GEIG
Groupe d'Expertise et d'Information sur la Grippe

Mercredi 9
novembre 2011
Salons de
l'Aéro-Club de France
6, rue Galilée, 75116 Paris

24e RENCONTRES SUR LA GRIPPE ET SA PRÉVENTION

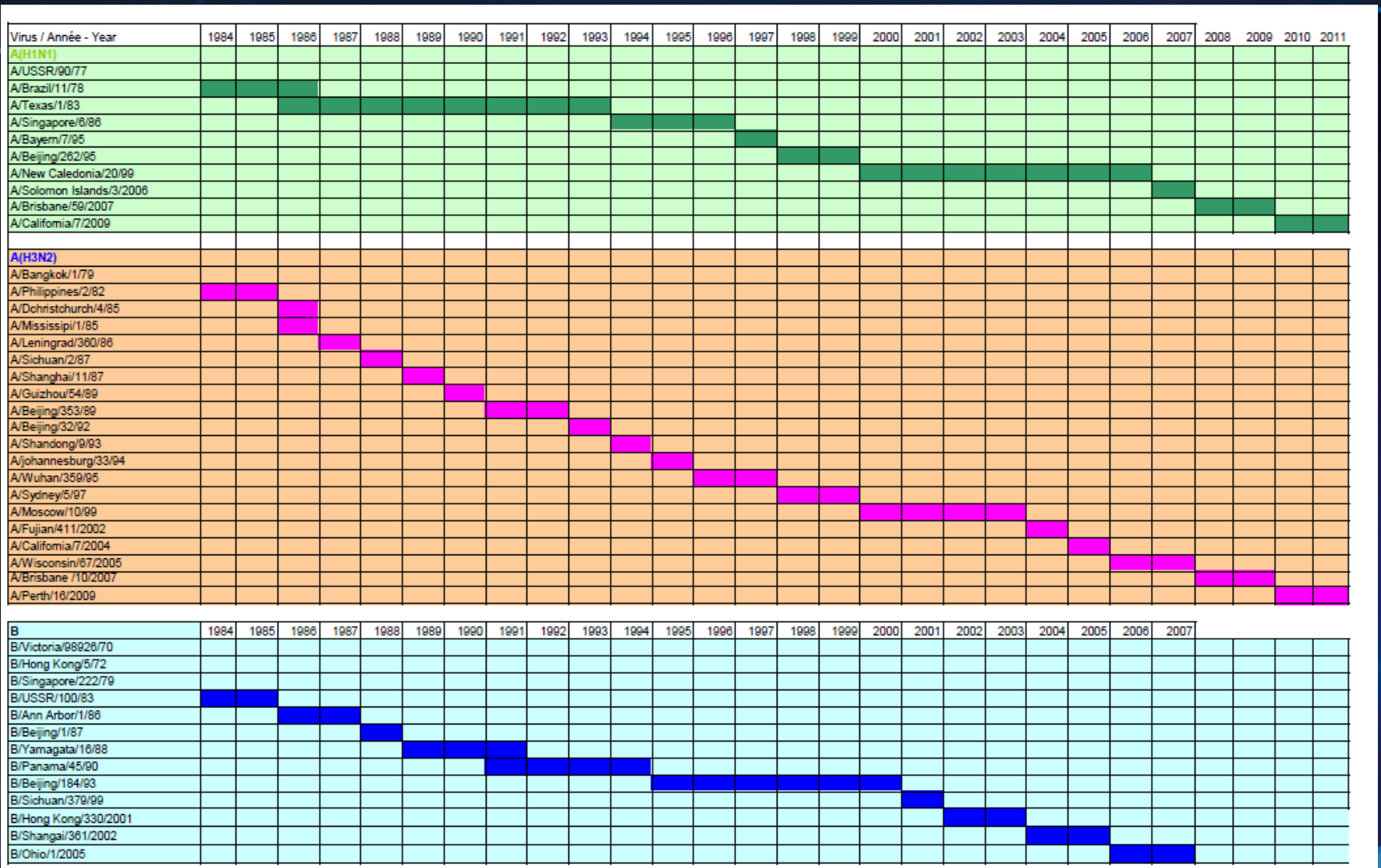
ATTENUATED INFLUENZA VACCINES

E NICAND

- Development of vaccines by reverse genetics (LAIV)
- Immunology of LAIV
- Avantages and disavantages

Vaccine strain selection

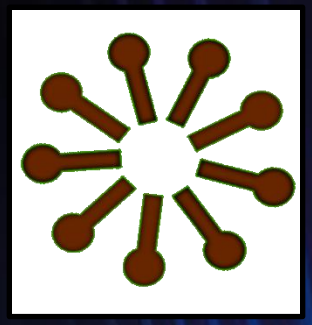
Northern Hemisphere (1984-2011)



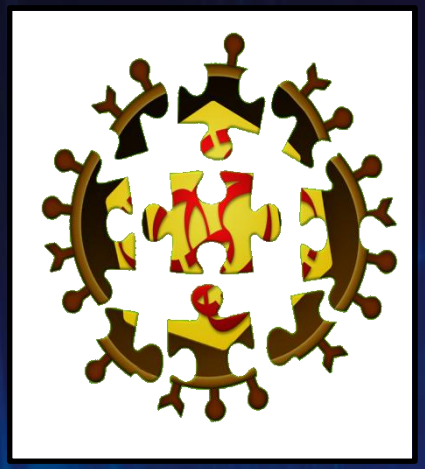
Current Influenza vaccines (TIV)

Sub-unit

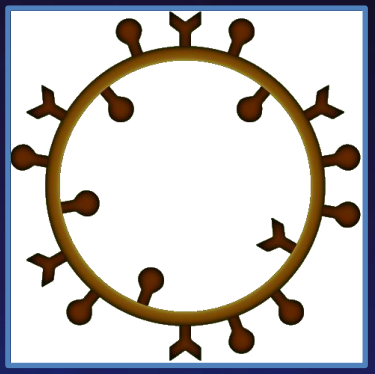
Surface antigens



Split virion inactivated



Virosomal



Adjuvant

Courtesy by Catherine Weil Ollivier

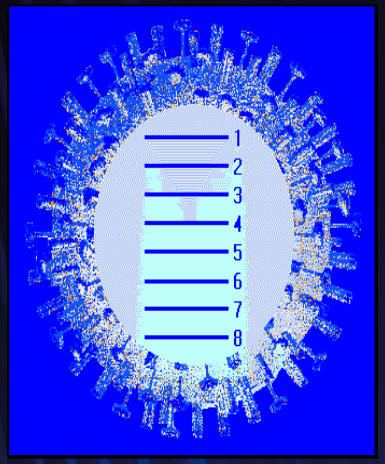
Live attenuated influenza vaccines

- Effective, safe, stable vaccines
- Local immunity in the upper respiratory tract (intranasal deliverance)
- Provide immunity similar to natural infection

- Update vaccines with the HA , NA genes (circulating strains)
- Attenuation phenotype: *ts*, *ca*, *att*
  - Master Donor strain

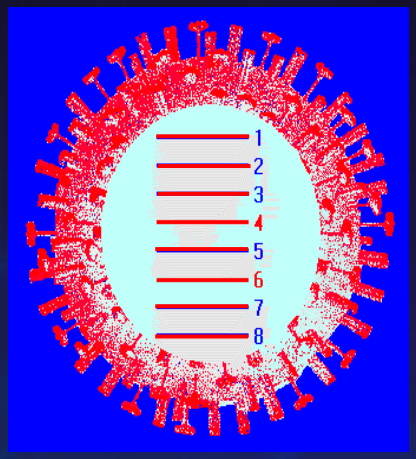
Host range variants

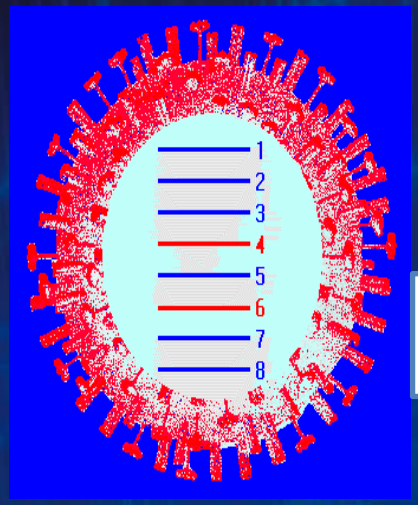
Master Donor strain



A/Ann Arbor/6/60
B/Ann Arbor/1/66

wt seasonal Influenza virus



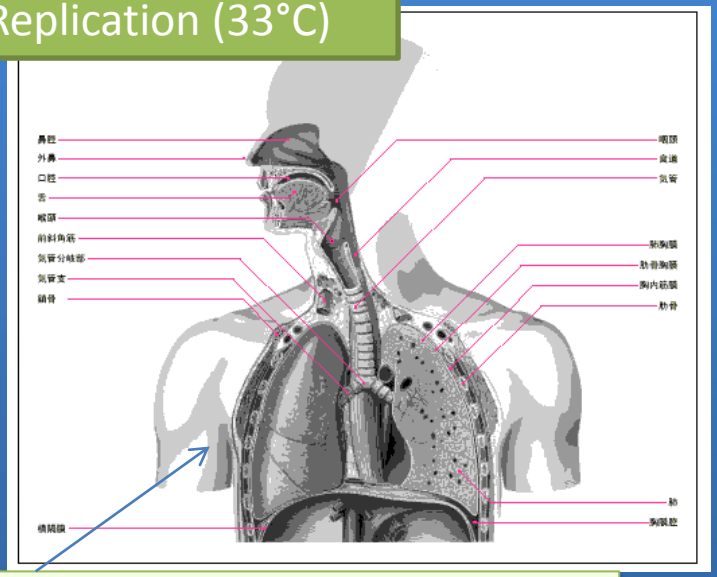


6:2 reassortant virus
PB2 gene: Host range restriction phenotype

Phenotype of 6:2 reassortant (*ts*)

- Temperature sensitive vaccines (*ts* phenotype)

Replication (33°C)



Restricted replication (37-39°C)

- Type A:
  - 39°C shut off temperature of replication
  - 10^{-2} permissive replication at 33°C
- Type B:
  - 37°C shut off temperature of replication
  - 10^{-2} permissive replication at 33°C

PB2 gene: 112+265+556 (aa)

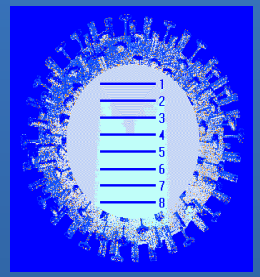
PB1 (391, 457, 581),NP genes (34)

Phenotype of 6:2 reassortant (*ca*)

- Cold- adapted vaccines

(*ca* phenotype)

Virulence



x 25°C -33°C

(32 stepwise passages)

Genetic basis of attenuation and stability of *ca* vaccines: 11 aa substitutions in 6 gene products

Table 1 Amino acid sequence comparison of influenza A wt and ca master donor strains

Gene product	A/Ann Arbor/6/60[a]			A/Leningrad/134/57[b]			
	Amino acid			Amino acid			
	Residue	wt	ca	Residue	wt	ca 17	ca 47
PB2	265[c]	N	S	478	V	L	L
				490	S	–	R
PB1	391	K	E	265	L	N	N
	457	E	D	317	M	–	I
	581	E	G	591	V	I	I
	661	A	T				
PA	613	K	E	28	L	P	P
	715	L	P	341	V	L	L
NP	23	T	N	341	I	–	I
	34	N	G				
M1	–			15	I	V	V
M2	86	A	S	86	A	T	T
NS1	153	A	T	–			
NS2	–			100	M	I	I

[a] From Cox et al. 1988 and Herlocher et al. 1996.

[b] From Klimov et al. 1992.

[c] Amino acids in bold are those associated with *ts* phenotype as defined in Jin et al. 2003.

Influenza vaccines generated by reverse genetics (Subbarao et al., CDC)

Phenotype of 6:2 reassortant

- Attenuated vaccine (*att* phenotype)

  Ferrets (level replication)

  - 10^{-3}

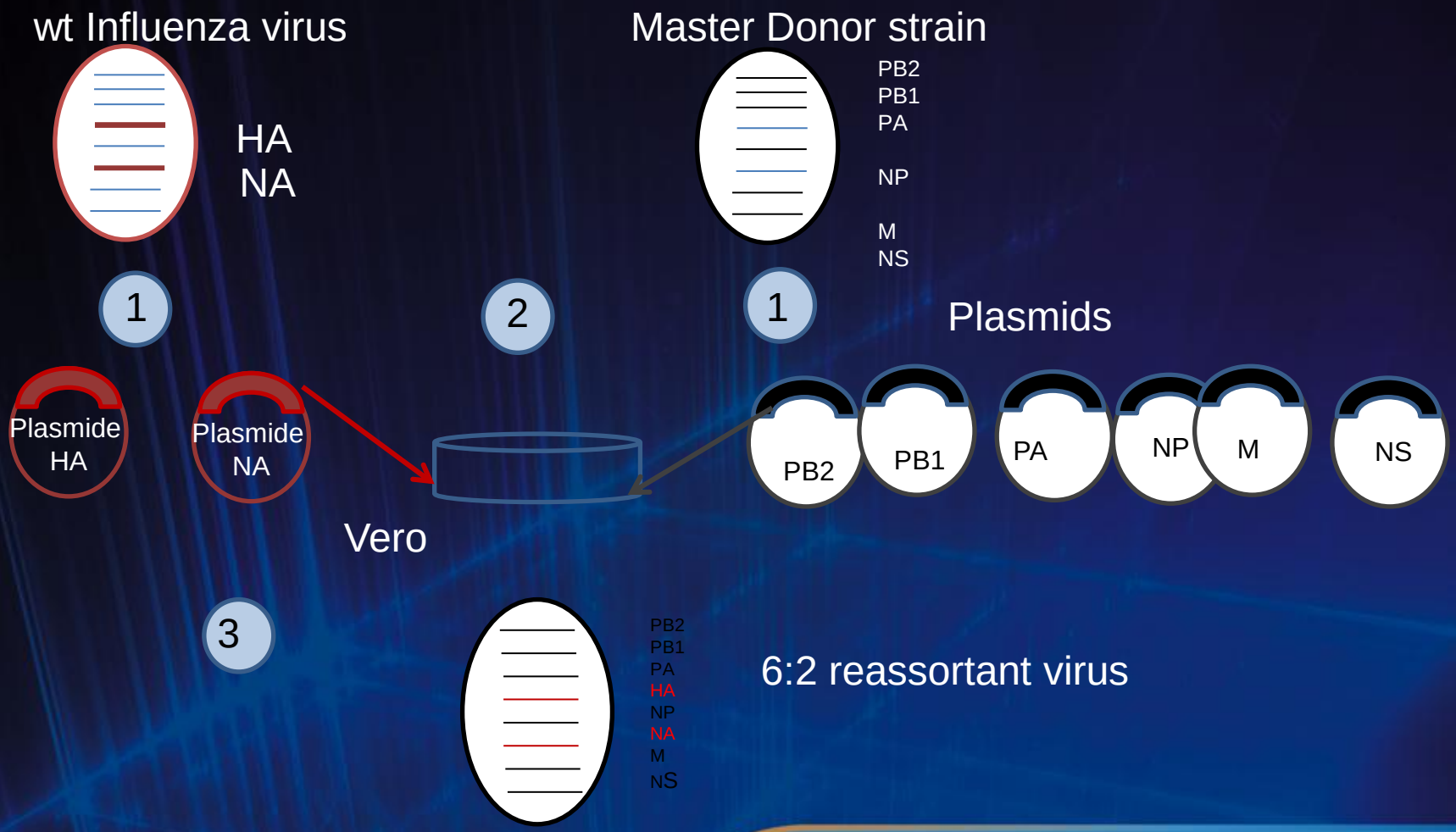
- Doses of immunogenicity and safety: 10^7 $TCID_{50}$

- Genetic stability
  - PB1,PB2,PA genes in *ca* or *ts* phenotypes (clinical studies in children)
  - 10^{-20} replication cycles

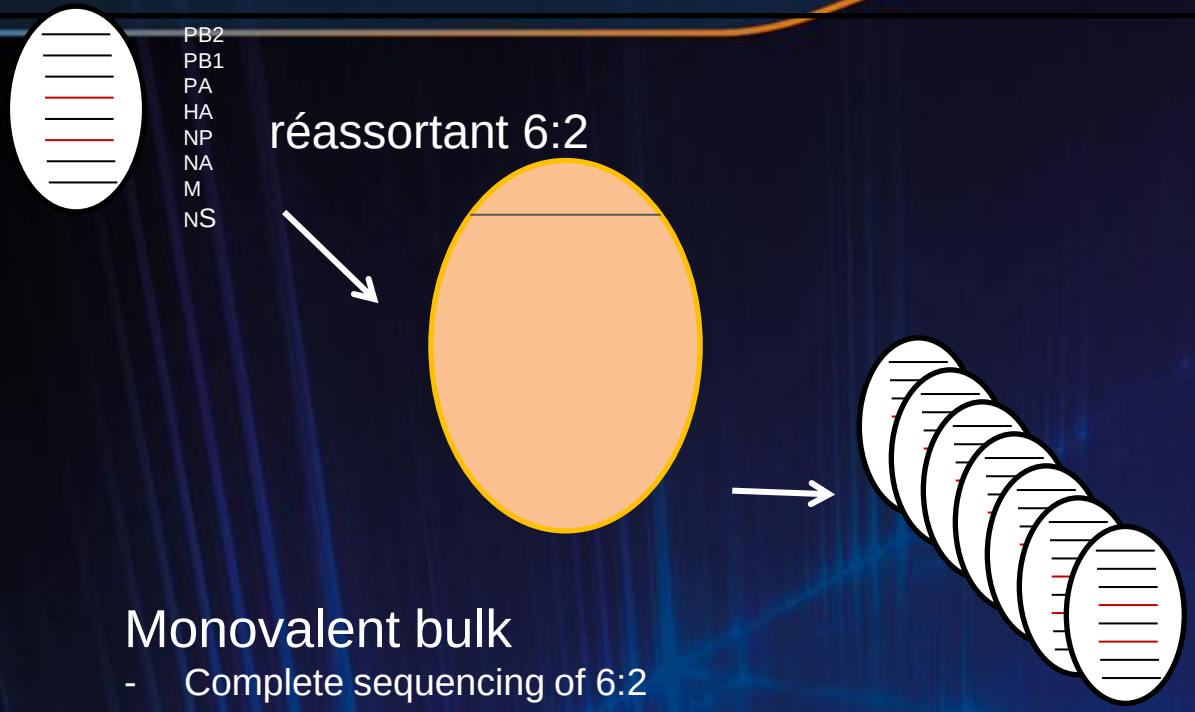
  (Kamps et al 2006)

Chen et al., Virology 2001

Reverse genetics application to influenza vaccine development



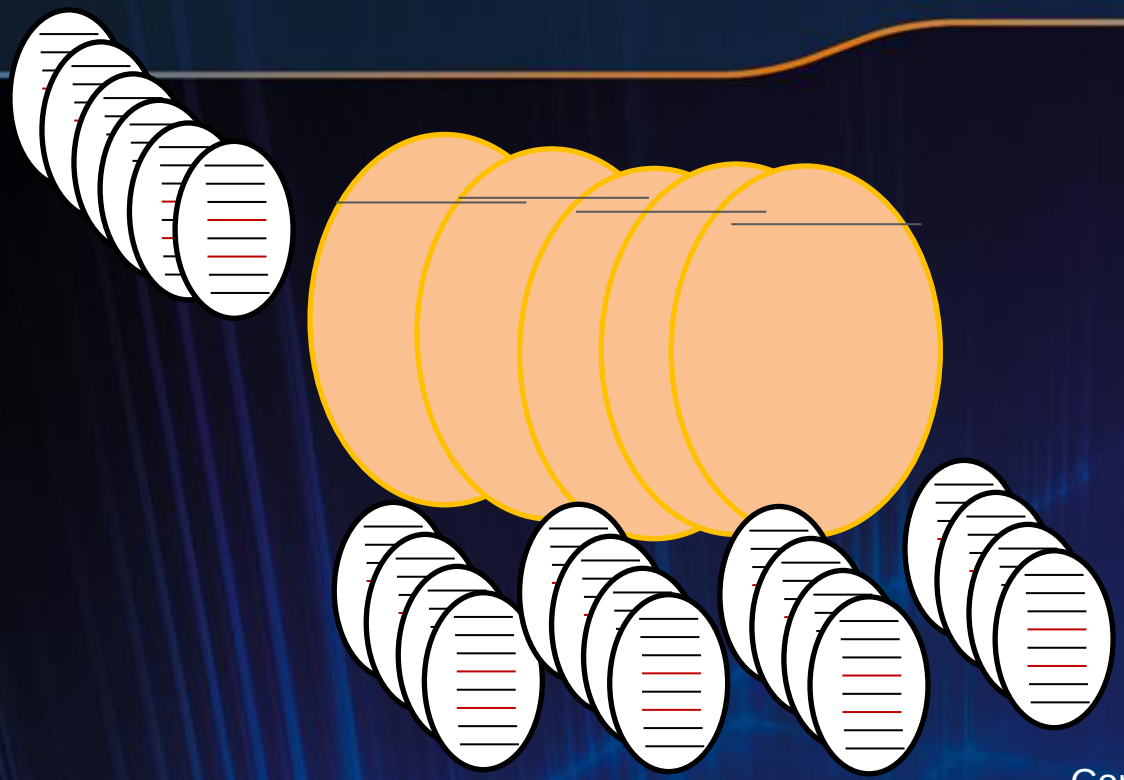
Manufacturing process of trivalent LAIV (1)



Monovalent bulk

- Complete sequencing of 6:2
- Characterization of Ha, N
- Attenuate tests(furet)
- Genetic stability(5 passages successifs)

Manufacturing process of trivalent LAIV (2)



Monovalent Bulks (-60%)

- Complete sequencing of 6:2
- Characterization of Ha, N
- Attenuate tests(furet)
- Genetic stability(5 passages successifs)

H1
H3
B

$10^{6,5-7,5}$ UFF

Immune response of LAIV

Immune response of trivalent LAIV

- Mucosal antibody response

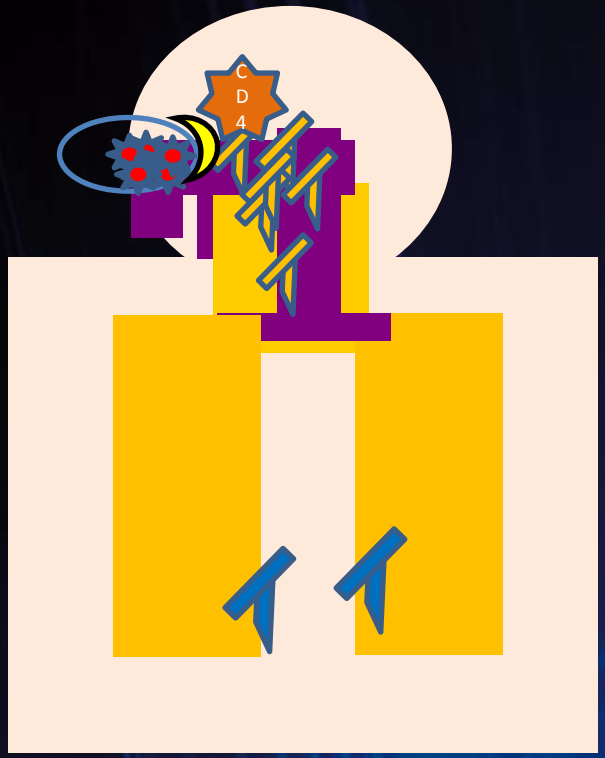


TABLE 3. Persistence of serum and nasal-wash HA antibodies induced by live cold-adapted or inactivated virus vaccines

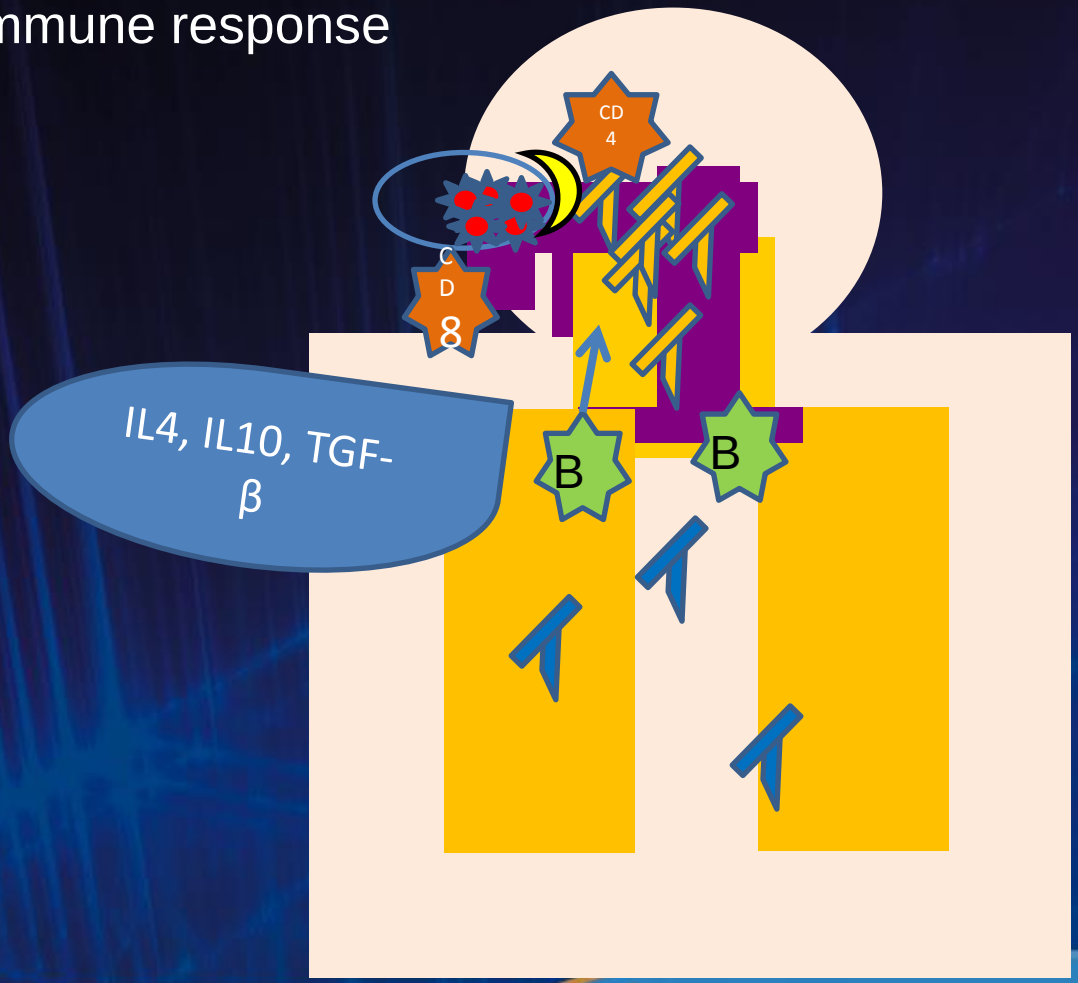
Vaccine administered	Proportion (%) of vaccine responders with persistently elevated antibody titers[a] at 28 weeks after vaccination			
	Serum		Nasal wash	
	IgG	IgA	IgG	IgA
Live H3N2	16/22 (73)	22/24 (92)	11/18 (61)	13/23 (56)
Inactivated H3N2	27/30 (90)	28/31 (90)	20/28 (71)	6/13 (46)
Live H1N1	11/14 (79)	12/16 (75)	12/14 (86)	6/16 (38)
Inactivated H1N1	18/18 (100)	15/15 (100)	16/17 (94)	2/6 (33)

[a] Persistently elevated titers were defined as a fourfold or greater increase between prevaccination and 28-week postvaccination titers.

Clements et al. J Clin Microbiol, 2003

Immune response of trivalent LAIV

- Cellular immune response



Advantages and disadvantages of LAIV

- Genetically modified organism:
  - Specificity to humans
  - No carriage of a toxic transgene
  - No replication in the environnement
  - well tolerance
- Reassortants between LAIV strains
  - Wt A/Sydney/5/97 and corresponding LAIV vaccine strains
    - 256 recombinant strains
    - 2^{8} potential combinations
    - Focus on RNP, PB2-PB1,PA,NP, NS,M

Reassortants between LAIV strains

Summary of reassortant virus phenotypes

Virus	Gene segment origin[a]								ts^b phenotype	npl^b phenotype	att^b phenotype
	PB2	PB1	PA	HA	NP	NA	M	NS			
Group I											
wt	PB2	PB1	PA	HA	NP	NA	M	NS	–	–	–
17	PB2	PB1	PA	HA	NP	NA	M	NS	–	–	–
Group II											
1	PB2	PB1	PA	HA	NP	NA	M	NS	–	–	*att*(+/–)
2	PB2	PB1	PA	HA	NP	NA	M	NS	–	–	*att*(+/–)
3	PB2	PB1	PA	HA	NP	NA	M	NS	–	–	*att*(+/–)
4	PB2	PB1	PA	HA	NP	NA	M	NS	–	–	*att*(+/–)
6	PB2	PB1	PA	HA	NP	NA	M	NS	–	–	*att*(+/–)
8	PB2	PB1	PA	HA	NP	NA	M	NS	–	–	*att*(+/–)
14	PB2	PB1	PA	HA	NP	NA	M	NS	–	–	*att*(+/–)
Group III											
16	PB2	PB1	PA	HA	NP	NA	M	NS	–	–	*att*
18	PB2	PB1	PA	HA	NP	NA	M	NS	–	–	*att*
32	PB2	PB1	PA	HA	NP	NA	M	NS	–	–	*att*
31	PB2	PB1	PA	HA	NP	NA	M	NS	–	–	*att*
30	PB2	PB1	PA	HA	NP	NA	M	NS	–	–	*att*
27	PB2	PB1	PA	HA	NP	NA	M	NS	–	–	*att*
Group IV											
7	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	–	*att*
9	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	–	*att*
10	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	–	*att*
13	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	–	*att*
29	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	–	*att*
25	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	–	*att*
26	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	–	*att*
23	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	–	*att*
19	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	–	*att*
Group V											
5	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	*npl*	*att*
11	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	*npl*	*att*
12	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	*npl*	*att*
15	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	*npl*	*att*
34	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	*npl*	*att*
33	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	*npl*	*att*
24	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	*npl*	*att*
28	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	*npl*	*att*
20	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	*npl*	*att*
21	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	*npl*	*att*
22	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	*npl*	*att*
vac	PB2	PB1	PA	HA	NP	NA	M	NS	*ts*	*npl*	*att*
	Associated phenotype (Table 1)										
	att *ts*	*att* *ts*	*att*		*att* *ts*		*att*				

[a] Gene segments is color coded to indicate wt (red) and vaccine (blue) as described in Table 2.

Buonagurio et al., Vaccine 2006

Advantages and disadvantages of LAIV

- Development of pandemic influenza vaccine: H5N VN 2004 *ca*,H5N1 HK 2003 *ca*

Table 3
Summary of virus shedding and immunological responses to $10^{7.5}$ $TCID_{50}$ of H5N1 VN 2004/AA *ca*.

Subject no.	Culture	rRT-PCR	HI Ab	Neut Ab	Serum IgG	Serum IgA	NW IgA
22	–	–	–	–	–	–	–
23	–	+	–	–	–	–	–
24	–	–	–	–	–	–	–
25	–	–	–	–	–	+	+
26	–	+	–	–	–	–	+
27	–	+	–	–	+	+	–
28	–	+	+	–	+	–	–
29	–	+	–	–	+	+	+
30	–	+	–	–	–	–	–
31[a]	–	–	–	–	–	–	–
32	–	+	–	–	+	+	–
33	–	+	–	–	–	–	–
34	–	+	–	–	–	+	–
35	–	+	–	–	–	–	–
36	+	+	–	–	+	–	–
37	–	+	–	–	–	+	–
38	+	+	+	+	+	+	+
39	–	+	–	–	–	+	–
40	–	–	–	–	–	+	–
41[a]	–	–	–	–	+	+	–
42	–	+	–	–	+	+	–
Total	2/21	15/21	2/21	1/21	8/21	11/21	4/21

Abbreviations used are as follows: No.: number; HI: hemagglutination inhibition assay; Neut: microneutralization assay; Ab: antibody; NW: nasal wash. Represents responses following either dose of vaccine.

[a] These subjects received only 1 dose of vaccine.

(n= 59 participants, 2 doses
Karron et al., Vaccine 2009

Advantages and disadvantages of LAIV

- Applications to generation of pandemic strains vaccine

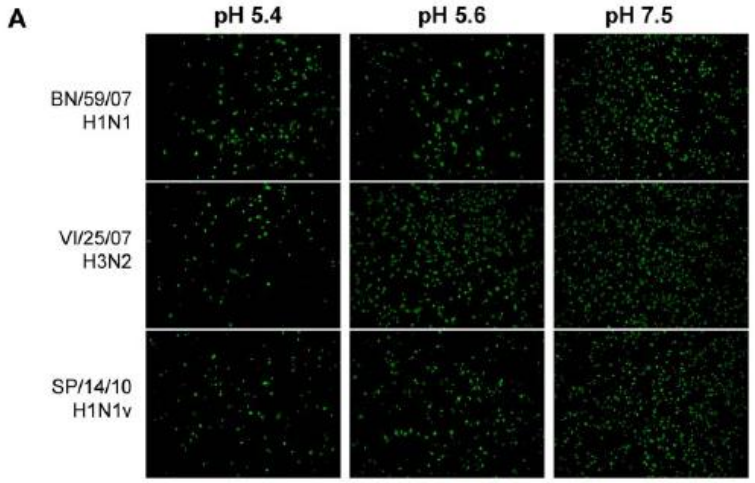
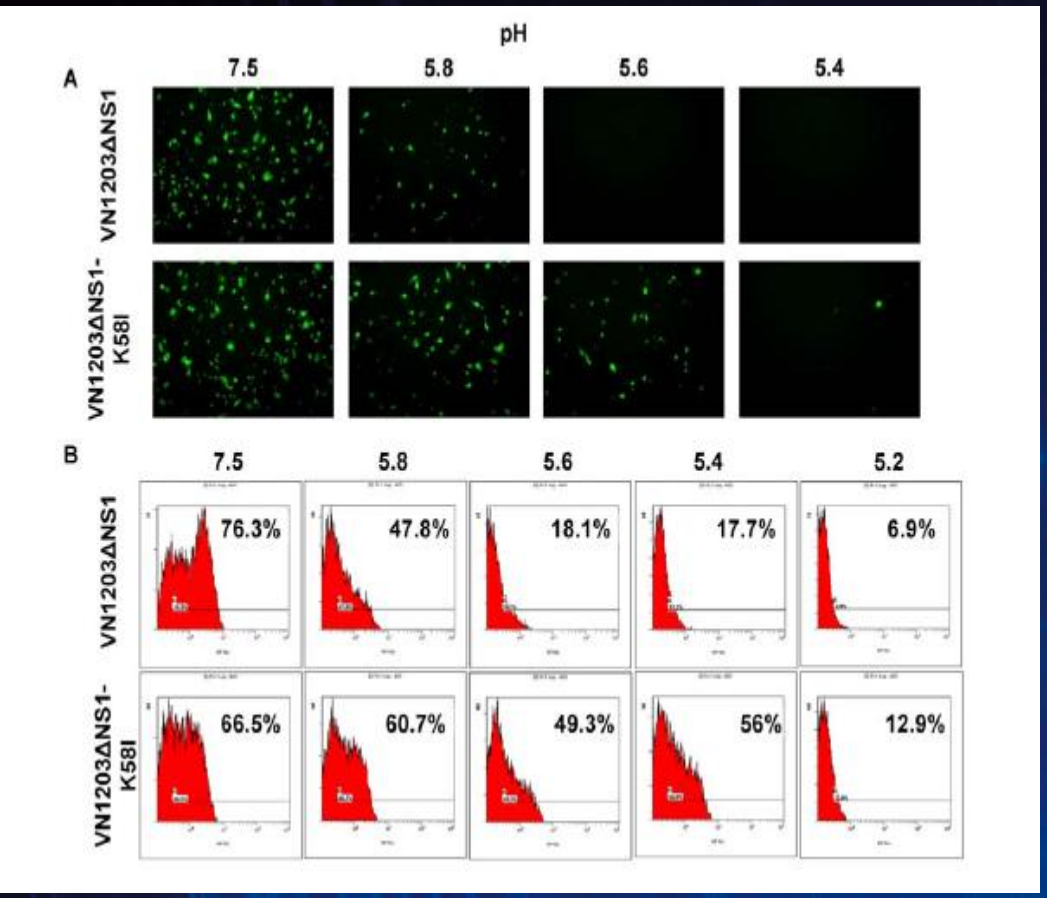


Figure 1. Infectivity of human and highly pathogenic avian viruses at an acidic pH in HNEpCs. Primary Human Nasal Epithelial cells were infected with human (A) epidemic BN/59/07 (H1N1), VI/25/07 (H3N2), SP/14/10 (H1N1v) or avian (B) highly pathogenic viruses VN/1203 (H5N1) and TH/01/04 (H5N1) at the indicated pH values. Influenza NP protein was visualized by immunostaining after incubating for 5 h.
doi:10.1371/journal.pone.0018577.g001

Krenn et al. PlosOne 2011

Infectious activity at acidic pH of LAIV candidate H5: mutation in HA gene (K58I)



- Influenza NP activity in nasal epithelial cells (mice)
- Replication efficiency in cell culture (MDCK)

Krenn et al. PlosOne 2011

LAIV

- Efficacy and effectiveness of intranasal LAIV:
  - Subsequent replication in cells of the upper respiratory tract
- Uptake vaccine formulation
- Limited genetic changes following replication in vaccine recipients without vaccine attenuation