Advances in the development of influenza virus vaccines

Interdisciplinary Technical Journal Club:
special series on Laboratory Animal Science

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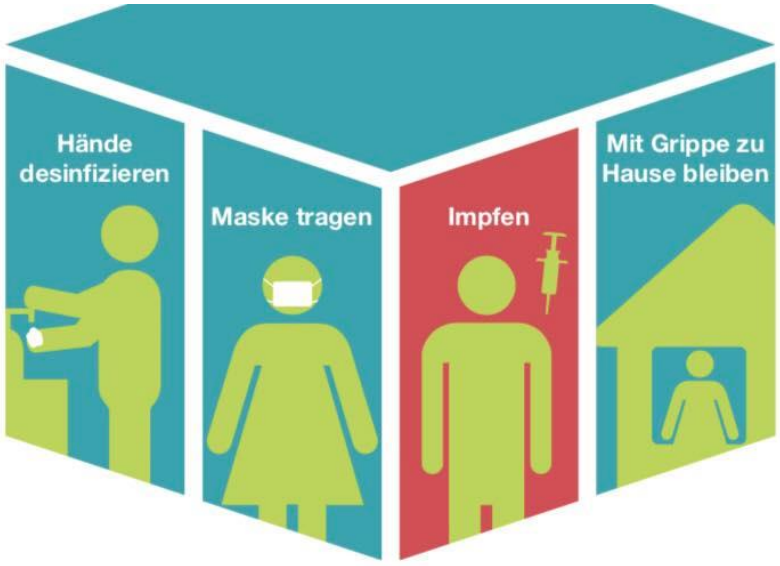
Influenza affects us all



USZ influenza campaign 2017/2018:

Fluarix® Tetra (egg based split vaccine):

- A/Michigan/20015(H1N1) pdm09
- A/Hong Kong/2014 (H3N2)
- B/Brisbane/2008 (Victoria line)
- B/Phuket/2013 (Yamagata-line)

- 5-15% of the human population are infected by the seasonal influenza each year
- Resulting in around 500‘000 death/year worldwide
- <10% of the world population is routinely vaccinated
- Global seasonal influenza virus vaccine production: 500 million doses

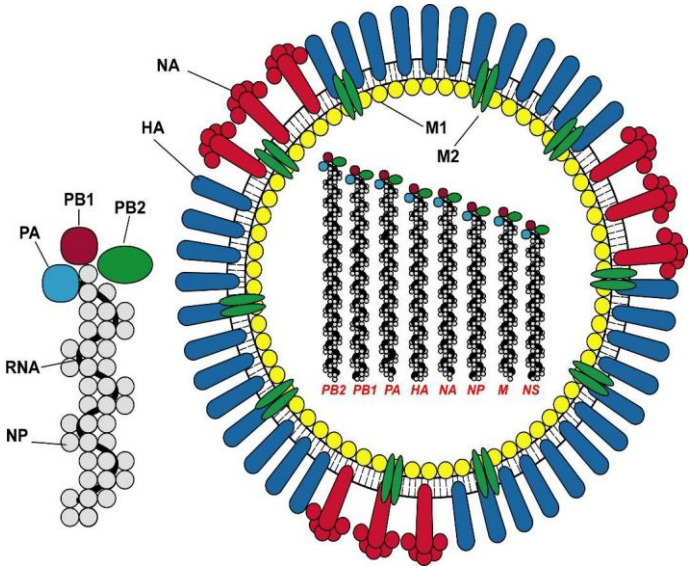
Primary target of the anti-influenza antibody response

Hemagglutinin (HA) = Major antigen

- Homotrimeric viral surface glycoprotein
- Binds to sialic acid on glycan structures of cellular receptors (attachment to cells)
- Mediation of fusion of viral and endosomal membrane (release of the viral genome)

Neuraminidase (NA)

- Homotetrameric viral surface glycoprotein
- Sialidase activity (transport, release)

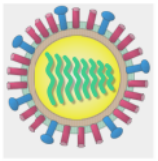


S. Sridhar, K. A. Brokstad and R. J. Cox, Vaccines, 2015

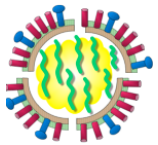
➔ Subtypes of influenza A based on combination of HA (18) and NA subtypes (11)
➔ A virus with circulation in humans: **A/H1N1**, A/H2N2 and **A/H3N2**
➔ Subtypes of influenza B: B/Victoria and B/Yamagata circulating in humans without animal reservoir

Orthomyxoviridae, negative-stranded RNA genome
Three genera are susceptible to infecting humans: A, B, C
Eight segment of viral DANN (A&B) encoding for: PB1, PB2, PA, M1, M2, NS NA, HA and NP

Established influenza vaccines

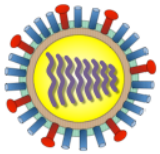


Inactivated: formalin and phenylmercuric nitrate



Split: Detergent and/or ether

Subunit: Split + Neuraminidase and Haemagglutinin + purification and enrichment



Live attenuated influenza vaccines (LAIVs): Temperature-sensitive and cold-adapted / replicate efficiently in the upper respiratory tract / but not in the lower respiratory tract (nasal spray)

Product name	Commercial name	Manufacturer
	Inactivated egg-based vaccines	
Influenza virus vaccine (trivalent)	Afluria	CSL Limited
Influenza virus vaccine (trivalent)	FluLaval	ID Biomedical Corporation of Quebec (a division of GlaxoSmithKline)
Influenza virus vaccine (trivalent)	Fluarix	GlaxoSmithKline Biologicals
Influenza virus vaccine (trivalent)	Fluvirin	Novartis Vaccines and Diagnostics Limited
Influenza virus vaccine (trivalent)	Agriflu	Novartis Vaccines and Diagnostics Limited
Influenza virus vaccine (trivalent)	Fluzone, Fluzone High-Dose, and Fluzone Intradermal	Sanofi Pasteur
Influenza virus vaccine (quadrivalent)	Fluarix Quadrivalent	GlaxoSmithKline Biologicals
Influenza virus vaccine (quadrivalent)	Fluzone Quadrivalent	Sanofi Pasteur
Influenza virus vaccine (quadrivalent)	FluLaval Quadrivalent	ID Biomedical Corporation of Quebec
	Egg-based live attenuated influenza vaccines (LAIV)	
Influenza A (H1N1) 2009 monovalent intranasal	No trade name	MedImmune LLC
Influenza virus vaccine (trivalent) intranasal	FLuMist	MedImmune LLC

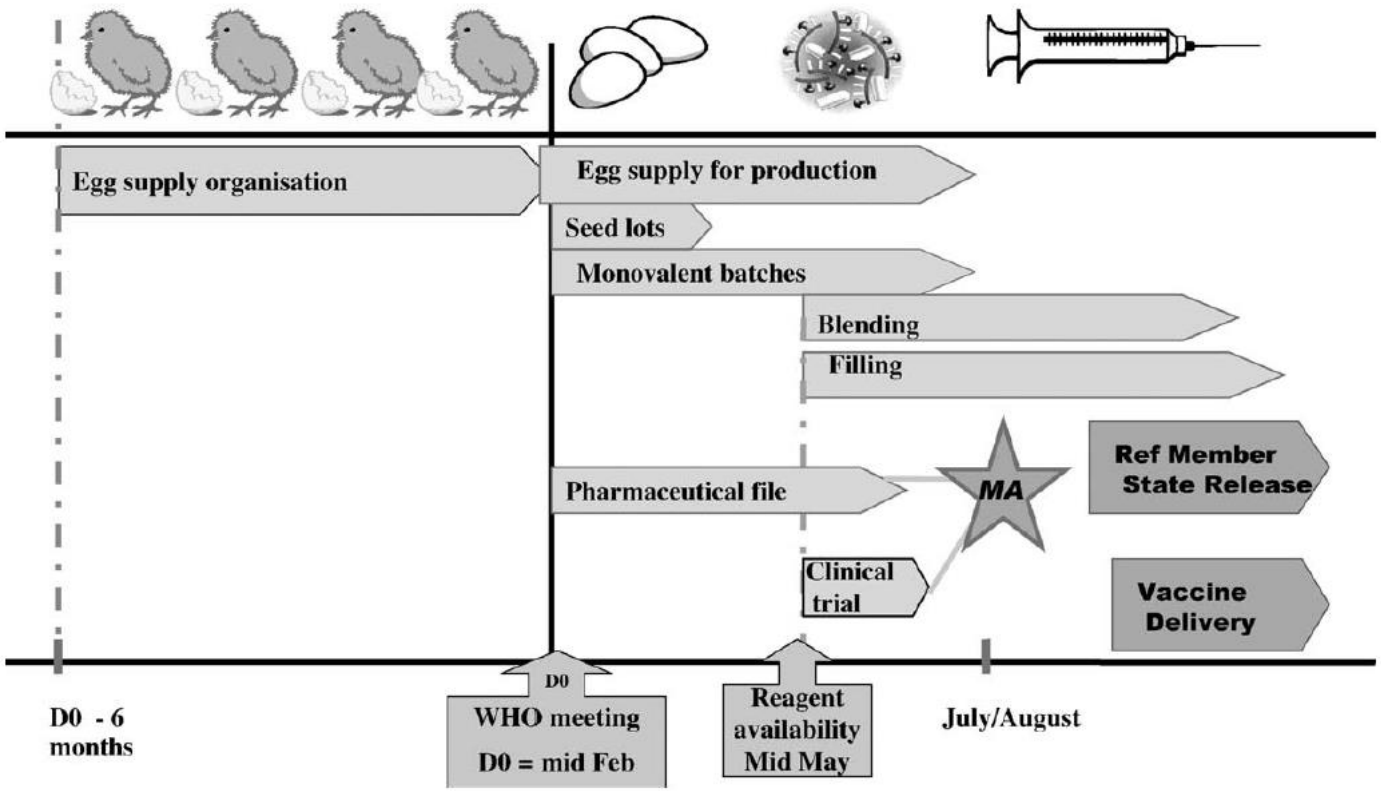
Trivalent: Antigens from three circulating strains: H1 and H3 for influenza A and one strain influenza B

Trivalent inactivated vaccine (TIV): Most common formulation of influenza vaccine, 15 µg of each component

Millan und Kamen, BioMed Reseach International, 2015

Seasonal influenza vaccine production timetable

Goal: Production of safe and effective vacines

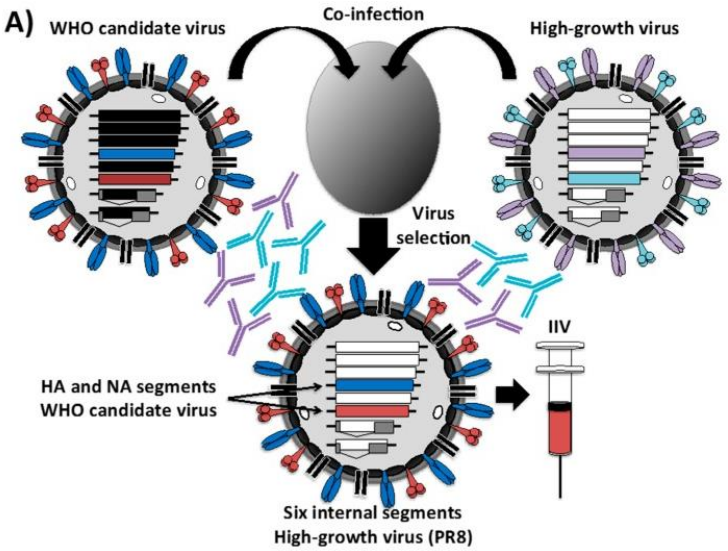


C. Gerdil, Vaccine, 2003

WHO surveillance

Colaborating center for onfluenza reference and research:
London, Atlanta, Melbourne, Tokyo, Memphis and Beijing

Generation of multivalent seeded strain



Nogales, Martinez-Sobrido, Int J Mol Sci 2016

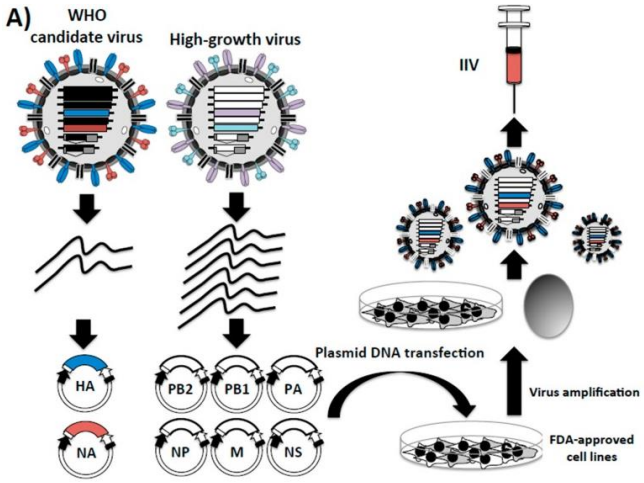
Genetic reassortment:

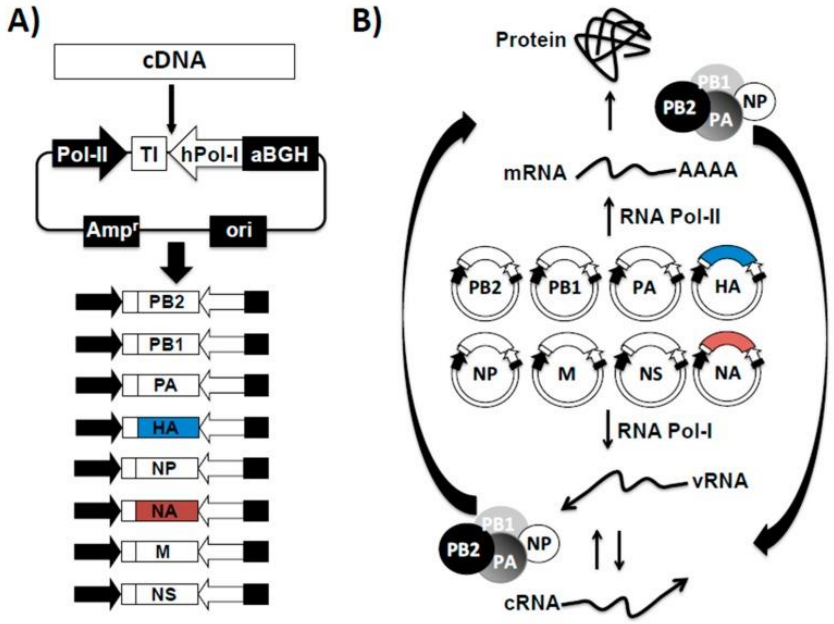
- Seasonal influenza H1N1 and H3N2
- H1N1/A/PR/8/34 strain with a high yield grow in embryonated egg
- Organisation of genome in eight negative sense, single-stranded viral RNA segments (vRNA)
- Co-infection in the allantoic cavity of embryonated eggs
- OR plasmid based **reverse genetic** (co-infection, cell culture)

➔**Generation of seed precursor virus: must be generated in eggs (regulations)**

Problematic: Isolates from cell culture might be reisolated from eggs and manufacturer may be de novo readapt to grow in mammalian cells

Reverse genetic approaches to generate influenza vaccines





- vRNAs are cloned into eight **bi-directional plasmids**
- Co-transfection into FDA-approved cell lines

→ **Rescue of the recombinant influenza viruses**

Bi-directional rescue plasmids (required for negative sense RNA viruses):

- Human Pol-I: Eight negative sense vRNAs
- CMV Pol-II: Eight viral mRNAs translated into the influenza proteins
- **vRNP: viral ribonucleoprotein complex** (vRNAs, NP, PA, PB1, PB2); transcription from viral promotors → mRNA → protein; vRNA → cRNA (amplification of vRNA)
- vRNAs + structural proteins → **new influenza virus**

Nogales, Martinez-Sobrido, Int J Mol Sci 2016

Very low vaccine effectiveness during the 2014–2015 Influenza season

Estimation of vaccine effectiveness in UK (week 40 2014 – week 3 2015)

	Cases (vaccinated : unvaccinated)	Controls (vaccinated : unvaccinated)	Crude VE (95% CI)	Adjusted[a] VE (95% CI)
All influenza (A and B)	65 : 247	177 : 825	−26.7% (−74.0 to 7.8)	3.4% (−44.8 to 35.5)
All influenza A	64 : 232		−32.2% (−82.2 to 4.0)	−0.7% (−52.0 to 33.2)
Influenza A(H3N2) only	61 : 210		−39.8% (−94.1 to −0.7)	−2.3% (−56.2 to 33.0)

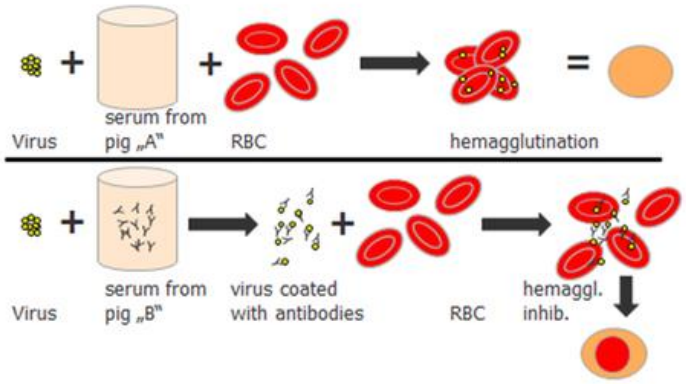
CI: confidence interval; VE: vaccine effectiveness.

[a] Adjusted for age group, sex, month, surveillance scheme and primary school area.

$$VE = \frac{ARU - ARV}{ARU} \quad (\times 100),$$

- VE = Vaccine efficacy,
- ARU = Attack rate of unvaccinated people,
- ARV = Attack rate of vaccinated people.

Influenza virus	Change in reactivity with A/Texas/50/2012 antiserum							
	‹4-fold		4-fold		›4-fold		Total	
	N	(%)	n	(%)	N	(%)	n	(%)
A(H3N2)	65	51.2	35	27.6	27	21.3	127	100



- Hemagglutination inhibition (HI) assay
- Fold difference in comparison with A/**Texas/50/2012 strain** (2014-15 norhern hemispheric vaccine, H3C.1)
- Influenza A (H3N2) virus isolates 2014-2015 from infected patients (n=127)
- A/Texas/antiserum: post infection anti-ferret sera

➔ 4-fold difference in HI assay is a significant antigenic drift (mutations)

➔ **Antigenic mismatch between the circulating strain and vaccine strain**

R G Pebody, Eurosurveillance 2015)

HA mutations in 2014-2015 Viruses

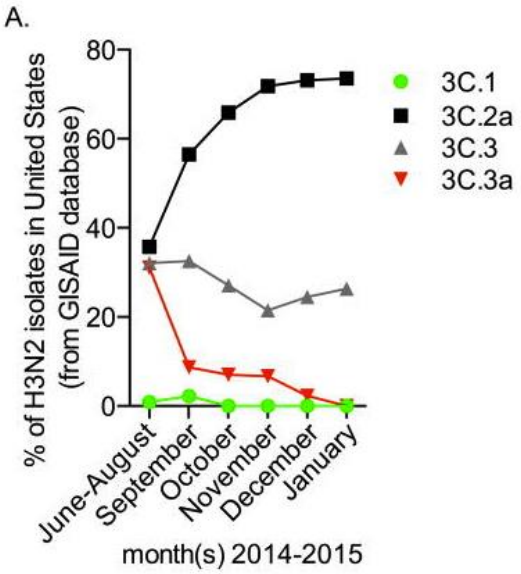
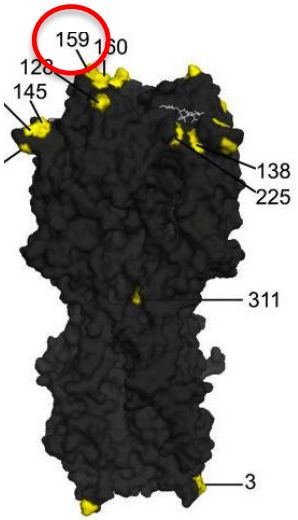
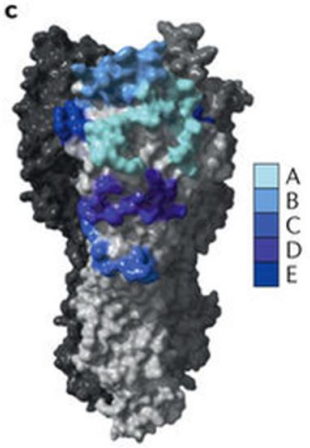


Table 1. HA Mutations in 2014–2015 H3N2 Viruses

Clades		
3C.2a	3C.3	3C.3a
L3I		
	T128A	T128A
		A138S
	R142G	R142G
N144S		
N145S	N145S	N145S
F159Y		F159S
K160T		
N225D		N225D
Q311H		

Shown are HA residues (H3 numbering) that differ between the A/Texas/50/2012 H3N2 vaccine strain and most clade 3C.2a, 3C.3, and 3C.3a viruses isolated during the 2014–2015 Northern Hemisphere influenza season.





Five classical antigenic sides

Percentage of viruses
That belong to each HA
Clade; Sequence deposit form
GISAID* database

HA Mutations in 2014-2015 Viruses/ New clades: 3C.2a, 3C.3 and 3C.3a

H3 component of 2014-2015: Texas/50/2012 strain (H3C.1)

H3 component of 2015-2016: A/Switzerland/9715293/2013 (3C.3a)

*GISAID: Global Initiative on Sharring all Influenza data

B. Chambers et al, Cell Reports, 2015

Mutant viral Texas/50/2012 panel

Goal: Determination which HA residues are responsible for the observed antigenic drift of 2014–2015 H3N2 strains

Significance of data: Proper selection of viral strains in future vaccine formulation

H3C.1 clade H3C.3a clade

Table 2. Analyses of Ferret and Sheep Anti-sera Raised against the A/Texas/50/2012 and the A/Switzerland/9715293/13 Strains

	Sera		
Viruses	Ferret α-A/Texas/50/12	Sheep α-A/Texas/50/12	Sheep α-A/Switzerland/9715293/13
A/Texas/50/12-WT	960	10,240	2,560
A/Texas/50/12-N128A	1,280	10,240	2,560
A/Texas/50/12-A138S	480	5,120	1,280
A/Texas/50/12-R142G	640	7,680	1,920
A/Texas/50/12-N144S+N145S	1,280	10,240	3,840
A/Texas/50/12-N145S	480	5,120	1,920
A/Texas/50/12-F159S	240	3,840	3,840
A/Texas/50/12-N225D	640	7,680	2,560
A/Switzerland/9715293/13	60	1,280	2,560

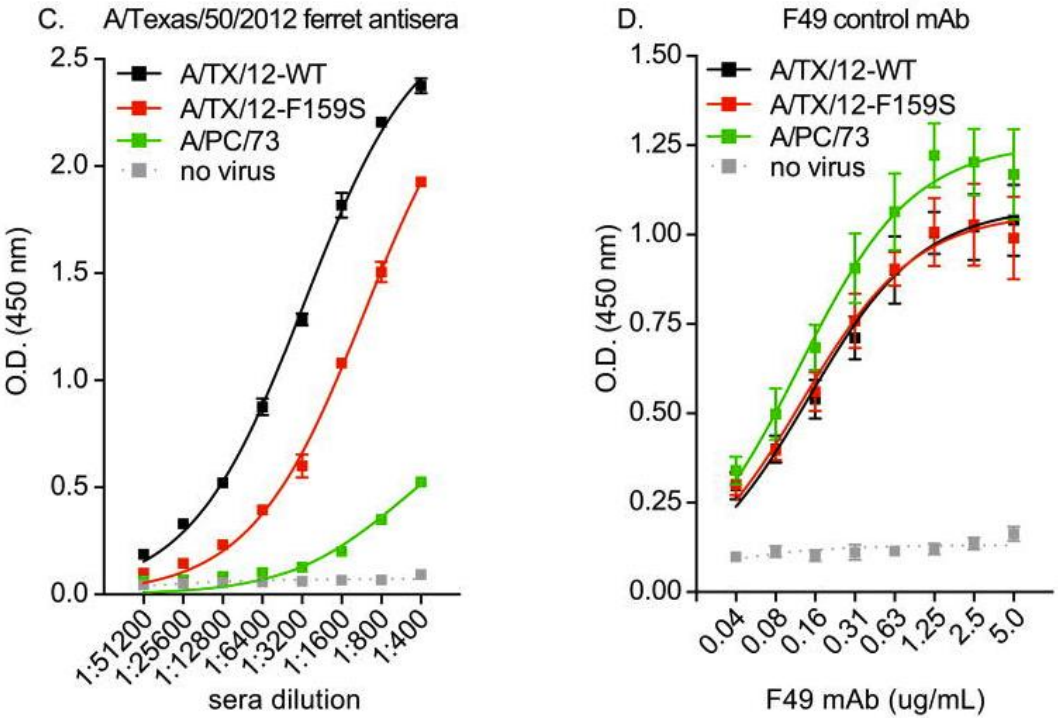
HAI assays were completed using antisera isolated from ferrets 19 days post-infection or sheep 28 days post-infection. Data are representative of three independent assays.

- Reverse genetic derived viruses: HA plasmids + plasmids from A/Puerto Rico/8/1934; transfection of 293T/MDCK co-culture;
- propagation in 10 day-old fertilized eggs
- HAI titer: invers of the highest dilution

➔ The F159S HA mutation result in an **assymetrical** antigenic change

B. Chambers et al, Cell Reports, 2015

Relevance of F159S HA mutation

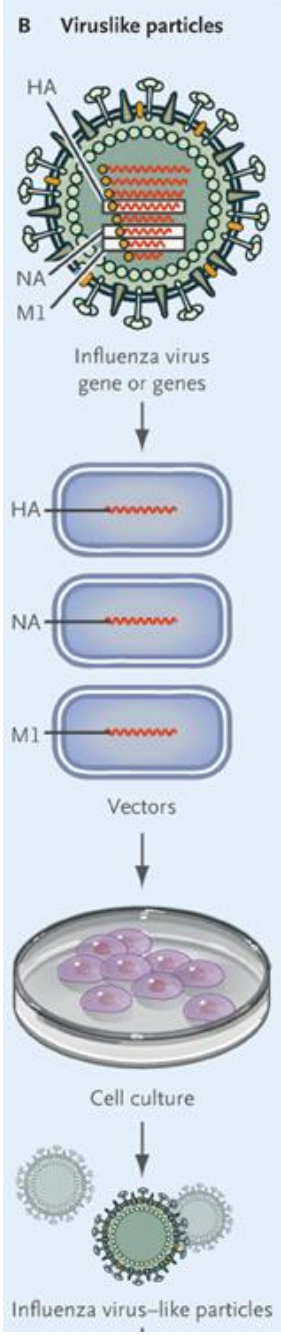


- ELISA plates coated with VLP A/TX/12-WT or Mutant
- Control: A/Port Chalmers/1/1973 HA (A/PC/73); 1973 H3 virus
- Anti-sera from ferrets infected with A/TX/12
- F49 mAb: recognize the conserved stalk region of the HA

Conclusion

➔ Demonstration that a mutation in the antigenic B side is primarily responsible for the mismatch

➔ Support of the WHO decision to update the H3N2 component



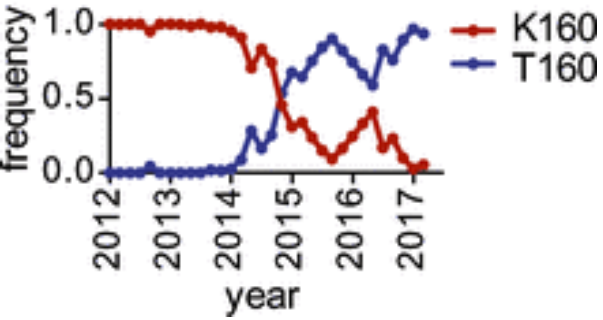
B. Chambers et al, Cell Reports, 2015

Relatively low vaccine effectiveness during the 2016-2017 influenza season

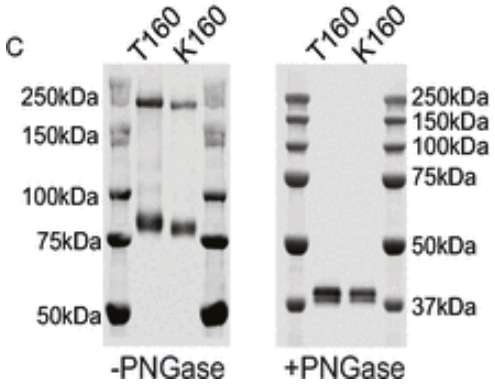
- Vaccine from 2016-2017 : 3C.2a H3N2
- Majority of viruses during influenza season: 3C.2a H3N2
- Relatively low VE : 43%

➔**Egg-adapted 3C.2a vaccine strain lacks the B glycosylation site; present on circulating 3C.2a H3N2 strains** (egg adapted strain posses a T160K reversion mutation; K160T strain grow poorly in egg)

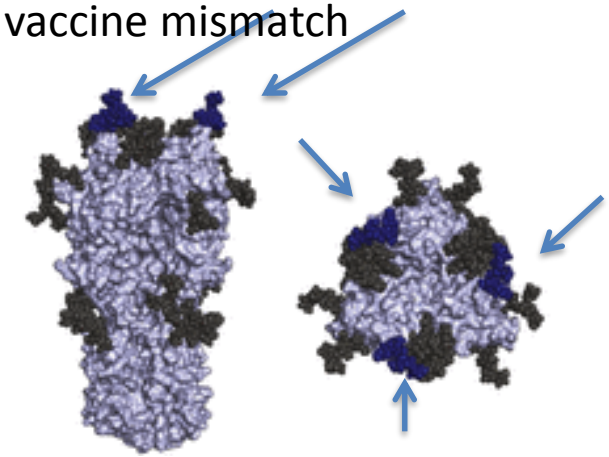
Goal: Determination weather the difference in glycosylation of HA antigenic site B of H3N2 vaccine strains and circulating strain contributes to vaccine mismatch



Appearance of K160T HA mutation GISAID database



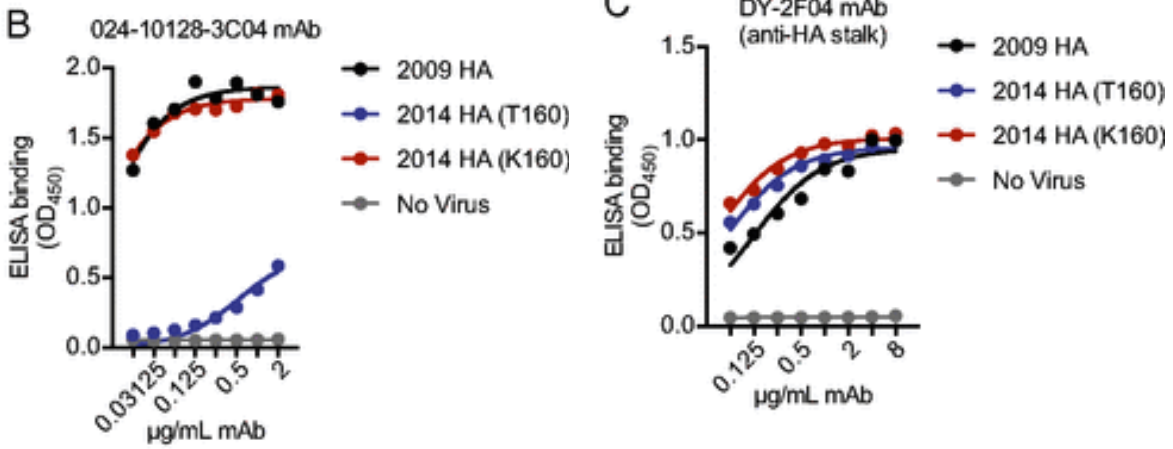
Western blot of homogenates from viruses generated with reverse genetic



Blue: New putative glycosylation sides introduced by the K160T mutation
Black: Other putative glycosylation sides

S. Zost, PNAS, 2017

H3N2 viruses with T160 HA are antigenically distinct



A

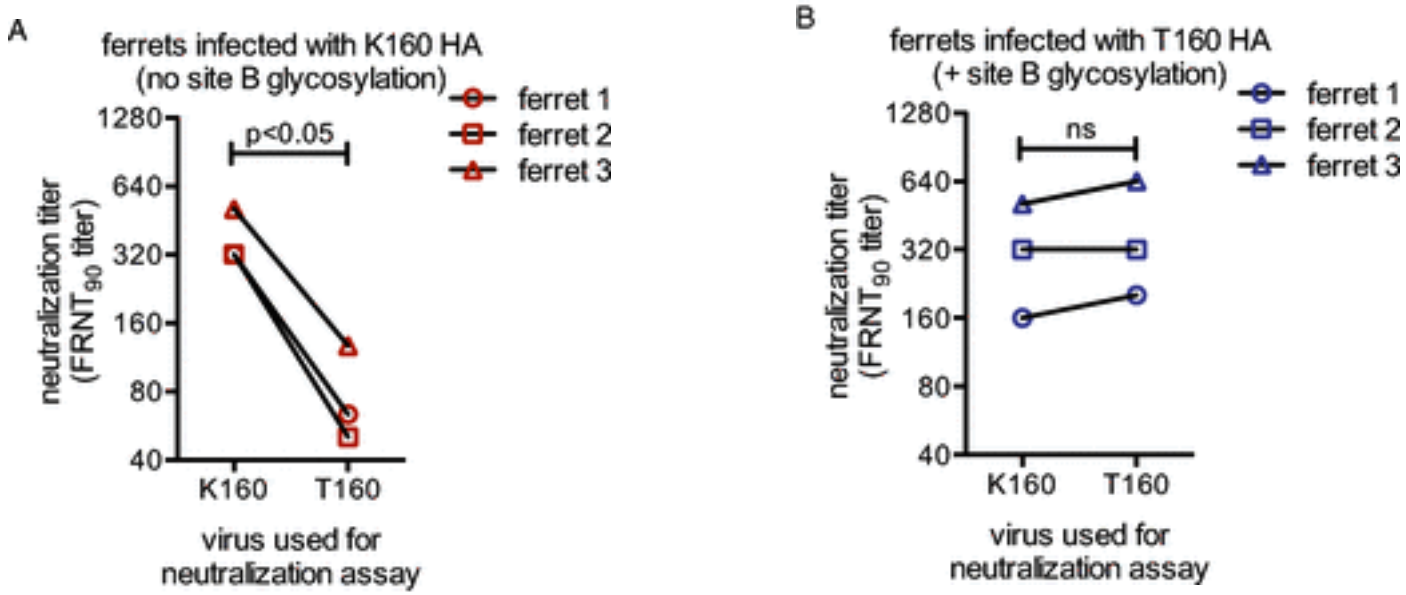
mAb	2009 HA	2014 HA (T160)	2014 HA (K160)
019-10117 1B02	100	0	0
017-10116 5G02	100	1	1
017-10116 5D02	100	1	1
013-10078 3G01	100	2	2
011-10069 5C01	100	0	9
009-10061 2A05	100	0	18
011-10069 3E06	100	0	36
034-10040 4E01	100	0	51
009-10061 2C06	100	0	38
011-10069 5D01	100	0	89
011-10069 2C01	100	0	79
008-10053 5E04	100	0	80
008-10053 5G01	100	0	77
019-10117 3F01	100	0	88
024-10128 3F04	100	3	90
019-10117 3A06	100	4	93
034-10040 4C01	100	4	72
024-10128 3C04	100	4	98
037-10036 5A01	100	5	91
011-10069 5G04	100	17	66
011-10069 5G01	100	24	60
009-10061 3B06	100	59	74
019-10117 3C06	100	62	90
008-10053 1G05	100	89	92
041-10047 1C04	100	94	105
028-10134 4F03	100	104	102

- ELISA plates coated with viral particle
- Human monoclonal antibodies isolated from donor peripheral blood mononucleat cells following vaccination with the 2010-2011 influenza vaccine

➔2016-2017 circulating influenza virus possess a new glycosylation site that affects antigenicity, this site is not present in 2016-2017 vaccine strain

S. Zost, PNAS, 2017

Different antibody response in ferrets



- Ferrets (n=3) infected with viruses, sera collected 28 d later
- FRNT assay: Inverse dilution of sera that reduced foci by 90%, pre-incubation of reverse-genetic transfection supernatant of each virus + diluted sera; Added to confluent monolayer of MDCK-SIAT1 cells; staining

➔ The new glycosylation site effectively “shields” antigenic site B

S. Zost, PNAS, 2017

Disadvantages of egg based influenza vaccine production

- Long vaccine production process (window for new virus variants to emerge)
- Human vaccine grown in eggs often possess adaptive and unintended mutations (increase viral attachment to chicken cells)
- High-level bio-containment facilities
- Antigenic proteins in eggs (allergic reaction)
- Scale is dependent on a large egg production

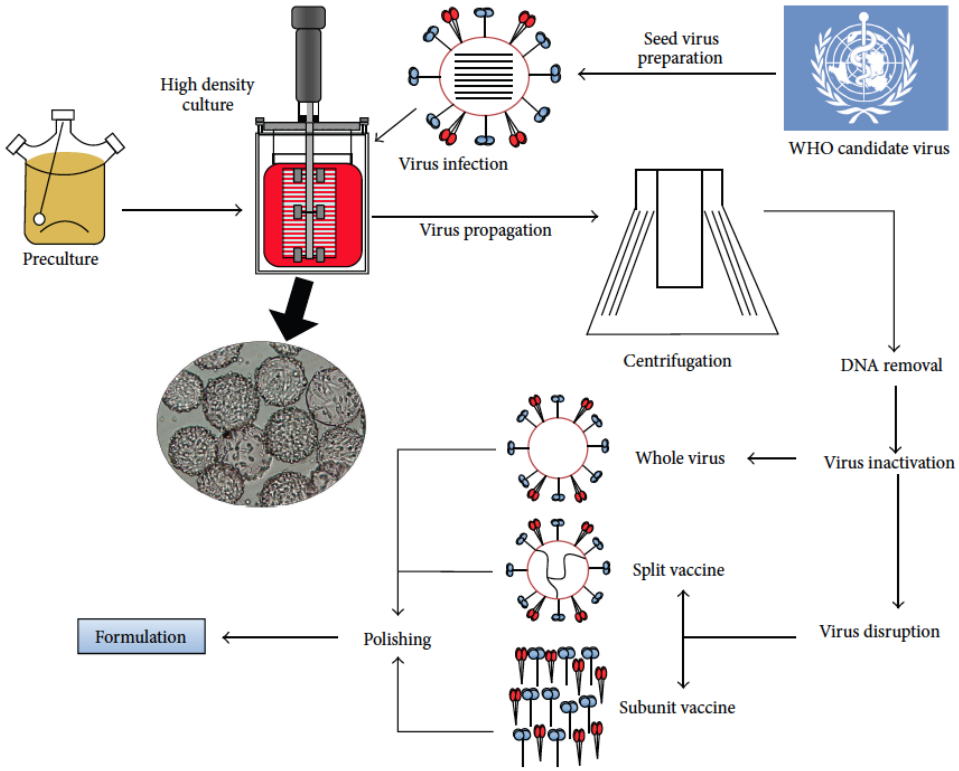
Allternatively established influenza vaccine production

Table 2: Licensed influenza vaccines produced using cell culture technology.

Cell-based vaccines				
Product name	Commercial name	Manufacturer	Cell platform	Commercially available
Influenza virus vaccine (trivalent)	Flucelvax	Novartis Vaccines and Diagnostics Limited	MDCK	EU/FDA
Influenza virus vaccine (trivalent)	FluBlok	Protein Sciences Corporation	Insect cells	FDA
Influenza virus vaccine (trivalent)	Preflucel	Baxter	Vero	EU
Influenza virus vaccine (H5N1)	Celvapan	Baxter	Vero	EU
Influenza A (H1N1) 2009 monovalent vaccine	Celvapan	Baxter	Vero	EU

Millan und Kamen, BioMed Reseach International, 2015

Cell-based inactivated influenza vaccine production



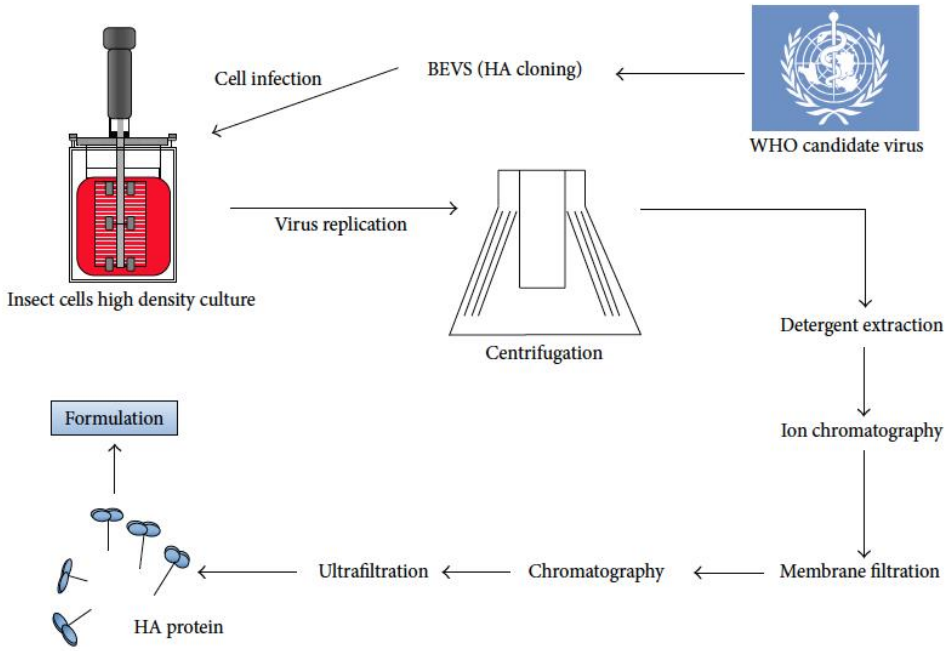
Cells: Madin-Darby canine kidney cells (MDCK); human embryonic retinal cells (PER.C6); monkey kidney cells (Vero); human embryo kidney cells (HEK293)

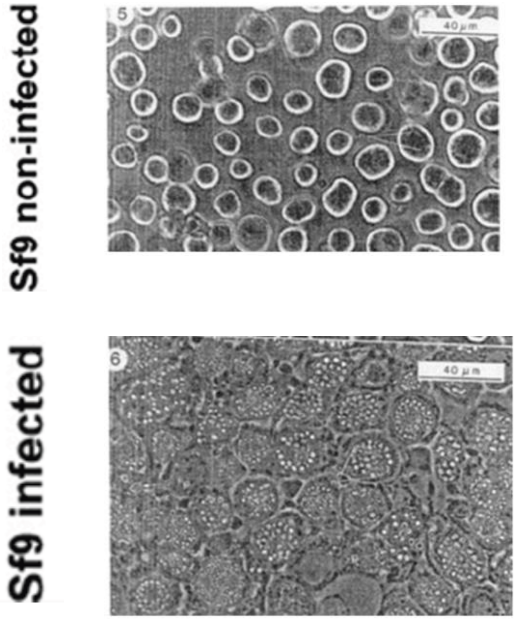
Optaflu/Flucelvax (Novartis):Trivalent subunit vaccine produced in MDCK cells form **egg-adapted i**nfluenza viral seeds.

Perflucel and Celvapan: Production in Vero cell

Millan und Kamen, BioMed Reseach International, 2015

Recombinant trivalent hemagglutinin (rHA) vaccine





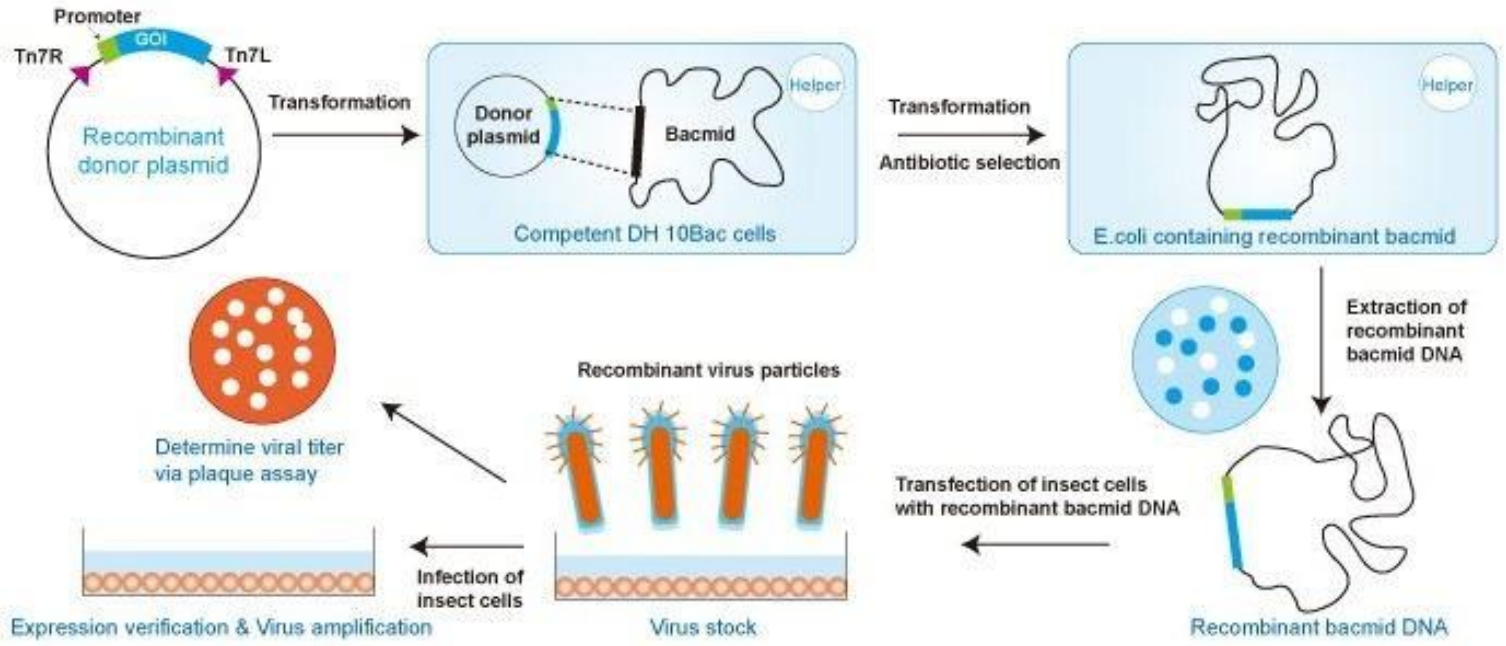
Millan und Kamen, BioMed Reseach International, 2015

Insect cell line	Source	Application
Sf9 and Sf21	*Spodoptera frugiperda*	All general types of recombinant protein expressi
Mimic™ Sf9	*Spodoptera frugiperda*	Expression of mammalian glycoproteins.
High Five™	*Trichoplusia ni*	Secretion of recombinant proteins. Shorter culture period.

FlubBlok: trivalent recombinant hemagglutinin vaccine

Produced in **insect cell culture** using the **baclovirus expression system**

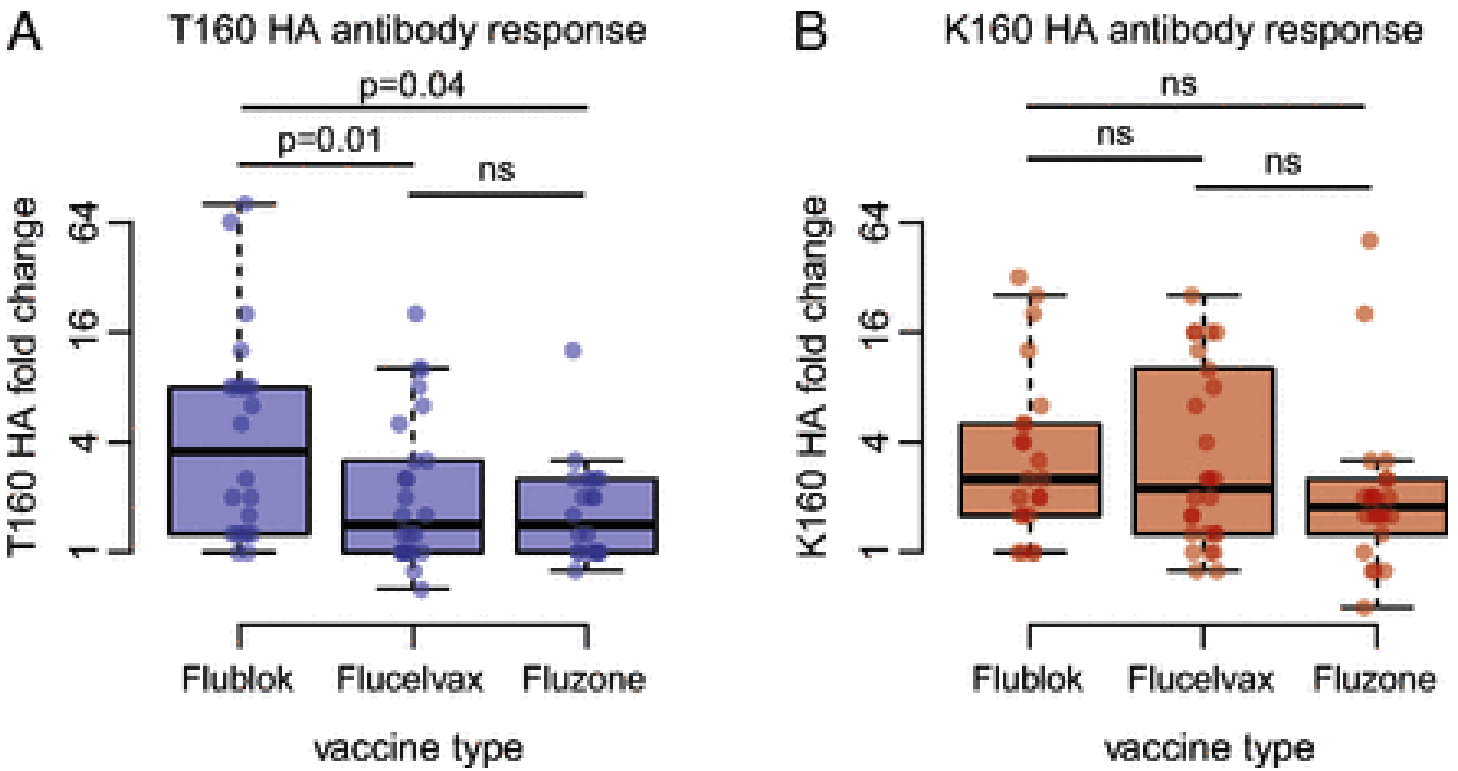
Production in insect cell with the baclovirus expression system



➔Capacity to produce many recombinant proteins at high levels
➔Provide eukaryotic protein processing capabilities
➔ Baclovirus system is well suited to avoide adoptive mutations

https://www.creativebiomart.net/baculovirus-insect-cell-expression-systems.htm

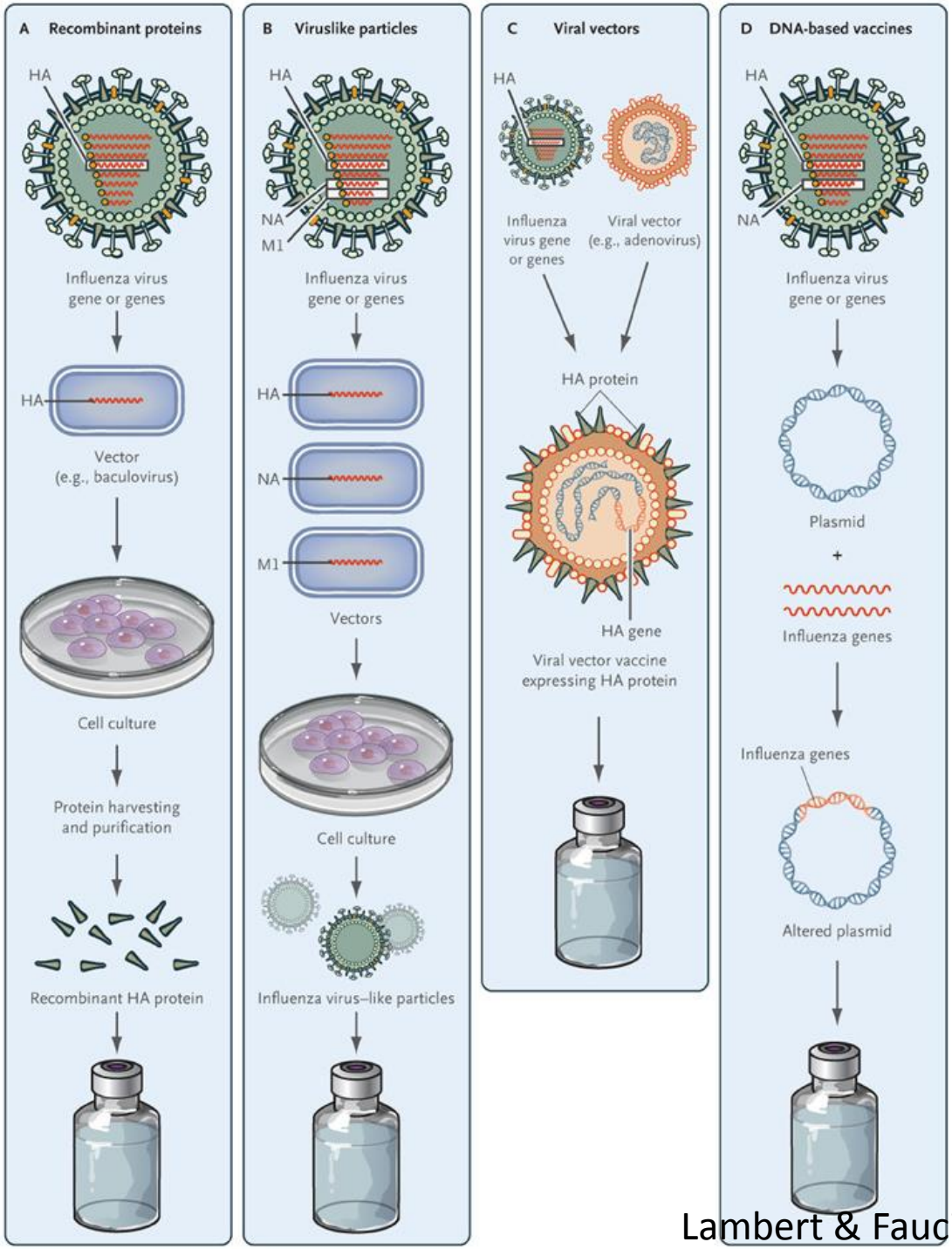
Increase in antibody response against T160 HA following vaccination with Flublok



Donors were vaccinated with seasonal influenza vaccines, sera were collected before and 28d after vaccination. FRNT (neutralization assay) with the use of viruses.

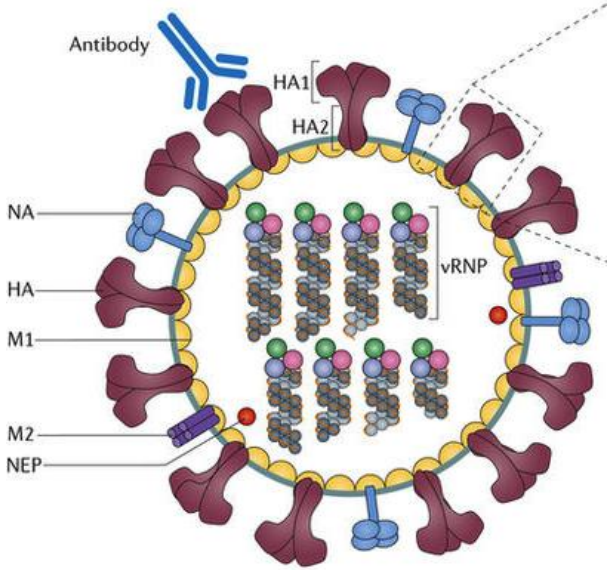
➔ Flublok induced higher fold change to T160 HA than did Flucelvax and Fluzone (Flublok vaccine possesses the T160 HA)

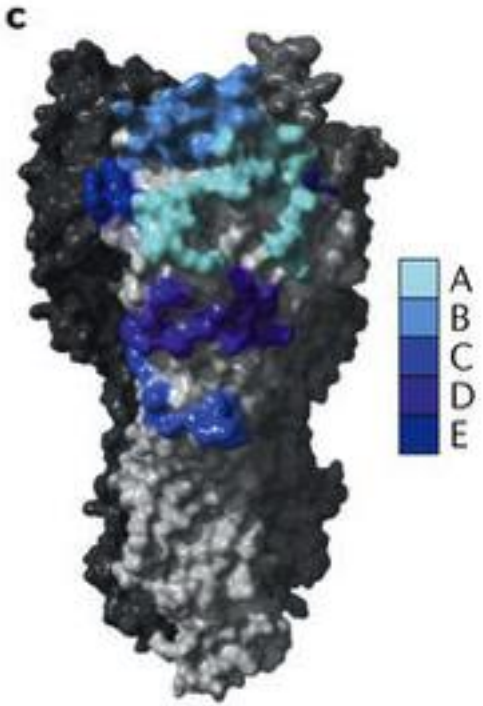
S. Zost, PNAS, 2017



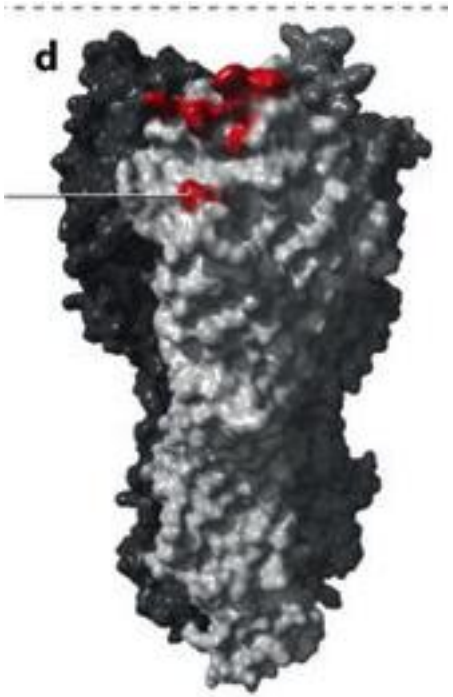
Lambert & Fauci, New Engl J Med, 2010

The hemagglutinin-stem is highly conserved





Five classical antigenic sides

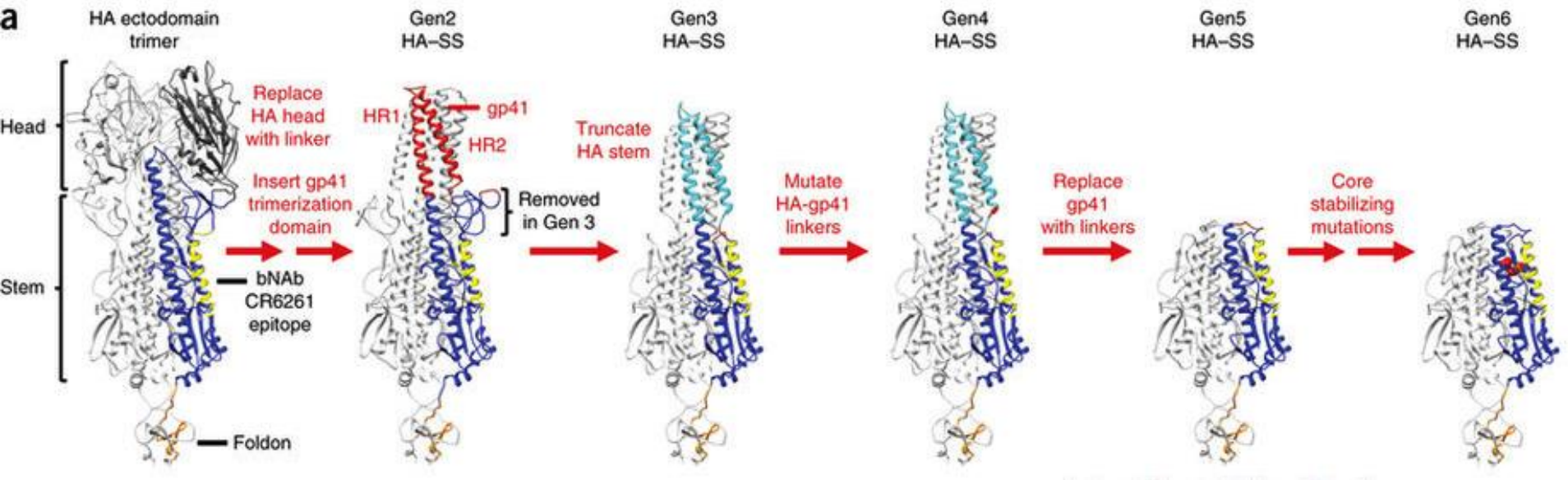


Immune escape Substitutions A/H3N2

➔Generation of a hemagglutinin-stem only immunogen

Petrova and Russell, Nature Reviews 2018

Structure-based removal of the HA head



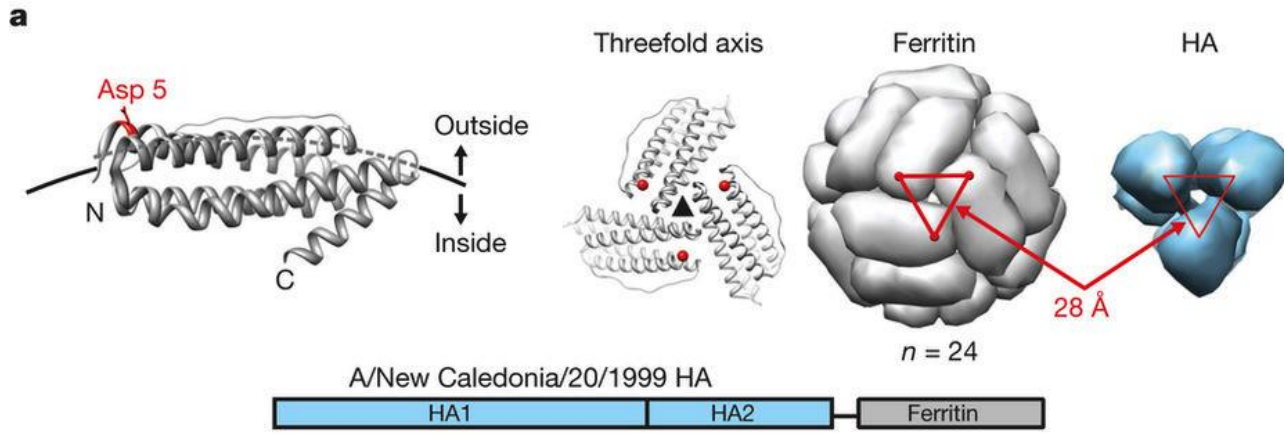
Goal: Structure based development of an H1 HA stem only immunogen

- Source**: H1N1** A/New Caledonia/20/1999; crystal structure, foldon trimerization domain as design templates
- Evaluation of the truncated mutant: Expression as soluble trimers (gel filtration), reactivity to stem-specific monclonal antibodies
- **Crystal structure:** stem epitope conformation was preserved, stem trimer subunit were splayed apart

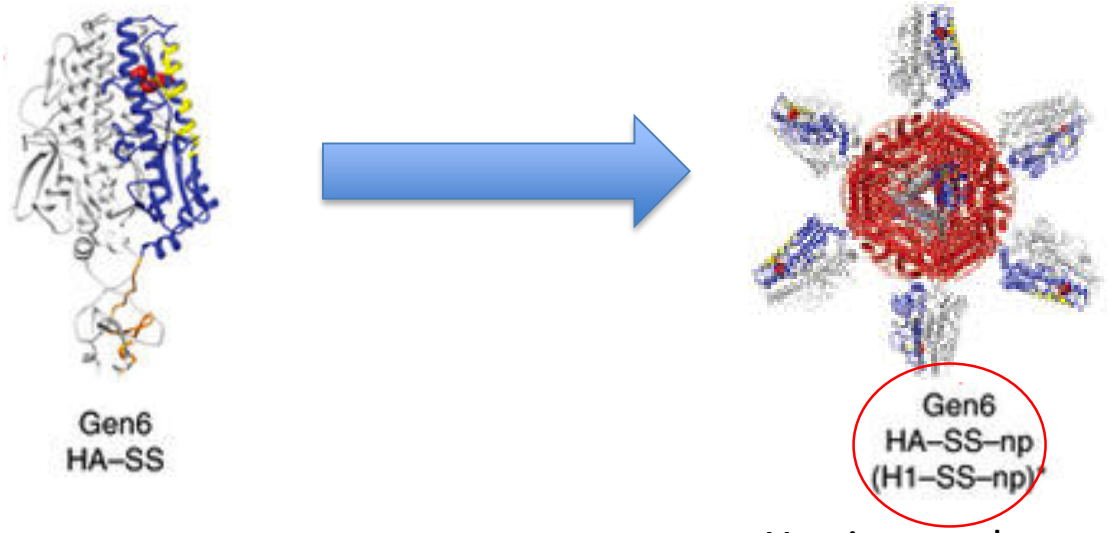
Overlapping PCR and site directed mutagenesis; freestyle HEK 293 or HEK 293 MGAT1$^{-/-}$

Yassine et al, nature medicine 2015

Ferritin nanoparticle displaying of HA stem

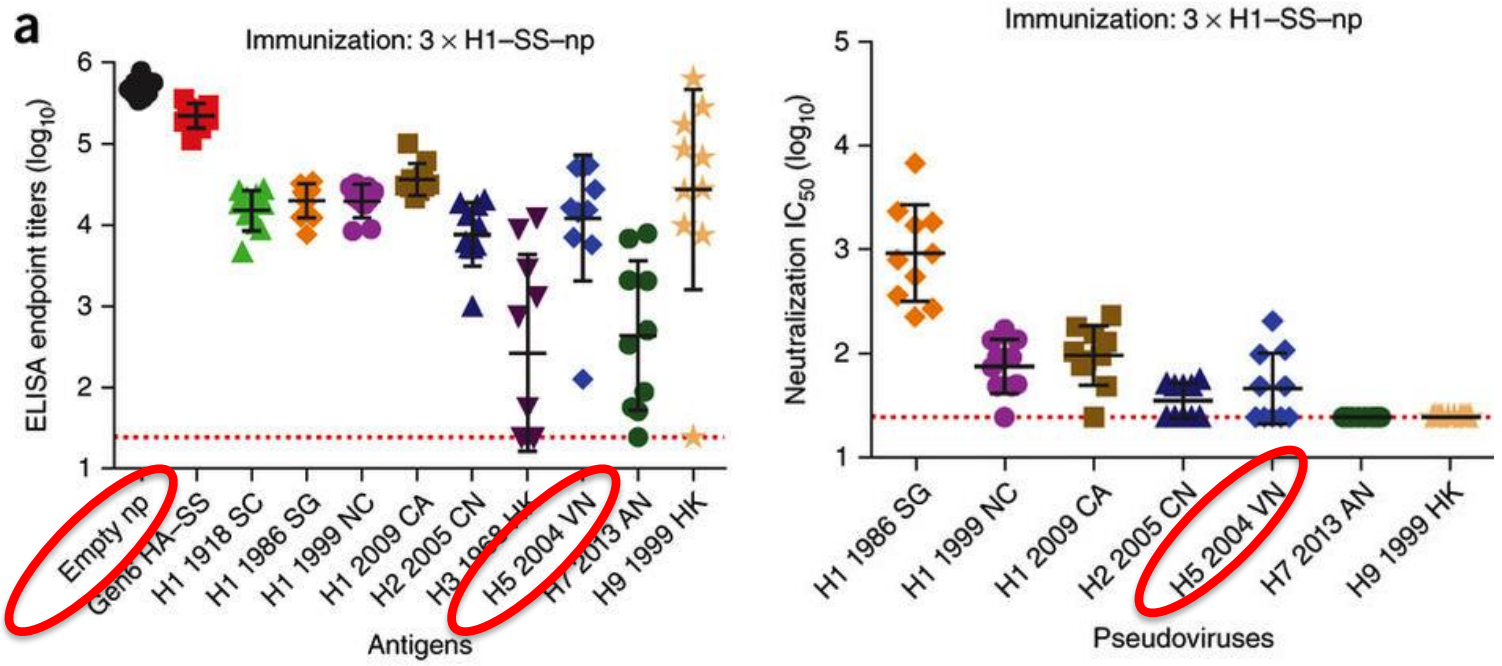


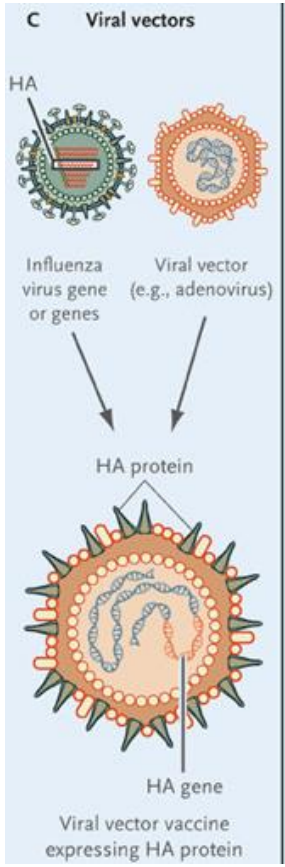
Kanekiyo et al, nature 2013



Yassine et al, nature medicine 2015

Immune response of immunized mice

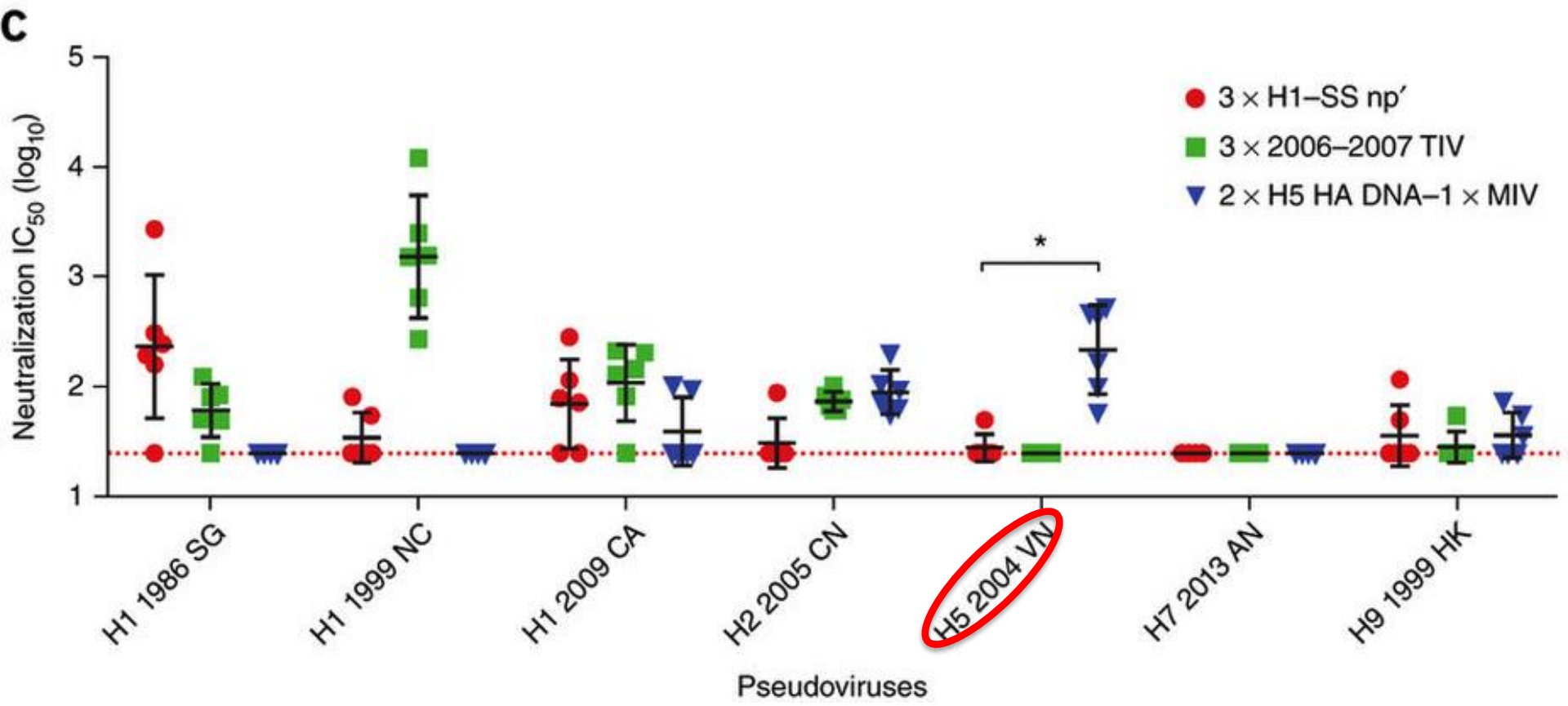




- Sera from BALB/c mice immunized with SAS-adjuvanted H1-SS-np
- ELISA with HA protein
- Neutralization IC_{50}: Reciprocal dilution required to inhibit 50% of pseudotyped lentiviral reporter (viral-vectors, recombinant HA-NA lentiviral vectors expressing a luciferase reporter, preincubation with antiserum)

Yassine et al, nature medicine 2015

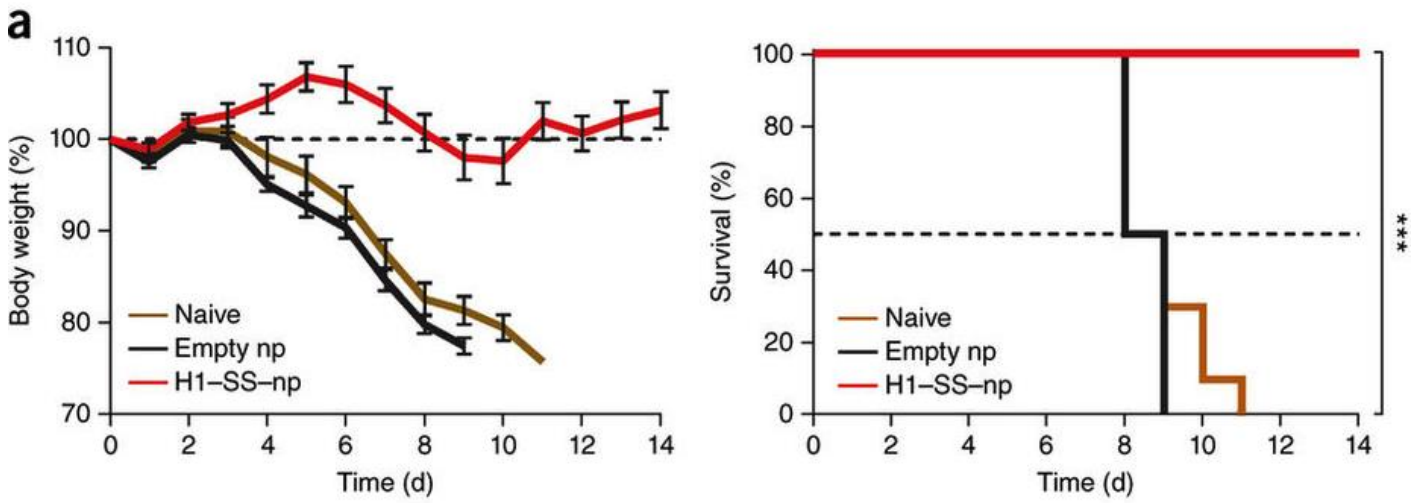
H1-SS np versus two other immunization regimes



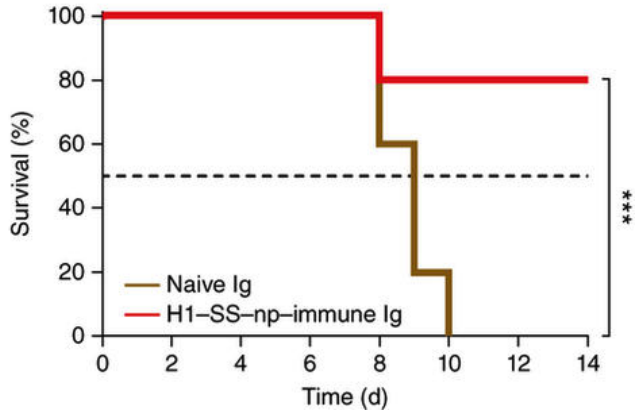
- Sera from ferrets immunized with three regimens
- H5 HA DNA 1 x MIV : H5 DNA priming + monovalent inactivated vaccine (MIV); H5N1

Yassine et al, nature medicine 2015

Protection against lethal H5 2004 VN in mice



BALB/c mice (n: 10) vaccinated three times; challenged 4 weeks post final vaccination with a lethal H5 2004 VN dose

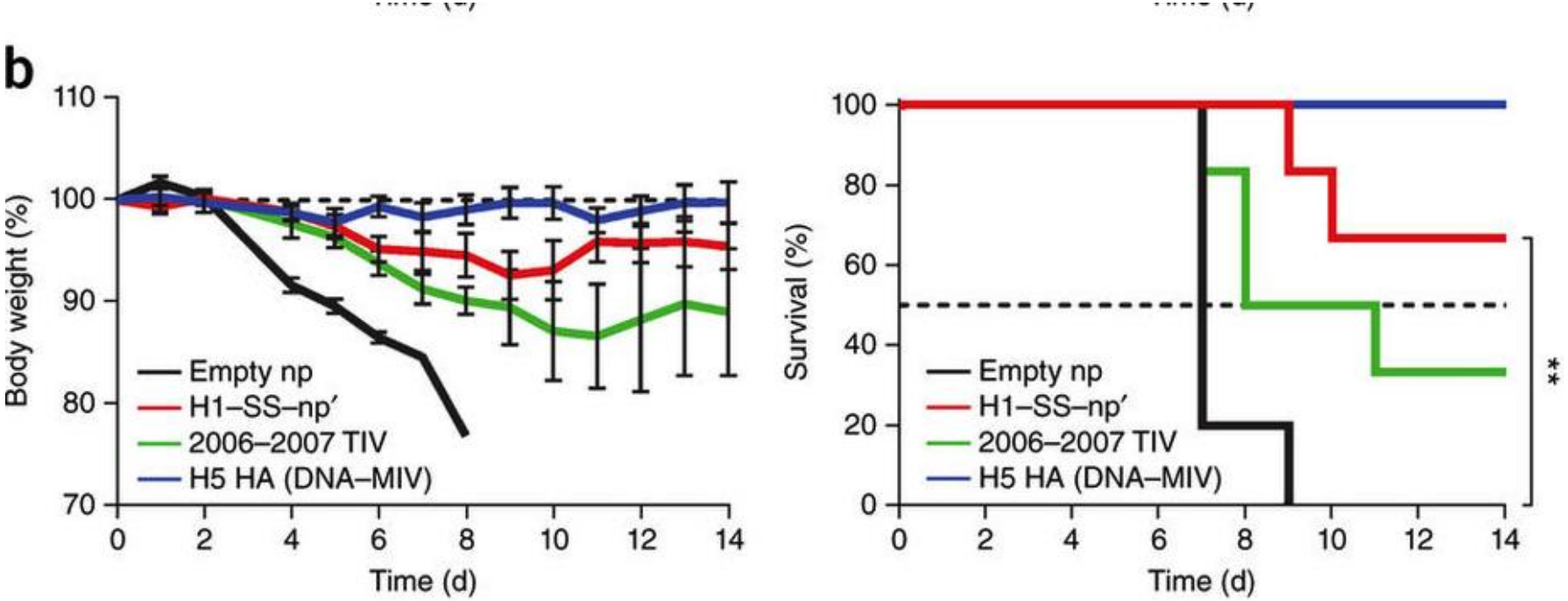


Passive immunization with 10 mg Ig from either naive or immune animals 24h before challenge with a lethal H5 2004 VN dose

➔H5N1 neutralization activity was negligible, however **heterosubtype protection** observed; other effector mechanism than in vitro neutralization

Yassine et al, nature medicine 2015

Protection against lethal H5 2004 VN in ferrets



Ferrets were vaccinated three times, 6 weeks post final vaccination challenge with a lethal dose of H5 2004 VN

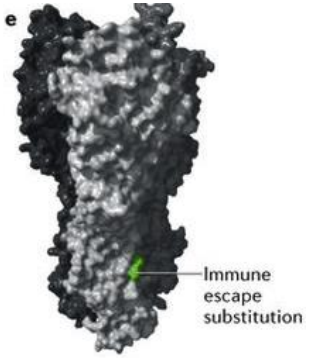
Yassine et al, nature medicine 2015

Stalk antibodies as broadly reactive antibodies

Summary Yassine et al, nature medicine 2015:

- Proof of concept
- Successful generation of HA stem-only nanoparticle vaccine immunogen
- Antibody mediated heterosubtype protection immunity against H5N1 disease in mice and ferretes

- High preservation of stalk
- Antigenic escape from antigens that target the stalk has not been widely reported
- However, this could be caused by a lack of selection pressure
- In in vitro experiments can be achieved



Substitutions to facilitate escape from broadly neutralizing anti-HA antibodies

Petrova and Russel, Nature Reviews 2018

Thank you for your attention!

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