Application of Reverse Genetics to Influenza Vaccine Development

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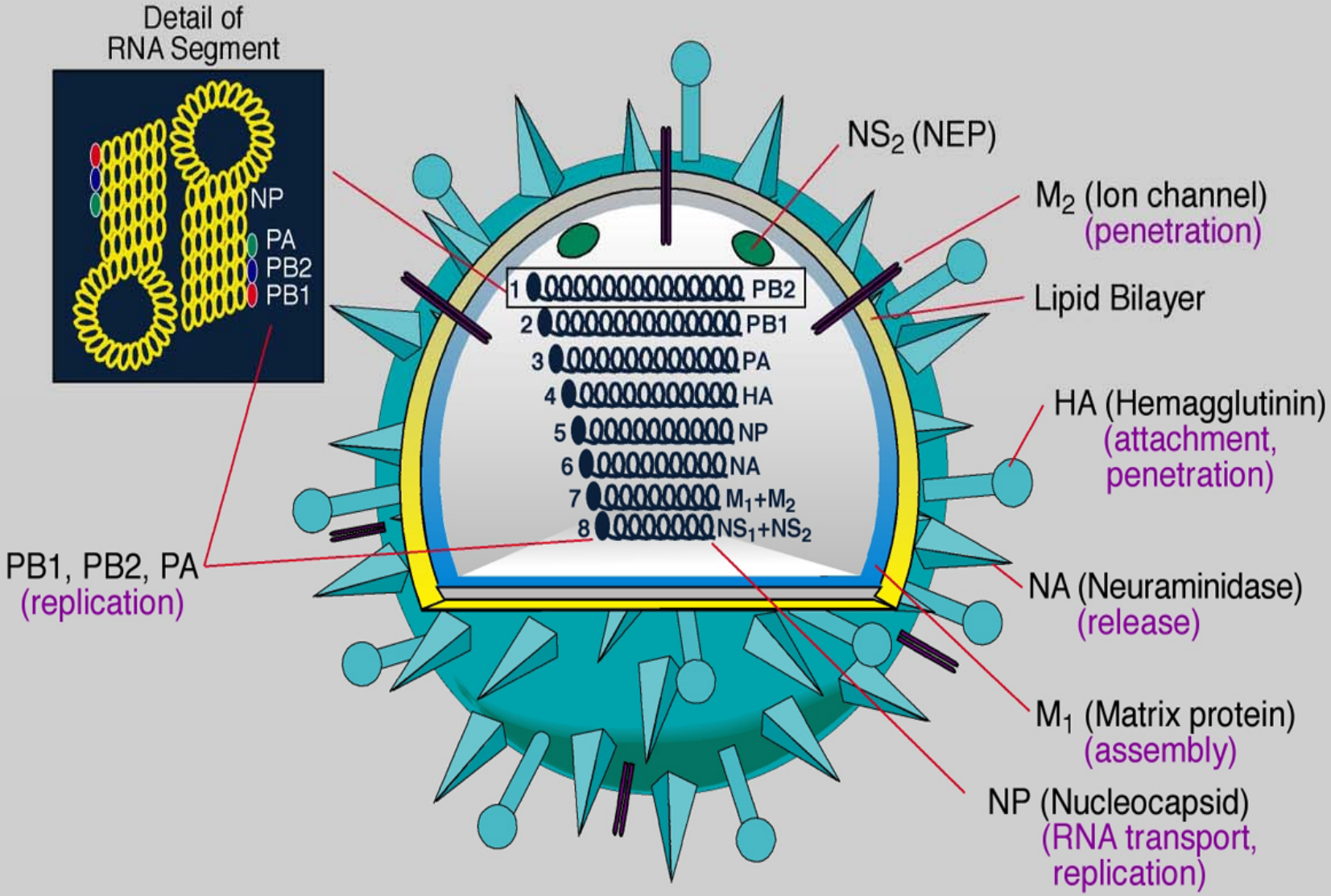
Proteins and RNA's of Influenza A Virus



Licensed Vaccines for Influenza

Principle: Induction of a protective immune response against the hemagglutinin gene.

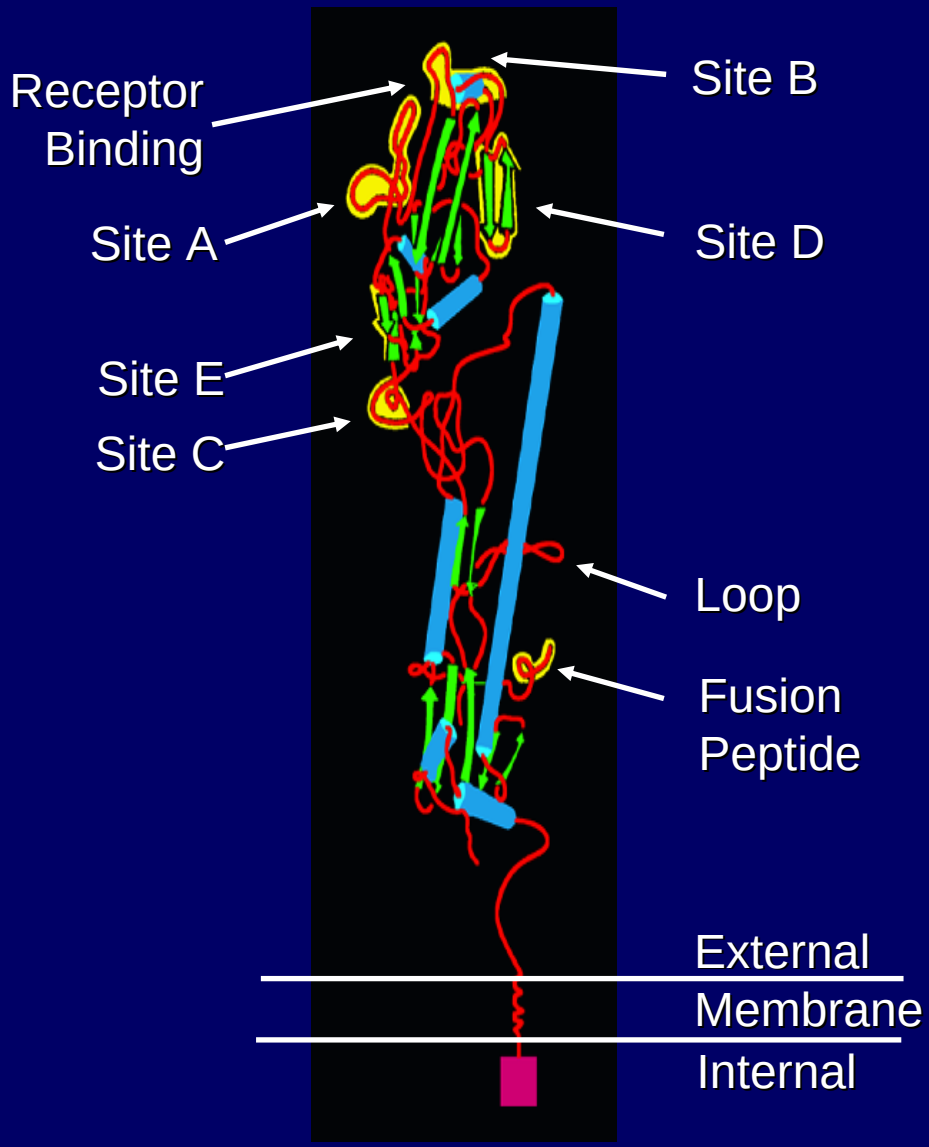
Trivalent vaccines containing A/H1N1, A/H3N2 and B strains

- Conventional inactivated vaccine: Reassortant viruses containing HA and NA from wt influenza A viruses and internal genes from A/PR/8/34 virus + wt influenza B virus.
- Live attenuated cold-adapted (ca) vaccine: Reassortant viruses containing HA and NA from wt influenza A and B viruses and internal genes from master ca strains A/Ann Arbor/6/60 or B/Ann Arbor/1/66, respectively.

Antigenic Drift

- Gradual alteration of the influenza surface proteins (mainly HA) within a subtype resulting in the inability of antibody to previous strains to neutralize new viruses.
- Antigenic drift results from point mutations in the HA and NA genes.
- The composition of the influenza vaccine has to be updated annually as a consequence of antigenic drift.

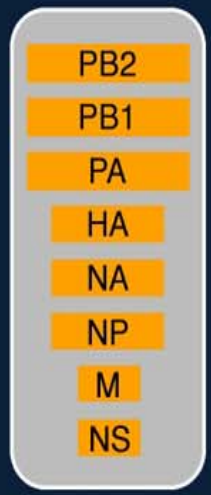
Antibody Binding Sites of HA



Wiley DC, Wilson IA, Skehel JJ. *Nature.* 1981;289:373–378.

Generation of Reassortant Influenza Viruses

Vaccine donor virus with phenotype of attenuation or high growth in eggs



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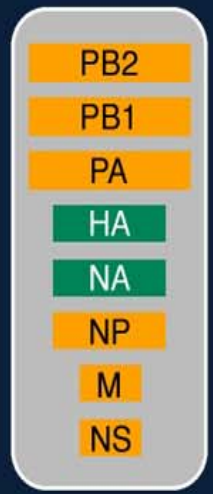
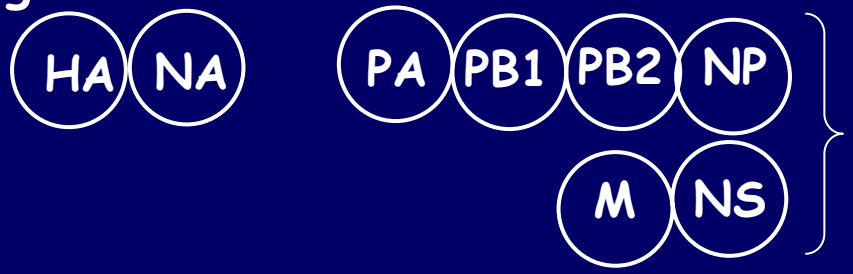
Circulating wild type virus



Reassortant vaccine virus with phenotype of attenuation or high growth in eggs

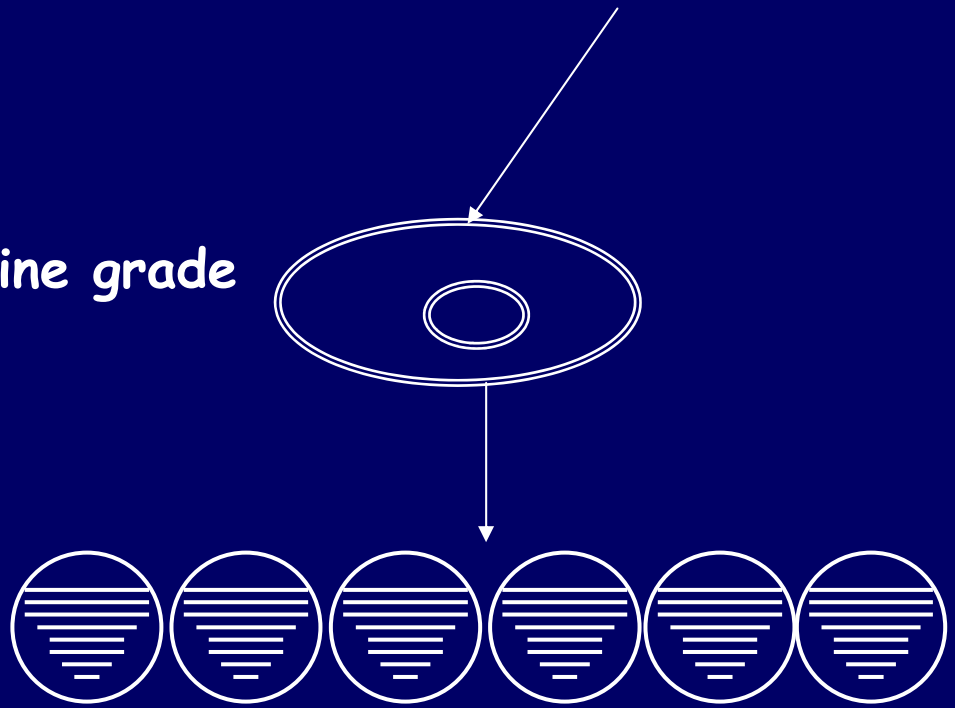
Human Influenza Vaccines Generated by Plasmid-based Reverse Genetics

2 plasmids encoding genes from circulating wt virus



6 plasmids encoding genes from a vaccine donor strain

Vaccine grade cells



Hoffmann et al 2002, Vaccine and PNAS

Biosafety Measures Used for Generation of Reassortant Influenza Vaccines

The reassortant virus is generated at the biosafety level recommended for work with the wild-type virus (BSL-2 for human influenza viruses).

Influenza A viruses in humans in the last century

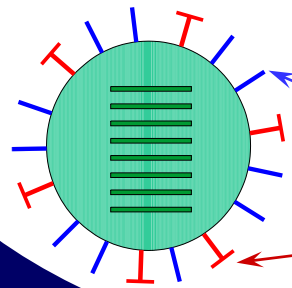
H1N1 → H2N2 → H3N2 → H1N1

Spanish Influenza — Asian Influenza — Hong Kong Influenza

1918 — 1957 — 1968 — 1977

?

Influenza A reservoir



15 HA subtypes

9 NA subtypes

Recent Outbreaks of Avian Influenza in Poultry

Year	Subtype	High pathogenicity?	Location	Birds killed
1983	H5N2	yes	Pennsylvania	17 million
1995	H5N2	yes	Mexico	?
1997	H5N1	yes	Hong Kong	1.6 million
1999-2000	H7N1	no	Italy	13 million
2002	H7N2	no	Virginia	4.7 million
2003	H7N7	yes	Netherlands	30 million
2004	H5N1	yes	Asia	>100 million
2004	H5N2	yes	Texas	?
2004	H7N3	yes	Canada	?

Avian Influenza Viruses Infecting Humans

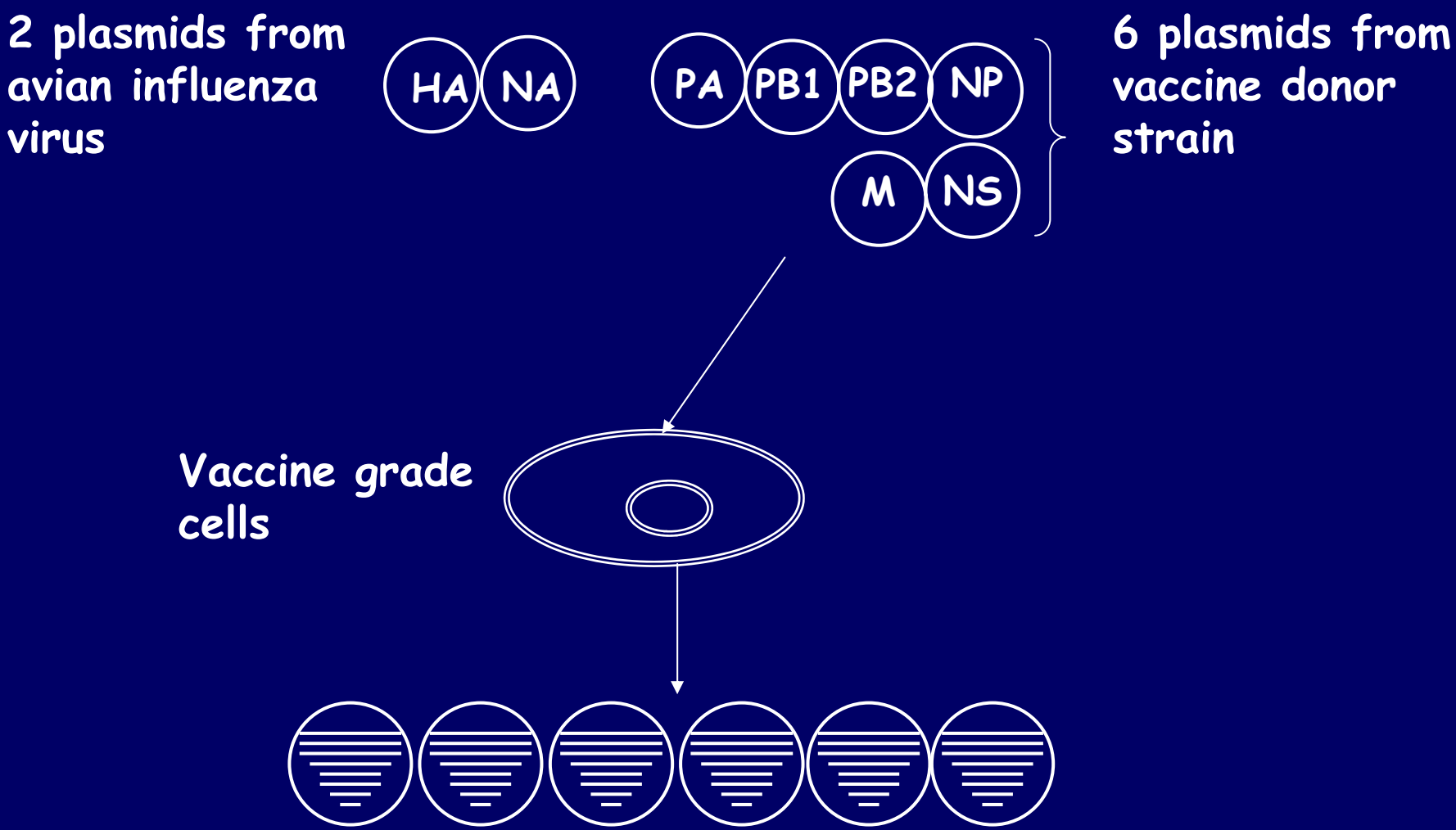
- H5
  - Hong Kong 1997: 18 cases, 6 deaths
  - Hong Kong 2003: 3 cases, 2 deaths
  - Vietnam, Thailand 2004: 39 cases, 28 deaths
- H7
  - Case reports
  - Netherlands 2003: 79 cases of conjunctivitis, 13 ILI, 1 death, 3 person-person transmissions
- H9
  - Hong Kong and Southern China 1999: 7 cases
  - Seroprevalence in poultry workers 1999
  - Hong Kong 2003: 2 cases

Potential Social and Economic Impact of an Influenza Pandemic

- Mathematical model* estimates for first year of a pandemic in absence of effective interventions:
  - 89,000 - 207,000 deaths in the U.S.
  - 314,000 - 734,000 hospitalizations
  - 18 - 42 million outpatient visits
  - additional 20 - 47 million illnesses
  - economic impact: $71- $166 billion

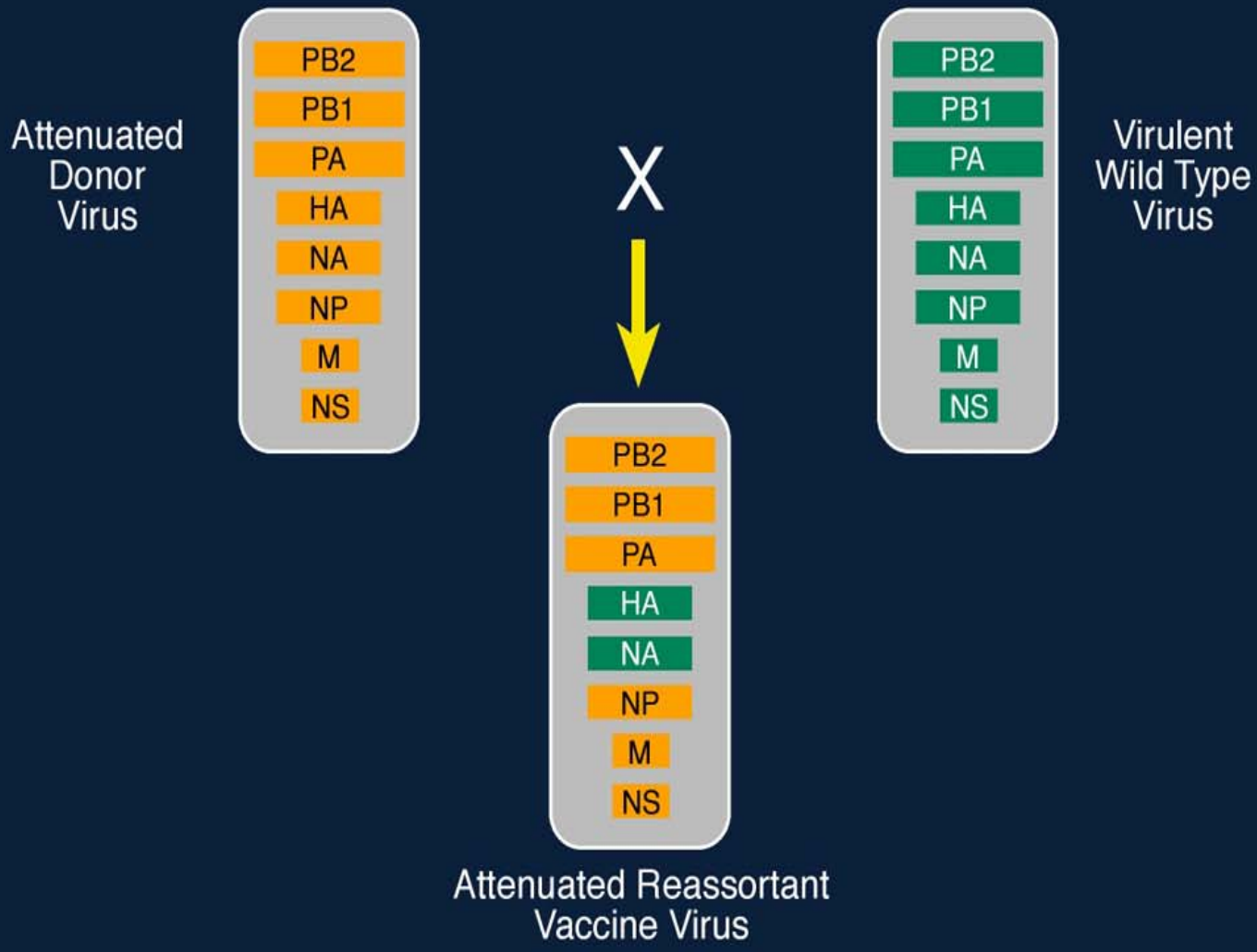
** Meltzer et al. Em. Infect. Dis. 1999; 5:659-71*

Pandemic Influenza Vaccines Generated by Plasmid-based Reverse Genetics



Hoffmann et al 2000

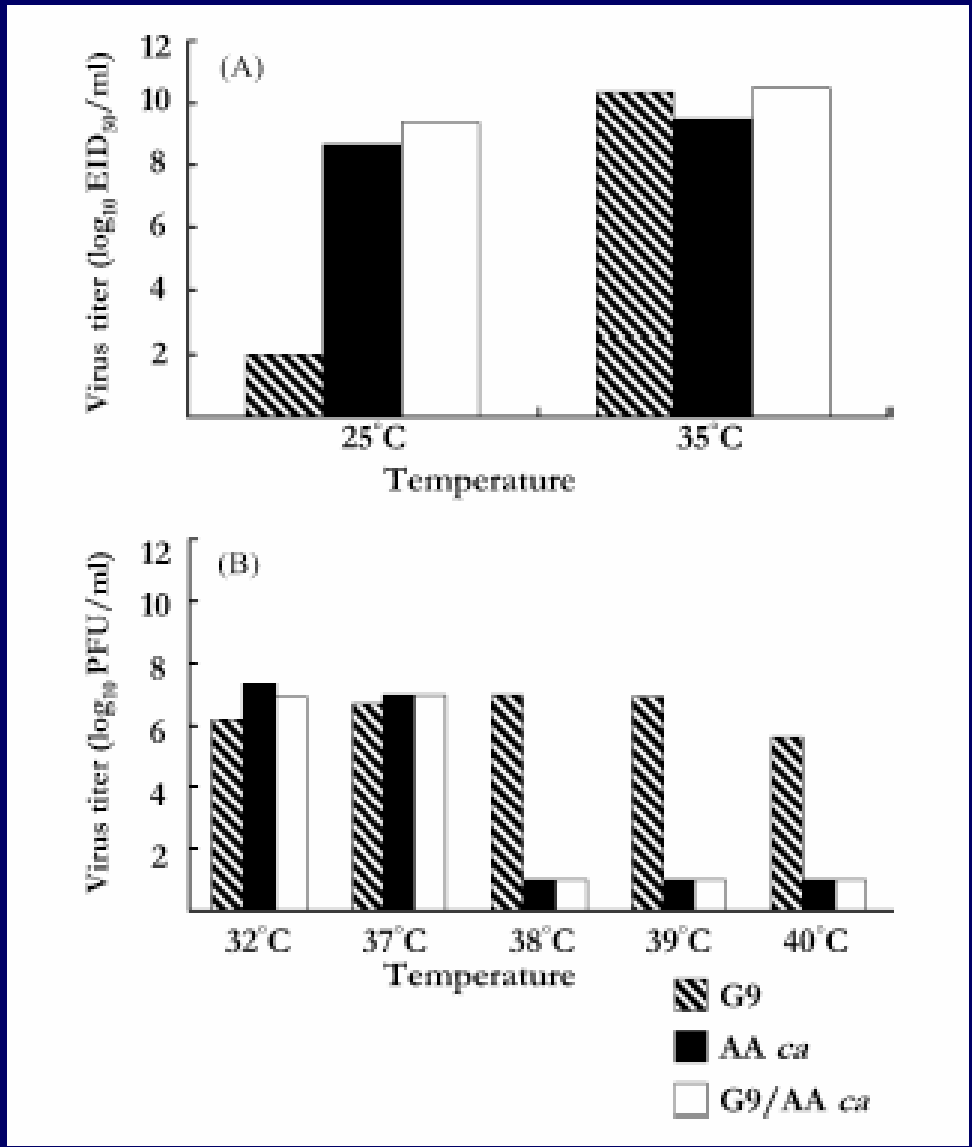
Strategy for Production of Live Attenuated Influenza Virus Vaccine (Genetic Reassortment)



General Approach to the Evaluation of Candidate Vaccine Viruses

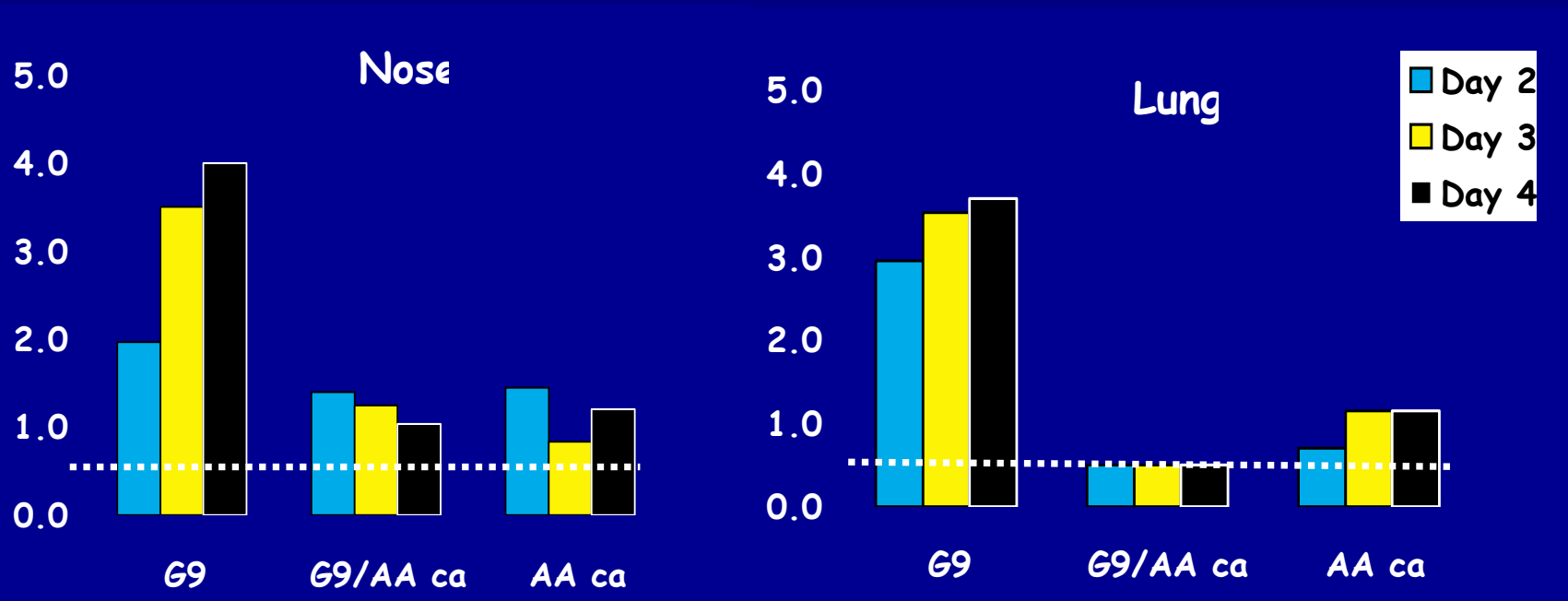
- Genotype and sequence the HA and NA genes
- Compare the antigenicity of the vaccine reassortant with the parent virus
- Pathogenicity in chickens (USDA)
- Pathogenicity and level of replication in mice or other suitable animal model
- Efficacy of protection from challenge with wild-type virus in mouse or other suitable animal model

ts and ca Phenotypes of the H9N2-AA ca Reassortant Virus



Chen et al. Vaccine 21: 4430-36; 2003

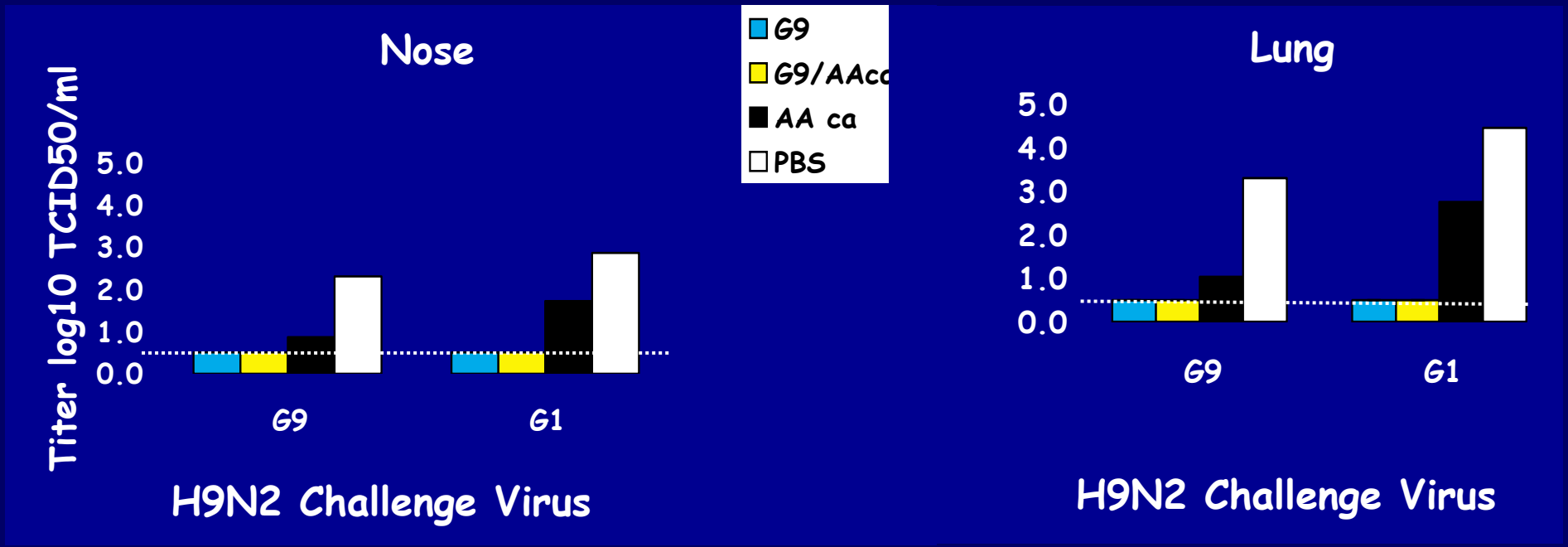
The AA ca Genes Attenuate the H9N2 Reassortant Virus in Mice



Mice were infected with 10^5 EID_{50} in 50 µl intranasally. Organs were harvested on days 2, 3 and 4 post-infection

Chen et al Vaccine 21: 4430-36; 2003

A Live Attenuated H9N2-G9/AA ca Vaccine Protects Mice against Replication of Homologous and Heterologous H9N2 Challenge Viruses



Mice were challenged with wt H9N2 viruses 4 weeks after intranasal infection with 10^5 $TCID_{50}$ of vaccine virus or controls

Chen et al Vaccine 21: 4430-36; 2003

Shedding of IV and IN Administered H9N2-AA ca Virus in Chickens

Route	Virus	Virus isolation from swabs				Antibody detected
		Oropharynx		Cloaca		
		Shedding	Titer	Shedding	Titer	
IV	H9N2 wt	8/8	4.3	8/8	4.2	8/8
	AA ca	0/8	<0.9	0/8	<0.9	5/8
	H9N2/AA ca	0/24	<0.9	0/8	<0.9	2/6
IN	H9N2 wt	7/8	4.7	7/8	1.5	8/8
	AA ca	0/8	<0.9	0/8	<0.9	0/8
	H9N2/AA ca	0/8	<0.9	0/8	<0.9	0/8

Chen et al Vaccine 21: 4430-36; 2003

The Influenza H5 Hemagglutinin Gene

Avirulent	….RETR*GLF
Highly pathogenic	….RKKR*GLF
1997 HK human isolates	…RERRRKKR*GLF
2003/4 HK human isolates	…RERRRKKR*GLF

The presence of multiple basic amino acids adjacent to the HA cleavage site increases the tissue range of the virus in birds

Modifications Engineered into the HA Gene of HK/491/1997

HK/491/1997 wildtype HA:

```
CCT CAA AGA GAG AGA AGA AGA AAA AAG AGA ↓ GGA TTA TTT
Pro Glu Arg Glu Arg Arg Arg Lys Lys Arg   Gly Leu Phe
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Modified HA:

```
CCT CAA AGA GAG ACT                     CGA ↓ GGA TTA TTT
Pro Glu Arg Glu Thr                     Arg   Gly Leu Phe
```

↓ Site of cleavage of into HA1 and HA2 domains.

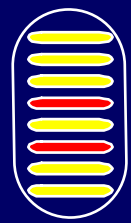
Subbarao et al Virology 305: 192-200; 2003

Strategy for the Generation of a Candidate Reassortant H5 Vaccine

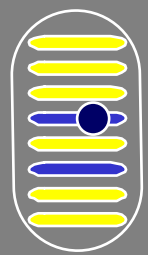
Modify the cleavage site of the H5 HA gene

N1 NA

Influenza Vaccine Donor Strain A/AA/6/60 ca OR A/PR/8/34



RERRRKKR → RETR



Reassortant modified H5N1 vaccine

Li et al JID 1999; 179: 1132-38; Subbarao et al Virology 2003

In Vitro Characterization of the H5N1/PR8 virus

Genetic Analysis:

- HA and NA genes sequenced
- HA gene lacks multibasic amino acid cleavage site motif
- Remaining genes characterized by RFLP and partial sequence analysis

Growth characteristics:

- Failure to plaque in MDBK cells in the absence of trypsin

Subbarao et al Virology 305: 192-200; 2003

Intravenously Administered H5N1/PR8 Virus Is Not Highly Pathogenic for Chickens

Virus	Mortality	Virus isolation on day 3				Sero conversion
		Oropharyngeal swabs		Cloacal swabs		
		Shedding /total	Mean Titer	Shedding /total	Mean Titer	
H5N1 wt	8/8	8/8	6.1	8/8	5.9	NA
PR8	0/8	1/8	0.94	6/8	2.9	7/8
H5N1/PR8	0/8	0/8	<0.9	0/8	<0.9	4/8

Subbarao et al Virology 305: 192-200; 2003

Intranasally Administered H5N1/PR8 Virus Replicates in Lungs But Is Not Lethal for Mice

Virus	Mean virus titer in lungs (log_{10} EID_{50})		LD_{50} (log_{10} EID_{50})
	Day 4	Day 6	
H5N1 wt	5.9	6.4	0.33
PR8	6.0	6.2	4.37
H5N1/PR8	5.7	5.5	>6

Subbarao et al Virology 305: 192-200; 2003

Immunogenicity and Protective Efficacy of Formalin Inactivated H5N1/PR8 Vaccine

Immunogen	HAI titer vs		Protection against challenge		
			Lung virus titer		
	Homologous	Heterologous	Homologous	Heterologous	Percent survival foll wt challenge
FI H5N1 wt	80	40	<0.8	0.9	100
FI H5N1/PR8	120	80	<0.8	<0.8	100
Live H5N1 wt	ND	ND	<0.8	<0.8	100
PBS	10	10	6.2	5.1	0

Subbarao et al Virology 305: 192-200; 2003

Biosafety Measures Used for Generation of Pandemic Influenza Vaccines

- The reassortant virus is generated at the biosafety level recommended for work with the wild-type virus.
- Risk assessment data are generated regarding *in vitro* and *in vivo* properties of the recombinant (modified?) candidate vaccine virus
- Based on risk assessment data, the biosafety level required for manufacture of the vaccine virus may be reduced