. HI (Haemagglutination inhibition test)
2. Microneutralization test

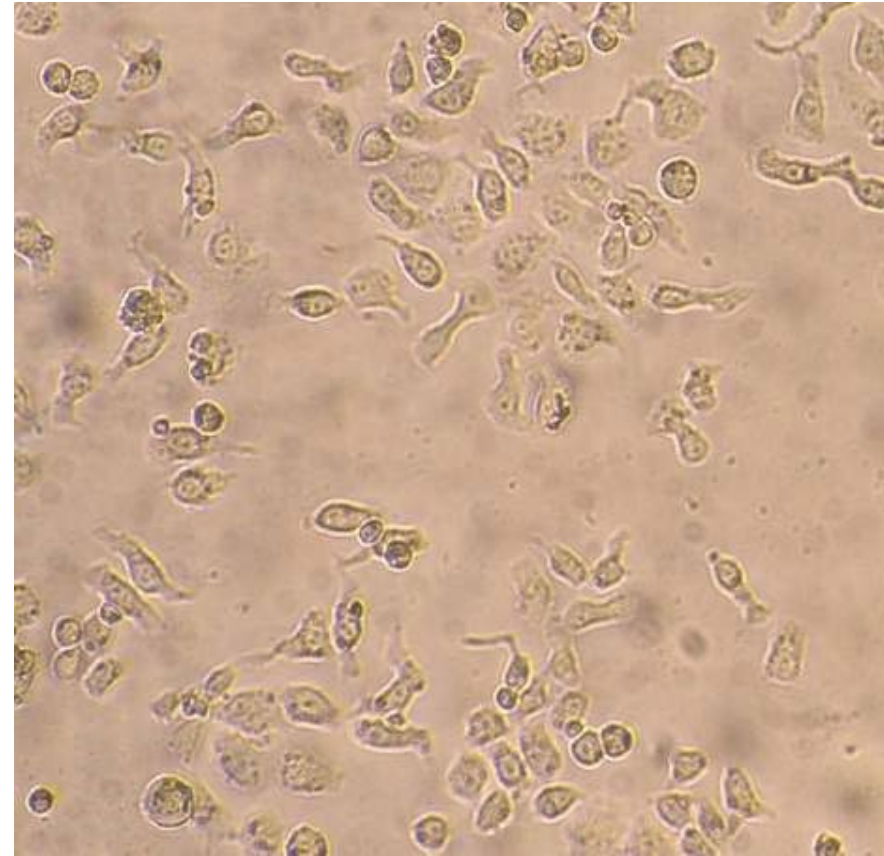
H5N1 virus strain

1. A/Hanoi 30408/2004 (Clade 1)
2. A/Hanoi 31461/2007 (Clade 2.3.4)

In-vitro Virus neutralization test



Anti-influenza IgY treated MDCK cell



Control IgY treated MDCK cell

Conclusion

- Eclogen FL IgY neutralized both clade 1 and clade 2 of H5N1 viruses

Evaluation of Anti-influenza IgY against new Pandemic H1N1/09 virus (New H1N1) by serological tests

Supervised by: Dr. Le thi Quynh Mai

Tested in : Laboratory of Special Viral Pathogen

Department of Virology

National Institute of Hygiene and Epidemiology Vietnam

In-vitro neutralization study to New Pandemic H1N1/09 virus

- **Types of IgY used:**
 1. Control IgY (Non-immunized egg)
 2. Anti-Human influenza H1N1 IgY
 3. Ecogen (Human and Pig H1N1 influenza IgY)

Method:

Microneutralization test

Virus strain used: HN31868 (New Pandemic H1N1 strain)

Microneutralization test results

| IgY sample | Neutralizing antibody titer |
|--|------------------------------------|
| Control IgY | - |
| Anti-Human influenza IgY | - |
| Ecologen (Human and Pig H1N1 influenza IgY) | 80 |

Conclusion

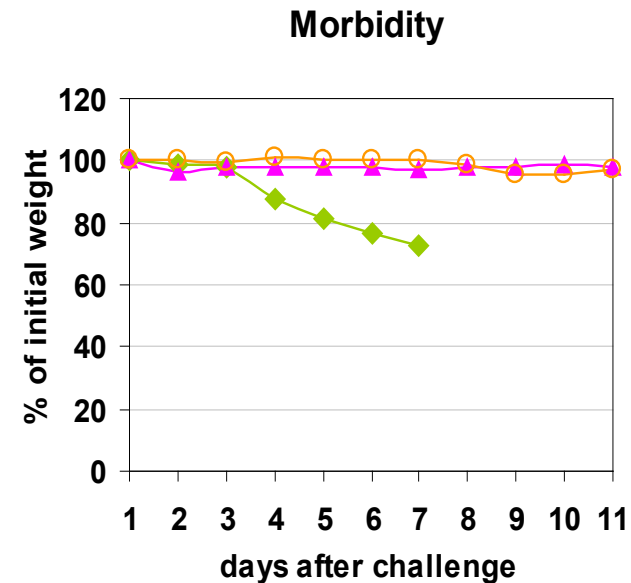
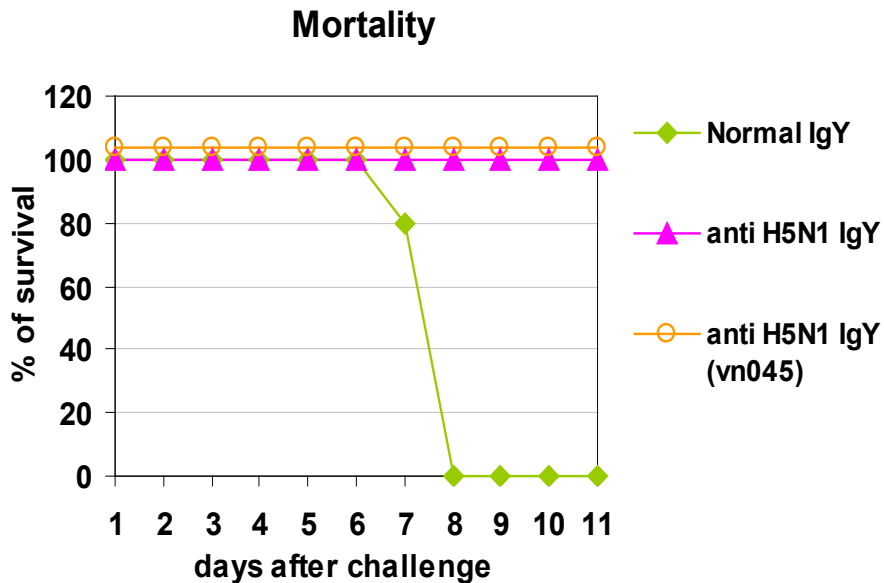
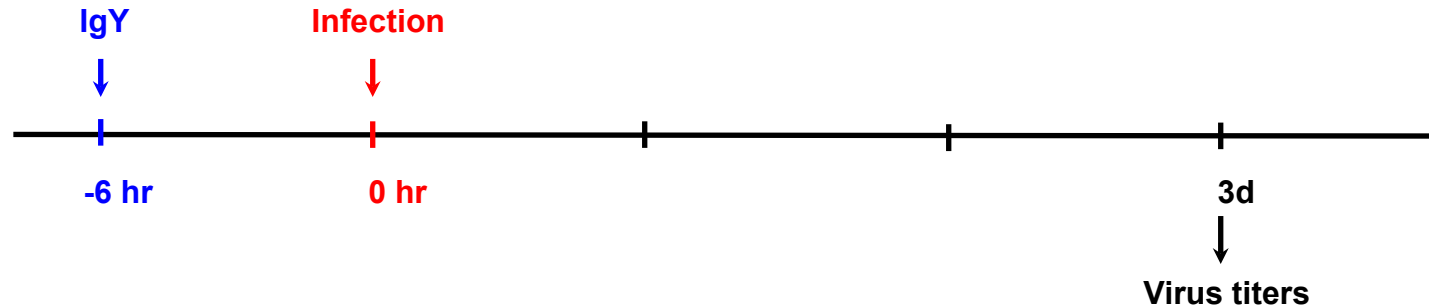
- **Anti-influenza IgY filter not only catches the virus but also neutralized the virus up to 99.99%.**
- **Ecologen neutralized H5N1 influenza virus (Clade 1 & 2)**
- **Ecologen neutralized new pandemic H1N1 influenza.**
- **Ecologen showed broad spectrum neutralization efficacy against H1N1, H3N2, and B type, the chicken influenza virus, and the new pandemic H1N1 influenza virus.**

Effect of Anti-influenza IgY in mouse model

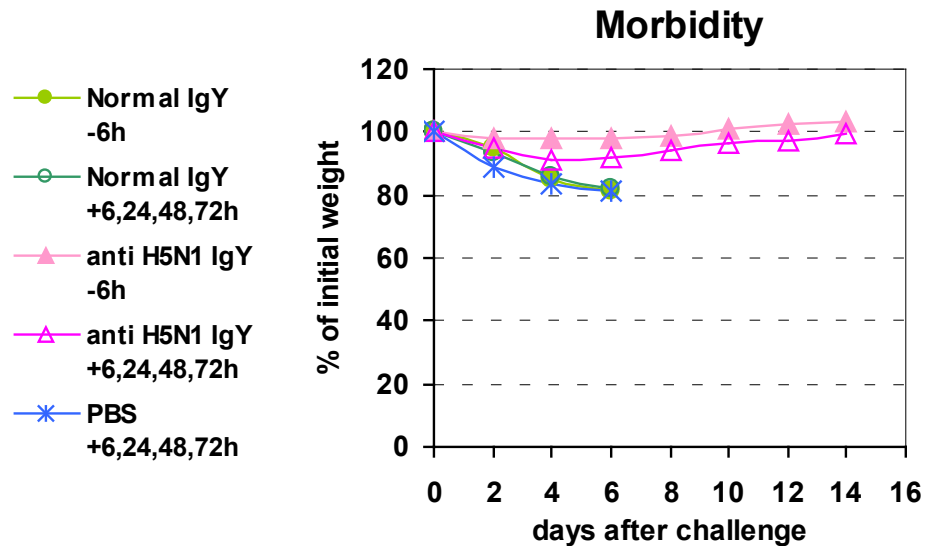
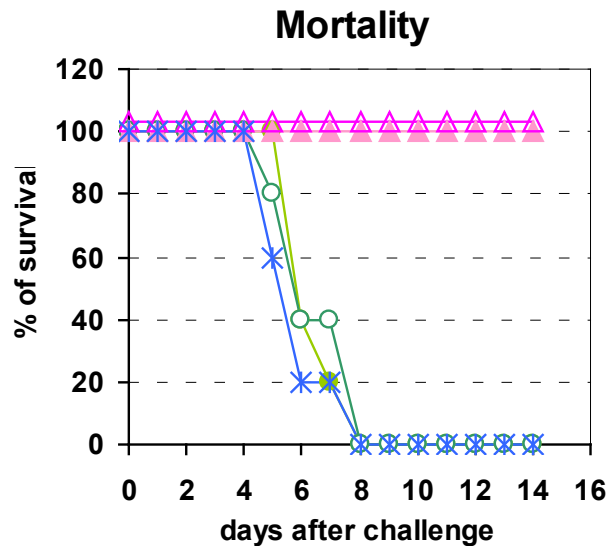
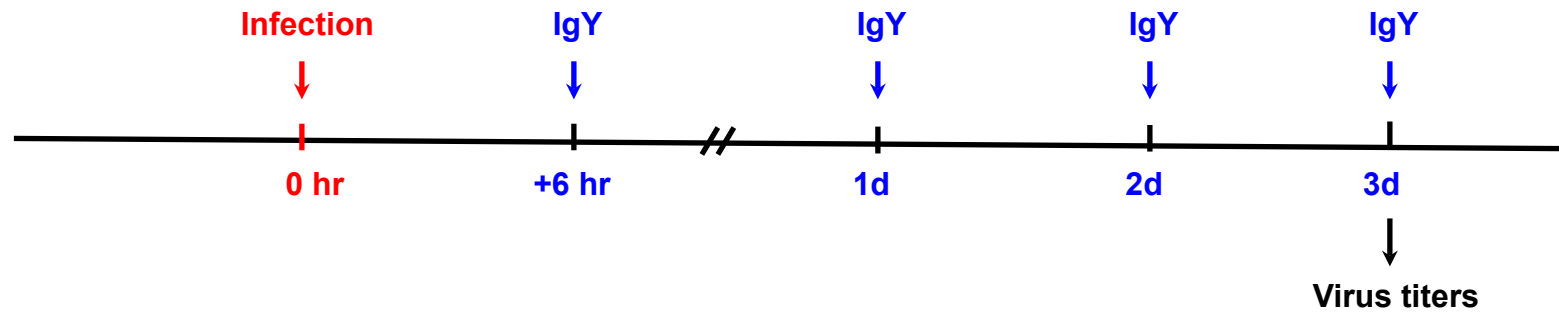
By Dr. Nguyen Huu Huan

International vaccine institute, Seoul

Trial 1: **Single pre-infection** intranasal administration protected mice from challenge with HPAIV H5N1 (A/Vietnam/1203/2004)

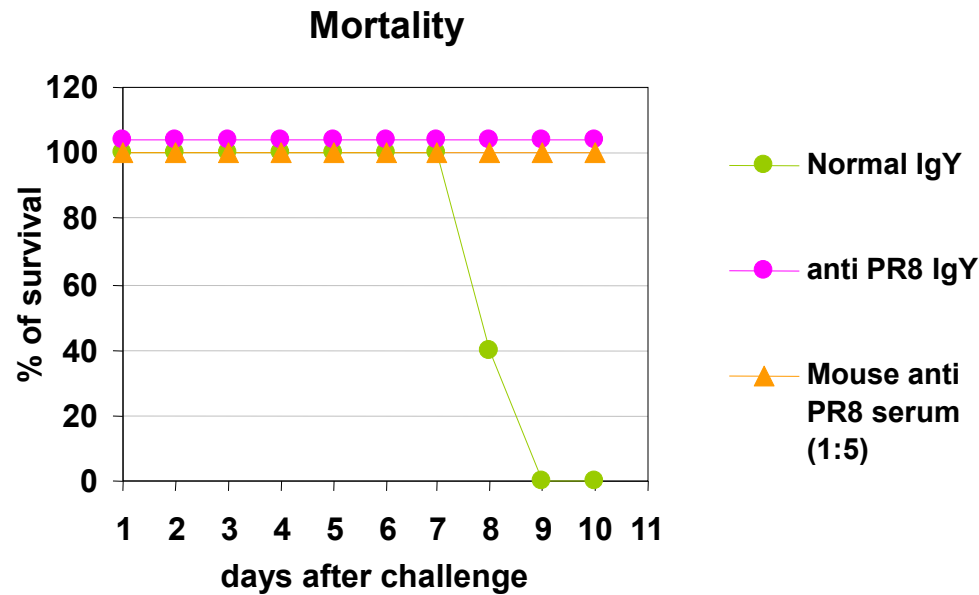


Trial 2: Multiple post-infection intranasal treatment



Trial 3: H1N1-specific IgY protected mice from challenge with H1N1 (A/PR8/34)

Single pre-infection treatment



Multiple post-infection treatments



Discussion

- Mixed influenza antigens used for mass vaccination of poultry offer an enormous source of valuable, affordable biological material for prevention and protection against potential H5N1 pandemic influenza.
- The approach could also be used to control seasonal influenza.

VISIONS

- Stockpile of H5N1-IgY for potential H5N1 pandemic.
- IgY for seasonal flu:
 - IgY development and production for specific virus targets is faster than for human vaccines
 - The prevention and treatment by IgY could have immediate effect
 - Suitable for immunocompromised individuals and/or elderly.