



# Influenza: virus evolution & vaccine strategy

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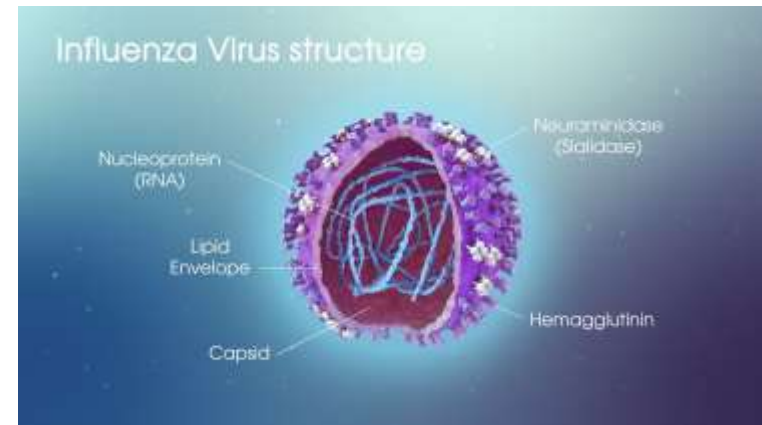
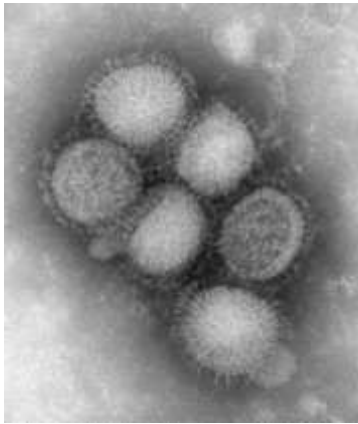
# What is Influenza?

- A contagious respiratory illness caused by **Influenza A or B viruses**.
- Symptoms: fever, chills, cough, headache, fatigue, sore throat and sneezing.
- Barring complications, Influenza usually last less than two weeks<sup>[1]</sup>.



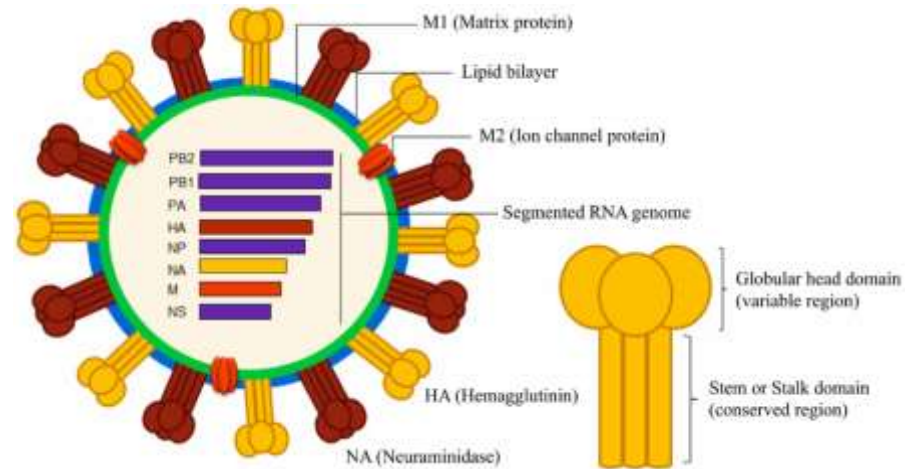
# Influenza virus

- Influenza viruses belong to the *Orthomyxoviridae* family, divided in types A, B, C, and D.  
Influenza A virus (IAV) has raised public health concern and been intensively studied.
- Enveloped, negative-sense, single-stranded RNA virus.



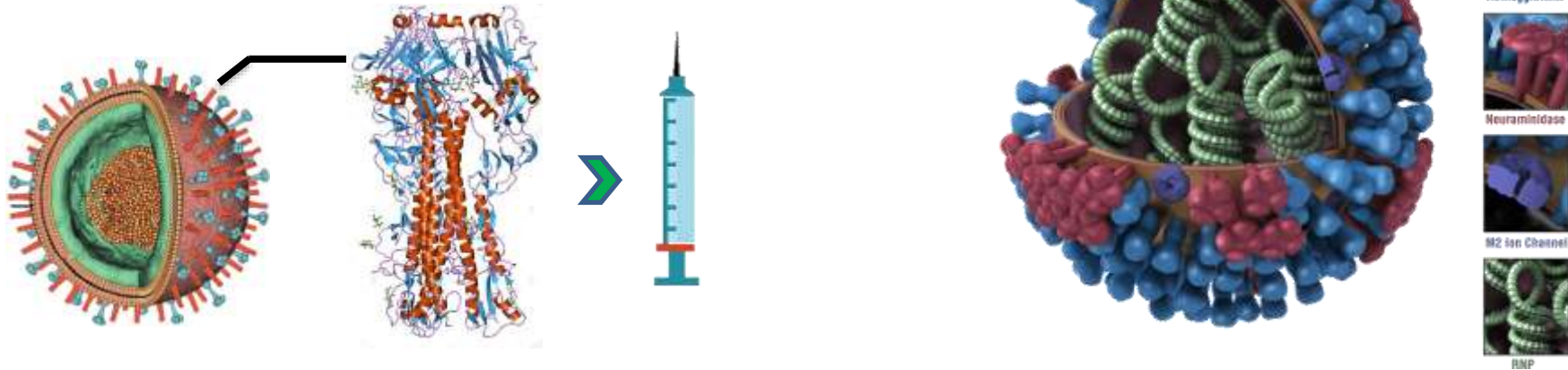
# Etiology

- Viral ribonucleoprotein (vRNP) includes Nucleoprotein (NP) and trimeric polymerase (PB1, PB2, and PA), responsible for transcription, replication and assembly<sup>[3]</sup>.
- Matrix protein (M) includes M1- major component of virion, and M2- integral membrane protein, ion channel.
- Non-structural protein (NS) includes NS1 and NS2, responsible for RNA transport, translation and splicing.



# Etiology

- Hemagglutinin (HA) and Neuraminidase (NA) are viral surface glycoproteins, responsible for receptor binding, virus entry, and virion release.
- HA & NA determine antigenicity and immunogenicity of IAV.
- The development of vaccines mainly focuses on HA & NA<sup>[4]</sup>.



# Influenza pandemics

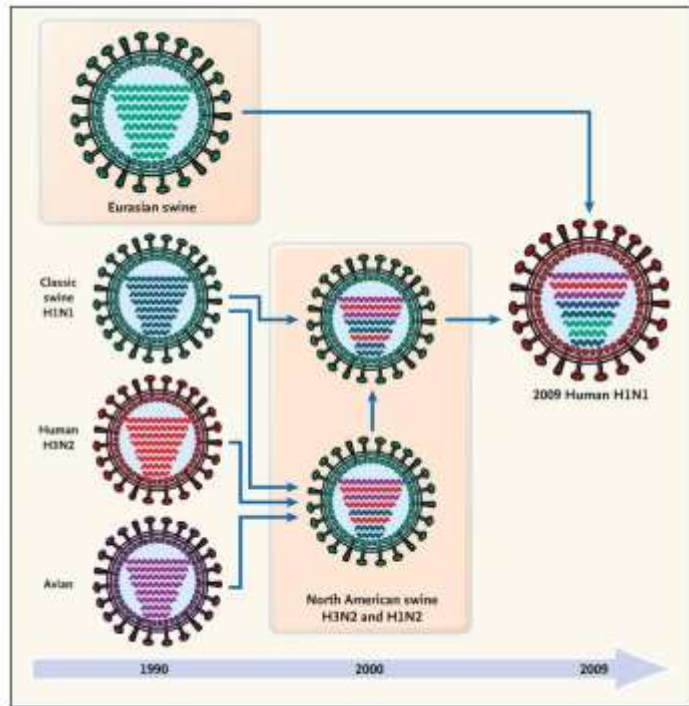


(estimated, in US.)

Year	Viral Subtype	Nickname	Deaths
1918-1919	H1N1	“Spanish” flu	675,000
1957	H2N2	“Asian” flu	70,000
1968	H3N2	“Hong Kong” flu	30,000
2009	H1N1	“Swine” flu	12,000

- 1<sup>st</sup>, 1918, H1N1 subtype.
- 2<sup>nd</sup>, 1957, H2N2 subtype.
- 3<sup>rd</sup>, 1968, H3N2 subtype.
- 4<sup>th</sup>, 2009, H1N1 subtype.
- Estimated by WHO, 1 billion flu-like cases, 3-5 million severe cases, and 0.65 million death cases worldwide, annually<sup>[6]</sup>.

# Viral evolution of pdm09



- Genomic analysis of pdm09 indicates close relationship to swine, human and avian reassortants.
- pdm09 is derived from a triple-reassortant event.
- The host, swine, functioned as a “mixing vessel”.

Figure 1. History of Reassortment Events in the Evolution of the 2009 Influenza A (H1N1) Virus.



# Viral evolution of Novel H7N9

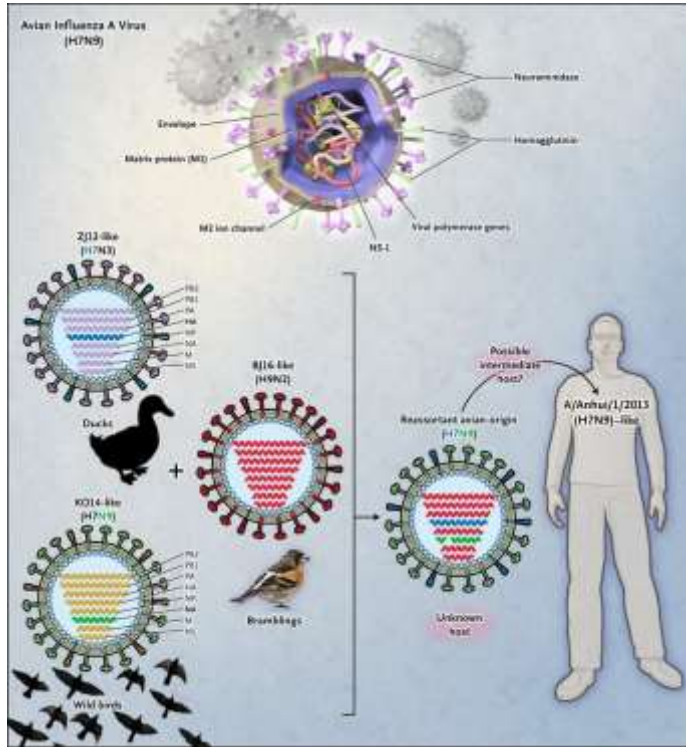


Figure 2. Hypothetical Host and Lineage Origins of the Gene Segments of the Novel Reassortant Human Influenza A (H7N9) Viruses.

- First reported case of H7 subtype cross-species transmission to human in Asia.
- A novel reassortant avian-origin influenza A (H7N9) virus was isolated and identified.
- Genomic analysis of novel H7N9 indicates all 6 internal genes from avian influenza A (H9N2) viruses, while HA & NA from another avian donors.

# Local situation of Influenza Activity in HK

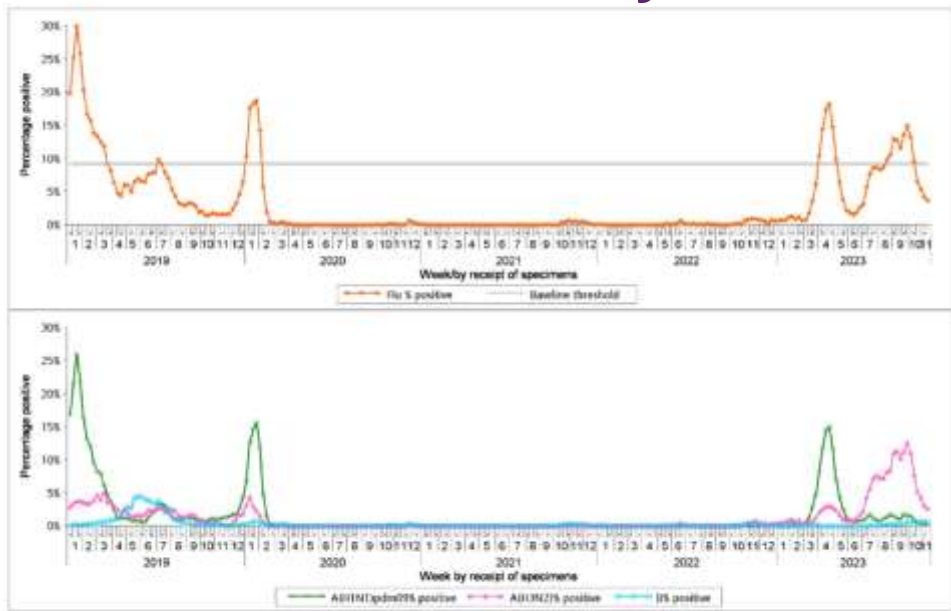
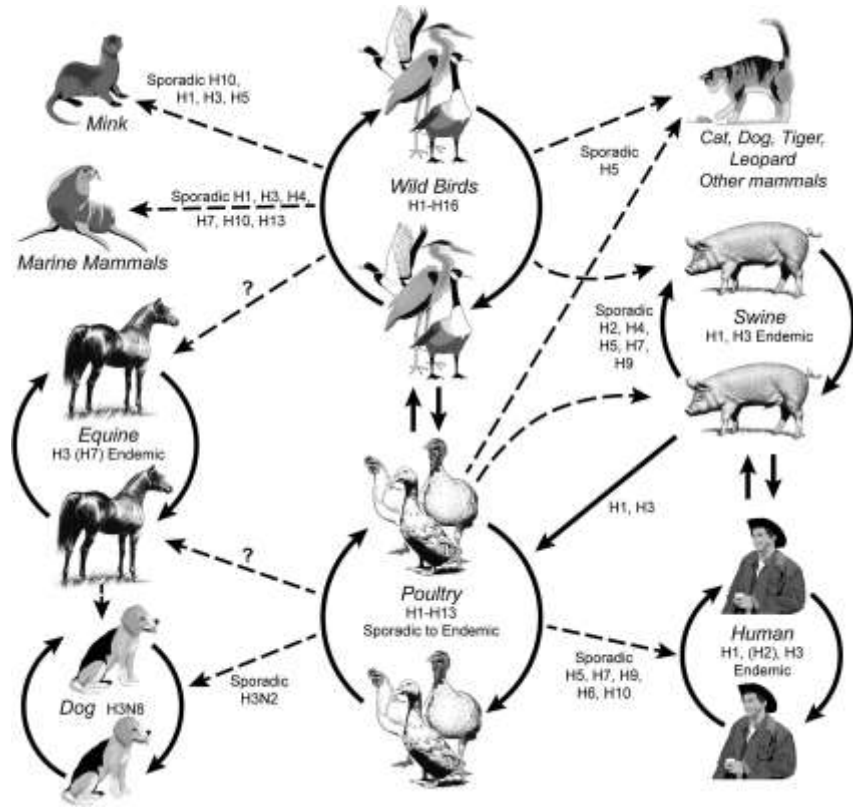


Figure 2.2 Percentage of respiratory specimens tested positive for influenza viruses, 2019-23 (upper: overall positive percentage, lower: positive percentage by subtypes)

- In HK, Week 45 (5-11 Nov), a total of 7,228 flu-like samples was collected.
- 267 (3.69%) positive for Influenza, including 23 (9%) H1, 194 (74%) H3 and 45(17%) B<sup>[7]</sup>.

# Influenza subtypes & hosts

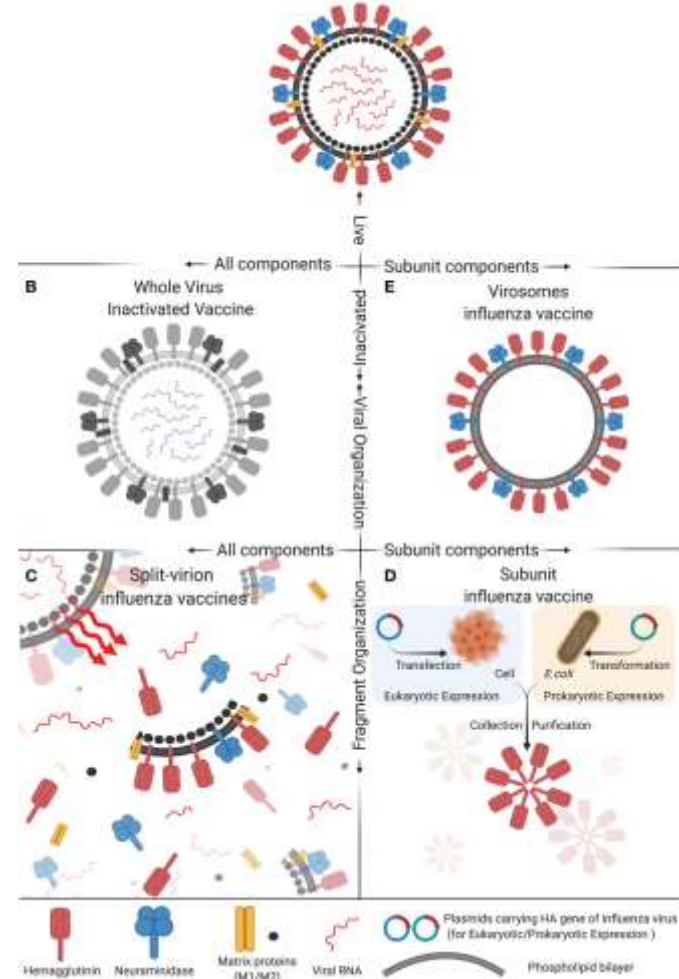


- Avian—  $\alpha$ -2, 3 Sialic Acid receptor
  - Human—  $\alpha$ -2, 6 SA receptor
  - Swine—  $\alpha$ -2, 3 +  $\alpha$ -2, 6 SA receptor
- ↓  
“mixing vessel”

# Vaccines



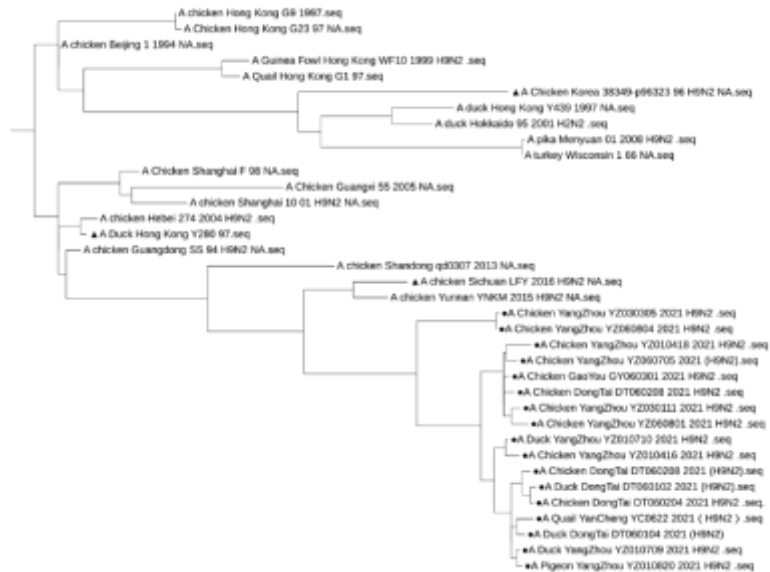
- The inoculation of vaccines is the most important prophylactic method.
- Inactivated vaccine is the most commonly used flu vaccine, traced back to 1940s.
- Others include live attenuated, VLPs, subunit influenza vaccines.



# Inactivated vaccine-H9N2

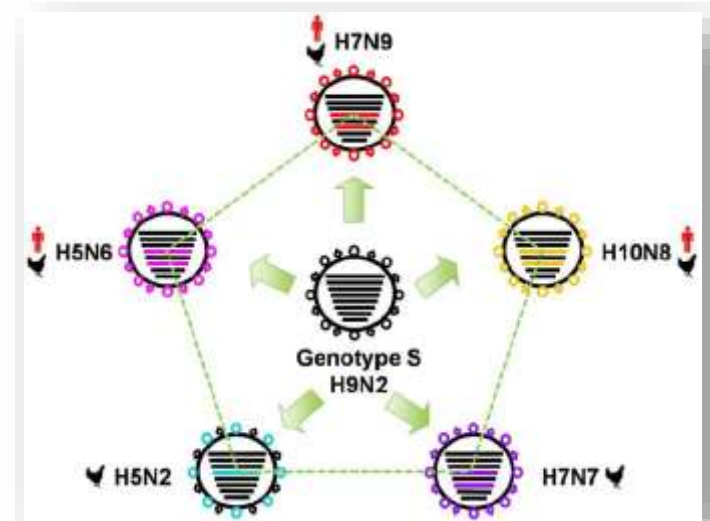
- H9N2 AIVs have 2 distinct sub-lineage with major antigenic difference.
- Develop an inactivated H9N2 vaccine with cross-lineage protection.

Tree scale: 0.01



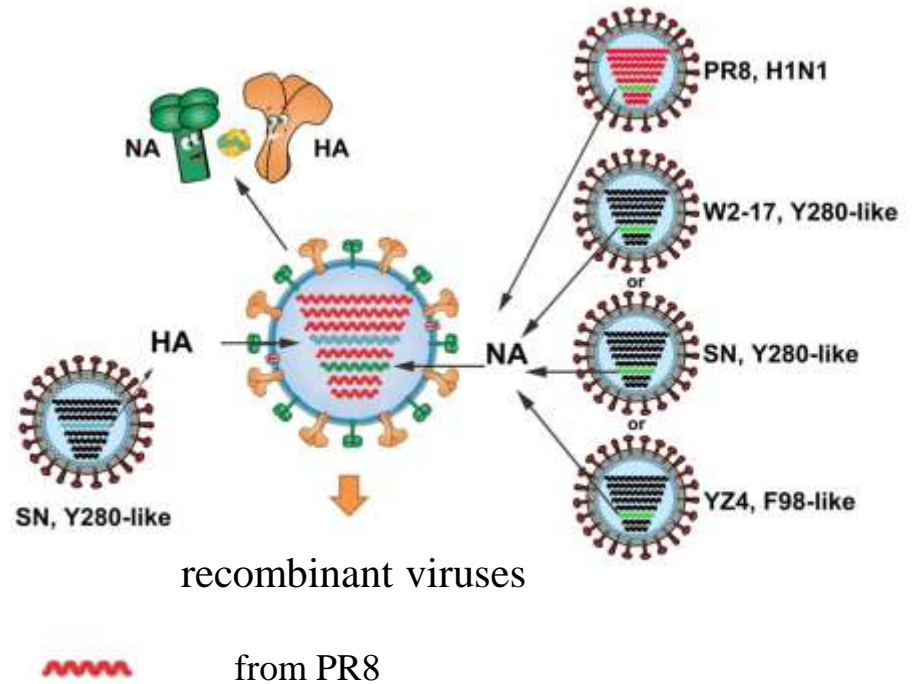
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Y290 Like



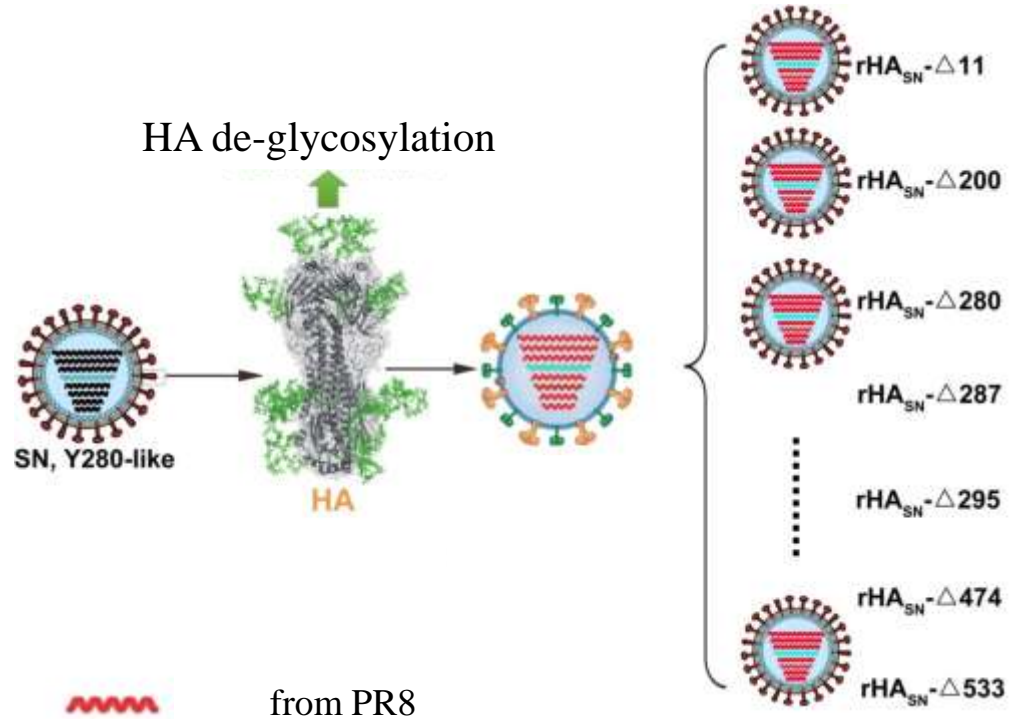
# Inactivated vaccine-H9N2

- Compatibility of HA & NA from different strains.



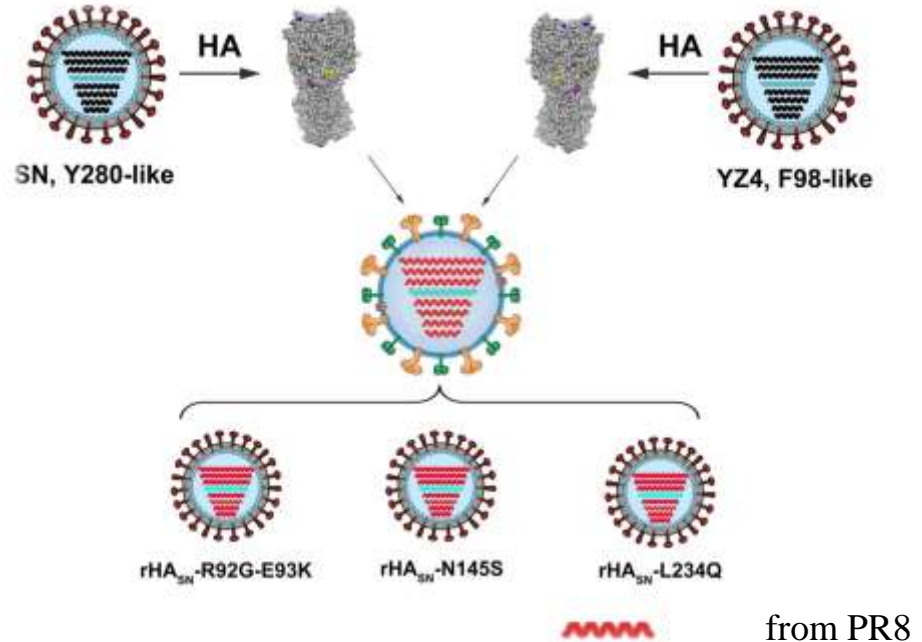
# Inactivated vaccine-H9N2

- Compatibility of HA & NA from different strains.
- The effect of de-glycosylation of HA.



# Inactivated vaccine-H9N2

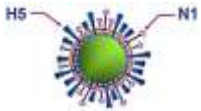
- Compatibility of HA & NA from different strains.
- The effect of de-glycosylation of HA.
- The substitution of protective antigenic epitopes in HA.
- Select optimal candidates by neutralizing (Nt) antibody titer and survival rate of virus challenge.





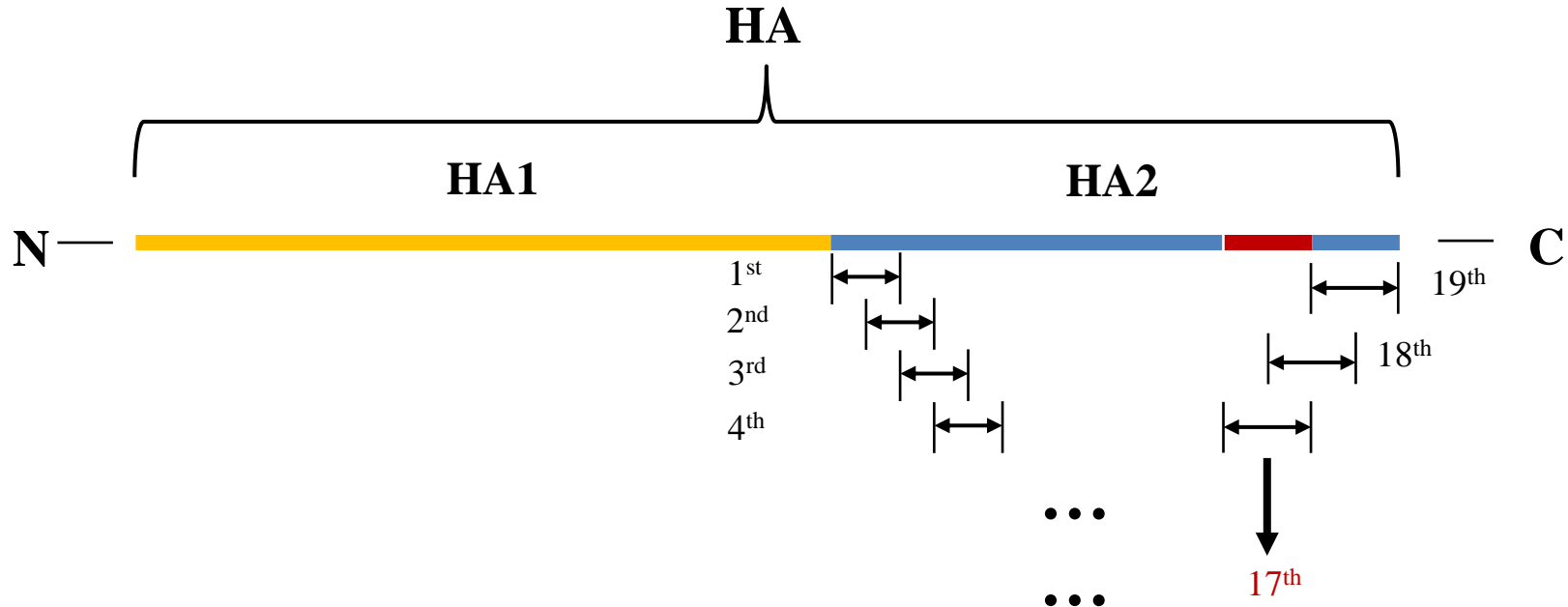
# Inactivated vaccine-H5N1

- Inoculation of inactivated vaccine can introduce Nt Abs.
- Serum can't be distinguished from whether infected or immunized.
- Develop a marked vaccine to Differentiate infected from vaccinated animals (DIVA).



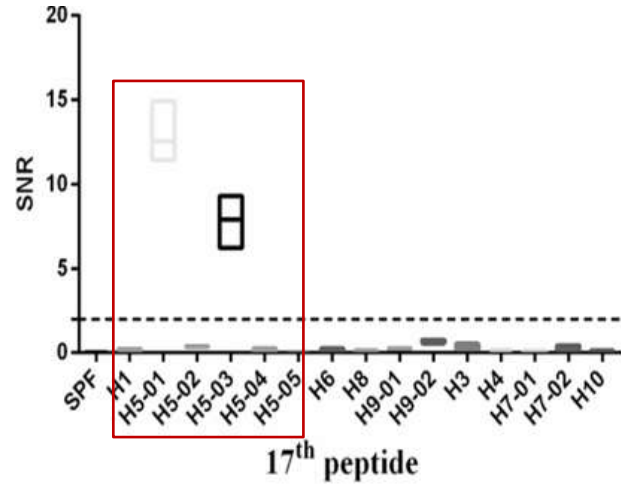
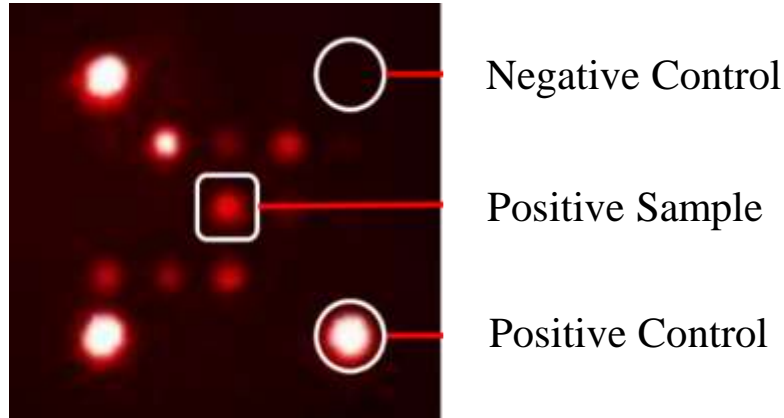
# Inactivated vaccine-H5N1

- IAV's HA consists of HA1 and HA2.
- HA2 is highly conservative among subtypes, making it a possible marker for DIVA vaccines.



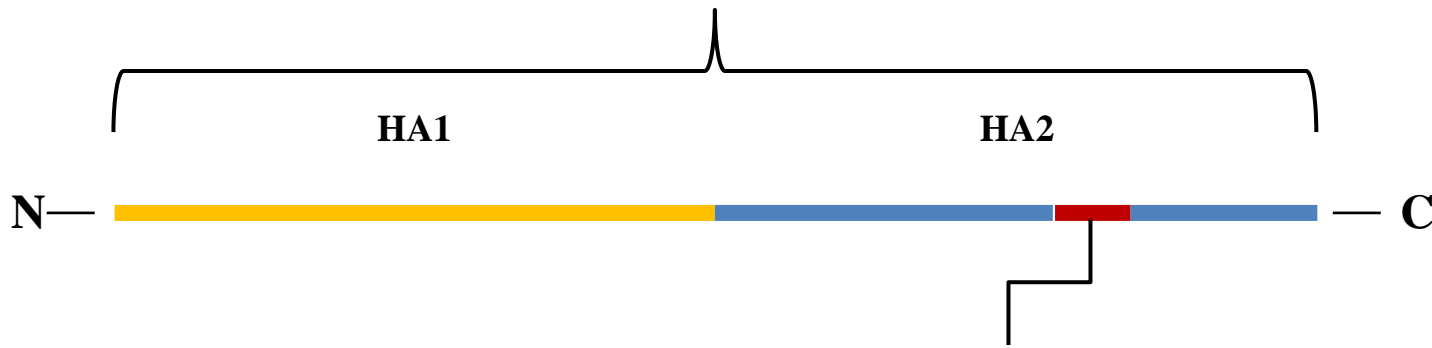
# Inactivated vaccine-H5N1

- H5 subtype AIV HA2 specific epitope H5-17<sup>th</sup>-peptide has been identified by Microarray chips.



# Inactivated vaccine-H5N1

- Epitopes have been designed and modified by Overlap-PCR.
- Vaccine candidates have been developed based on chimeric HA.



Parental strain: DTC

H5: QYSEEARLKREEISGVKLES

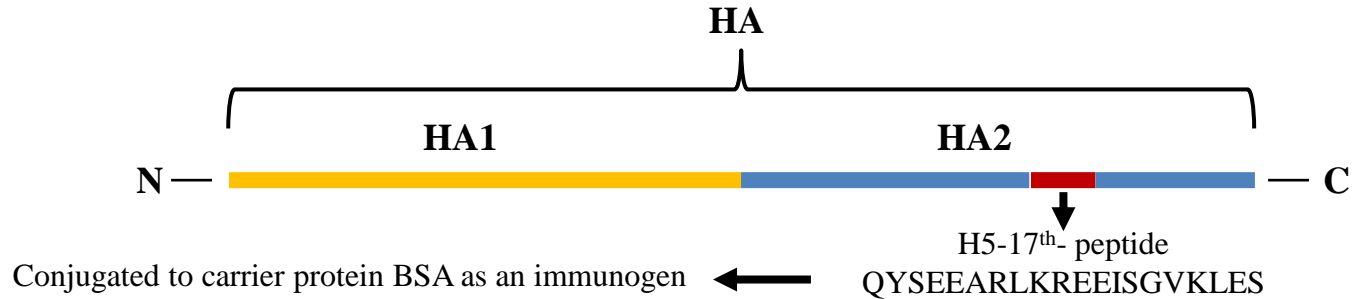
Candidate: DTC-Lo-171

H1: KYSEESKLNREEIDGVKLES

Candidate: DTC-Lo-173

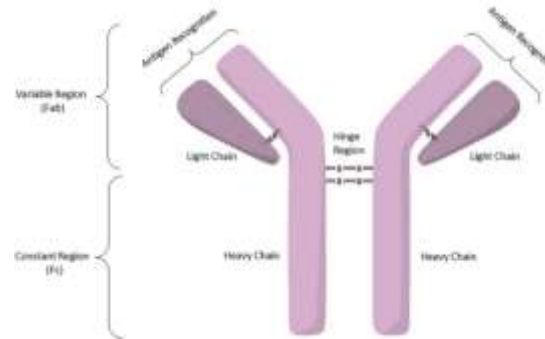
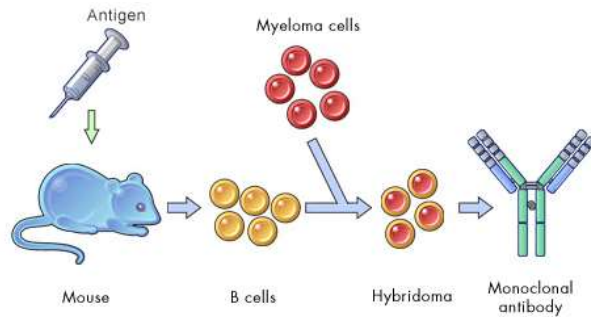
H3: IYRDEALNNRFQIKGVELKS

# Inactivated vaccine-H5N1



Routine immunization on BALB/c

Prepare McAbs from Hybridoma

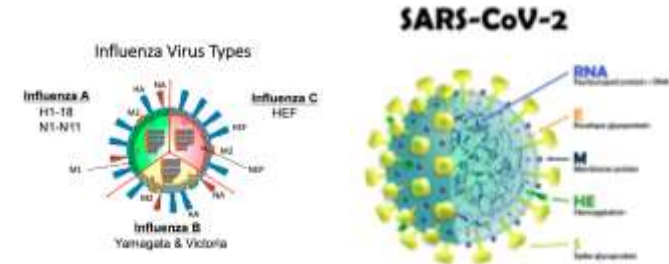


# Inactivated vaccine-H5N1

- A Competitive ELISA method has been established based on McAb.
- Specificity and broad-spectrum reaction ability of established Competitive ELISA method has been further evaluated.
- Optimal candidates have been selected by neutralizing (Nt) antibody titer and survival rate of virus challenge.
- DIVA characteristics of H5 marked vaccine candidates have been further evaluated.

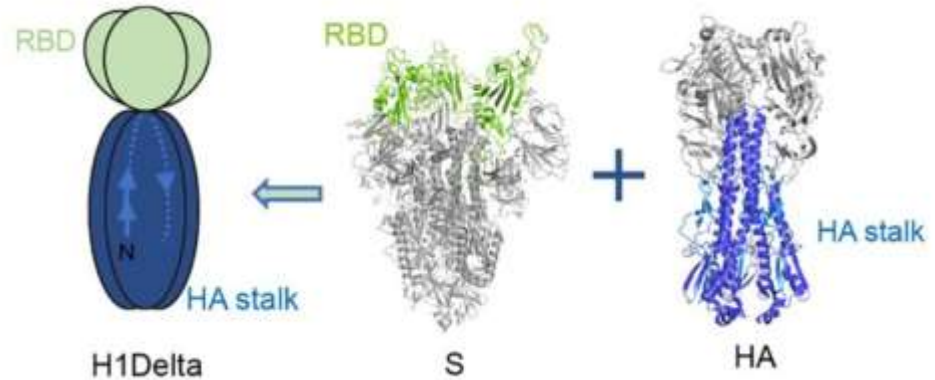
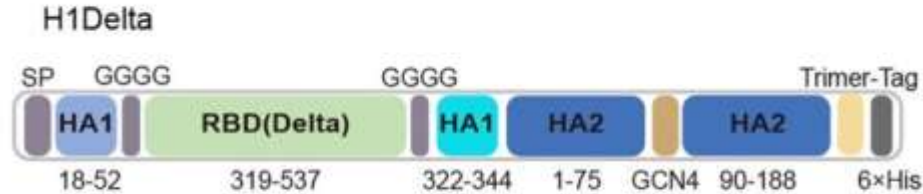
# Novel vaccine

- Subunit vaccine: Rational design of an influenza-COVID-19 chimeric protective vaccine with HA-stalk and S-RBD. By George Fu Gao.
- COVID-19 & Influenza has raised great public health concern.
- Their pathogens: SARS-CoV-2 & IAV are both enveloped RNA virus.
- The Spike (S) protein & HA protein are both major targets for Abs.
- Two-in-One Vaccine strategy: COVID-19 & Influenza.



# Novel vaccine

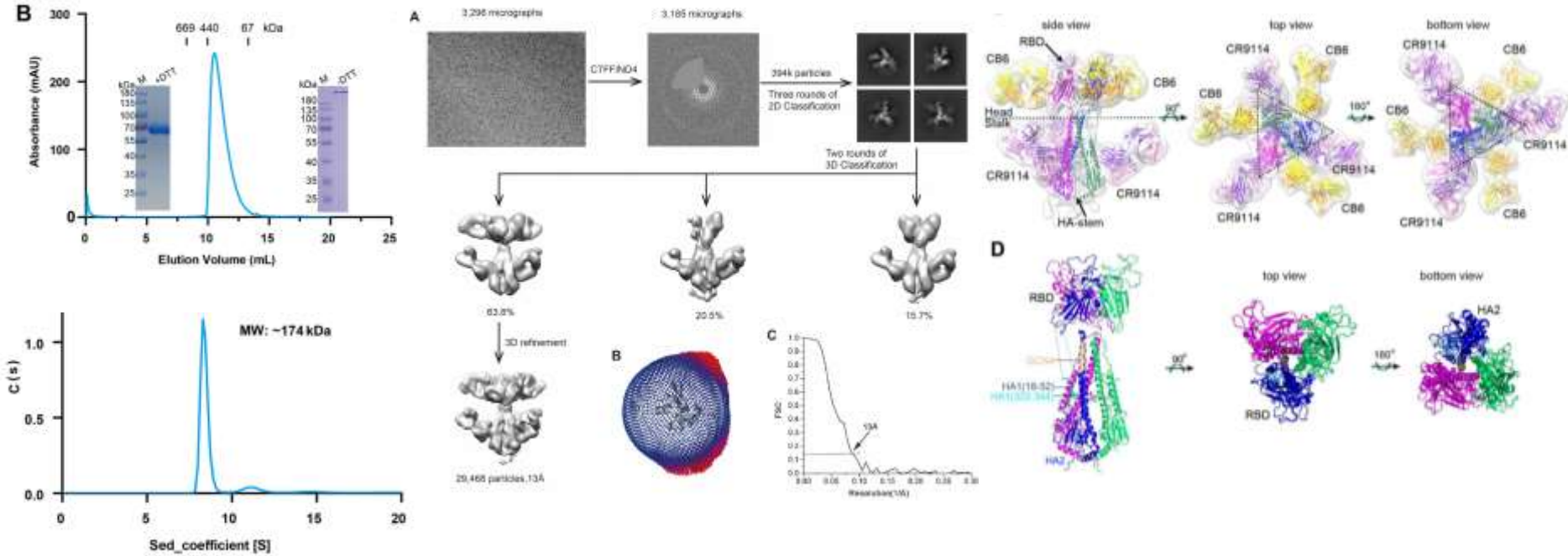
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- SP: Signal Peptide; GGGG: Linker.
- GCN4 & Trimer-Tag is for trimer formation; His-Tag is for purification.
- HA1(18-52, N-terminal)—Delta-RBD—HA1(322-344, C-terminal)—HA2—Tag.

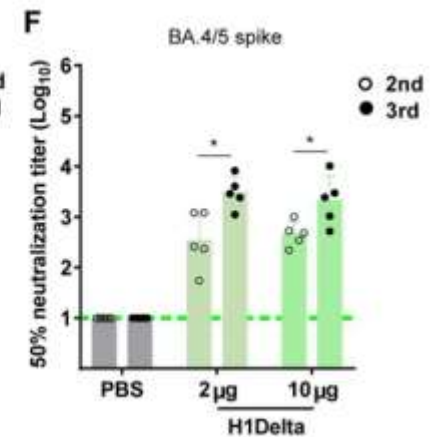
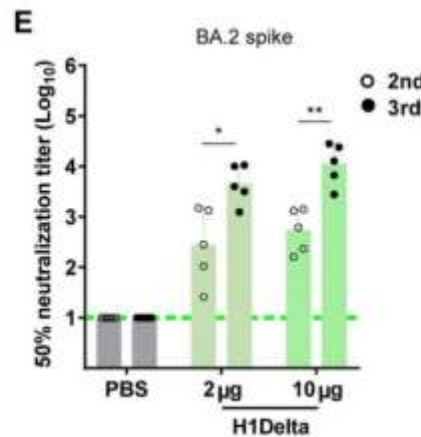
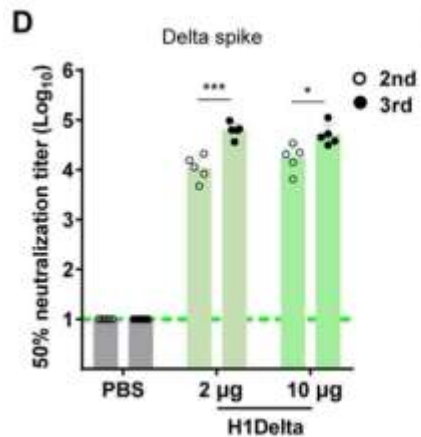
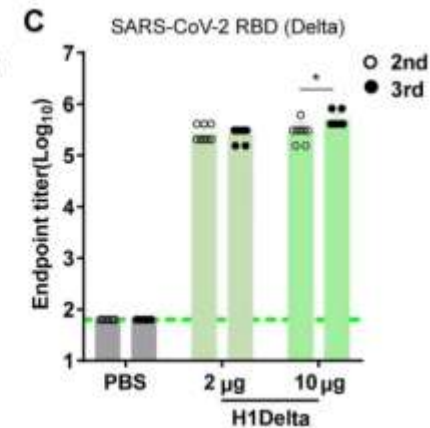
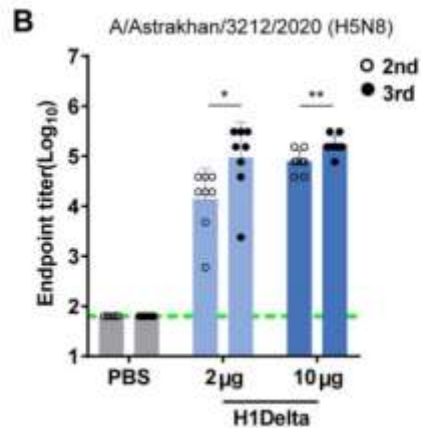
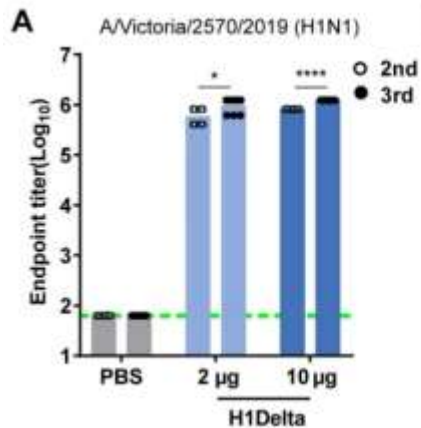
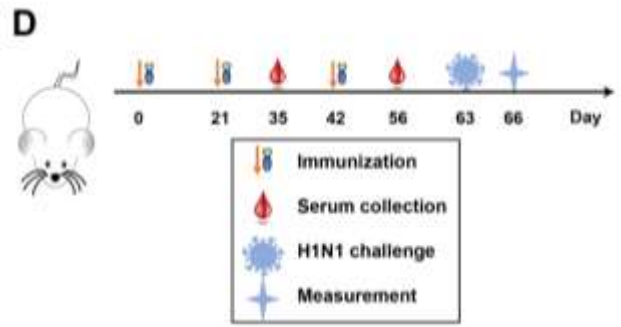


# Novel vaccine



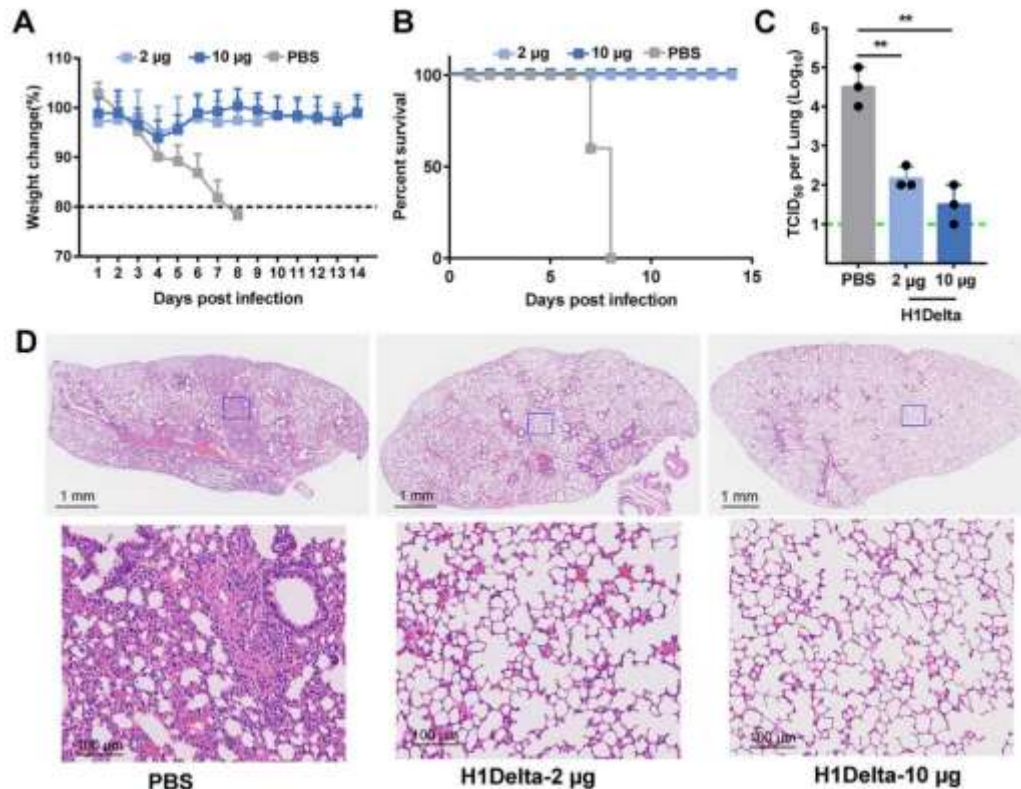
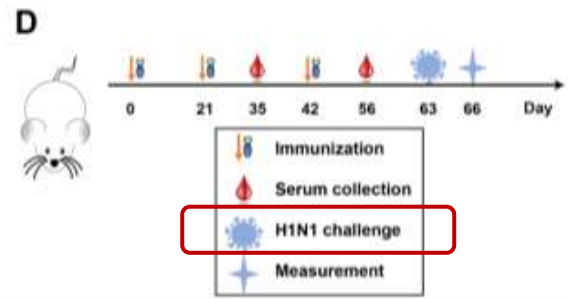
- Confirm the size and trimer formation of H1Delta.

# Novel vaccine



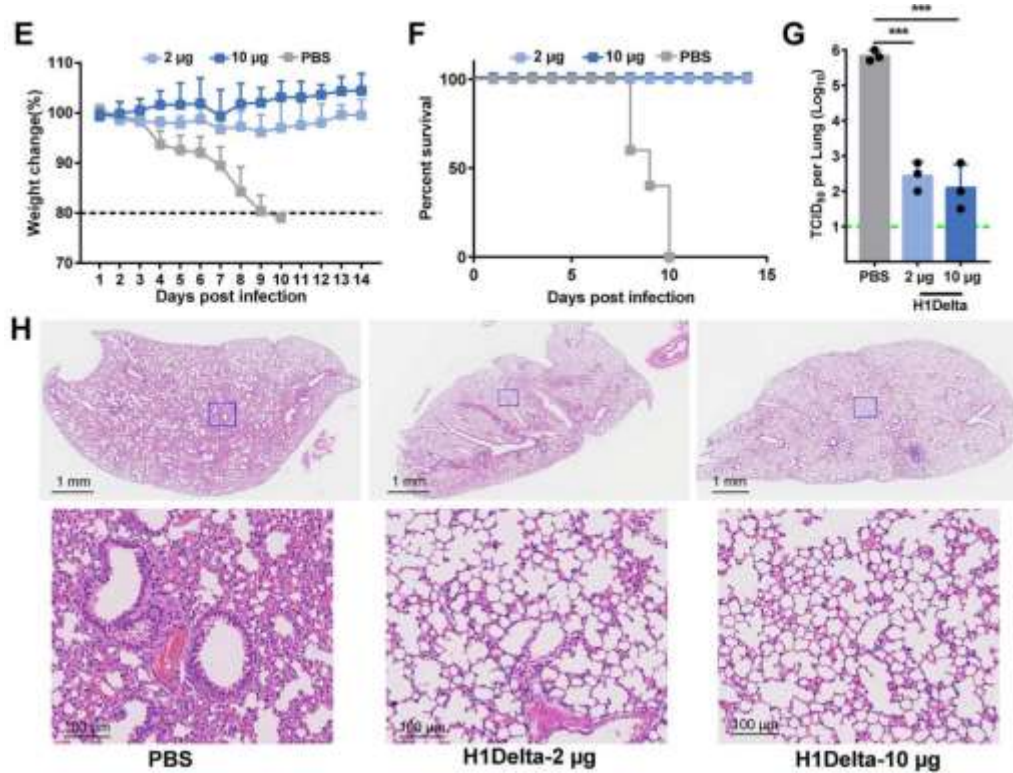
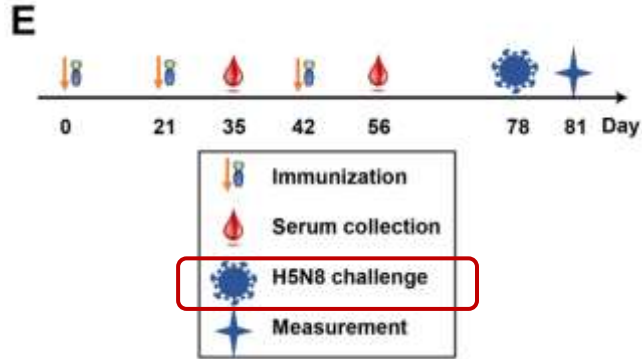
- Immunogenicity of the H1Delta vaccine in mice.

# Novel vaccine



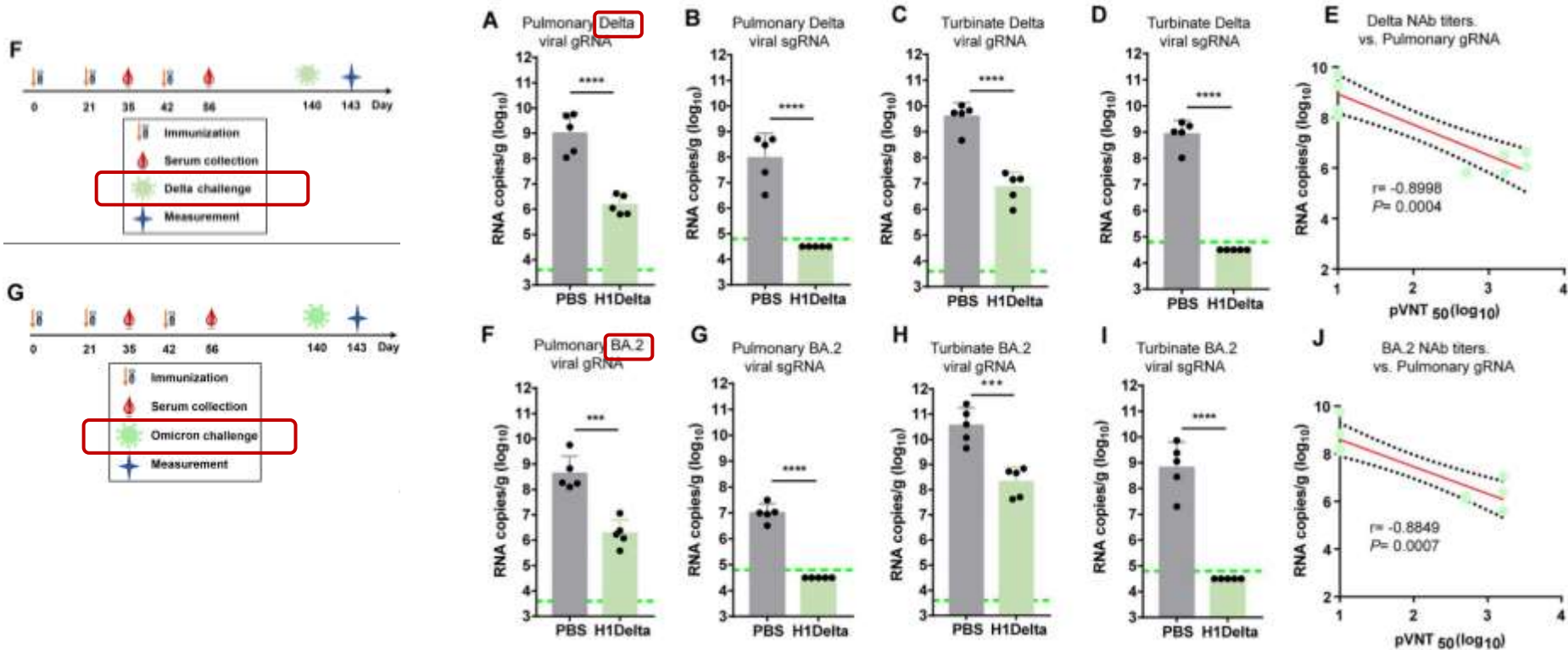
- Immune efficacy of the H1Delta vaccine against H1N1 challenge in mice.

# Novel vaccine

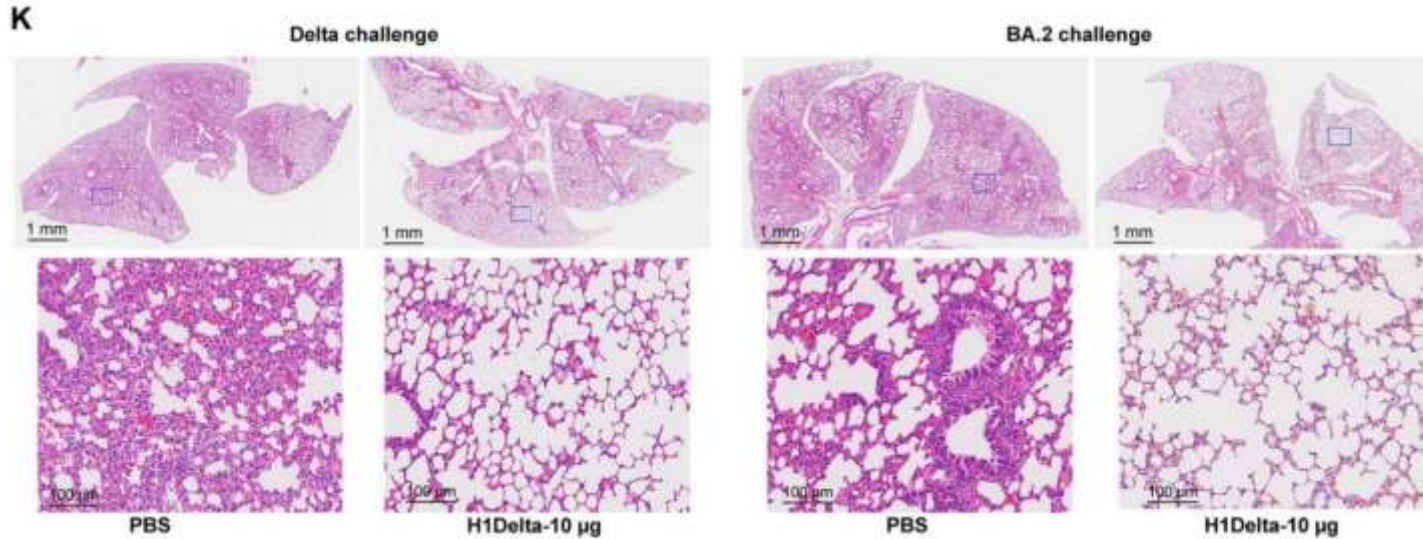


- Immune efficacy of the H1Delta vaccine against H5N8 challenge in mice.

# Novel vaccine



# Novel vaccine



- Immune efficacy of the H1Delta vaccine against Delta+Omicron challenge in mice.

# Novel vaccine

- Comprehensively, the H1Delta has a correct formation, induces good binding and Nt Abs, 100% prevents lethal challenge of H1N1 and H5N8 in 2  $\mu\text{g}$ /10  $\mu\text{g}$  dosage in mice.
- H1Delta extremely significantly decreased viral copies in Delta+Omicron challenge. Without pathological changes in lungs.
- Other studies revealed that RBD Fc dimer, tandem-repeat dimer, HR (heptad-repeat sequence)-induced trimer could induce high titers of Nt Abs<sup>[13,14]</sup>.
- Using polymer formation of antigen as vaccine candidates is better than monomer.

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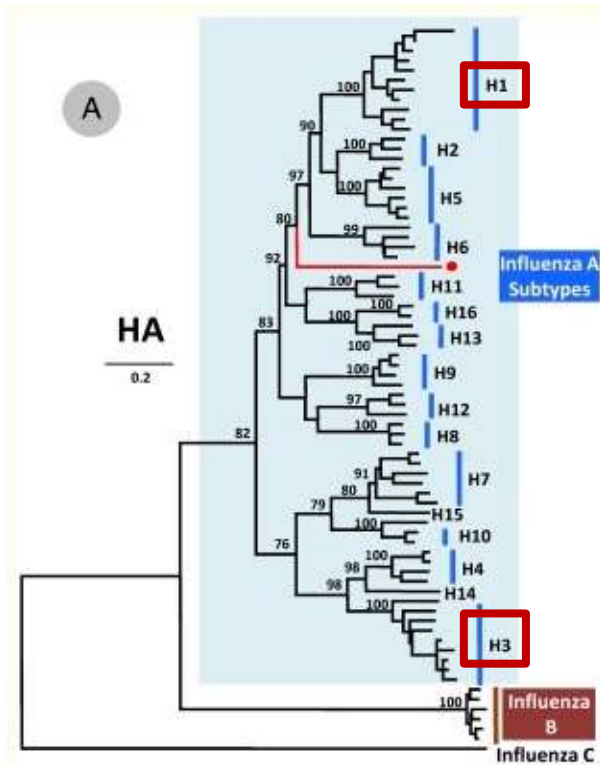
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Thank you

# Influenza phylogenetic tree



- Antigenic drift, results in new clades and lineages.
- Antigenic shift, results in recombination events which lead to new subtypes.

# H5 subtype AIV evolution

Tree scale: 0.01

