St. Jude Director's Rounds:
02 June 2006

Influenza from Genome to Vaccine

Robert G. Webster, PhD
Clayton Naeve, PhD
Jie Zheng, PhD
Richard Webby, PhD

www.Cure4Kids.org

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Influenza from Genome to Vaccine

Introduced by
William E. Evans, PharmD
Director
St. Jude Children's Research Hospital

www.Cure4Kids.org

Danny Thomas Lecture Series – Director's Rounds

Influenza from genome to vaccine

Rapid response. Robert Webster (left) and Richard Webby work to trace the dramatic evolution of swine flu in North America.

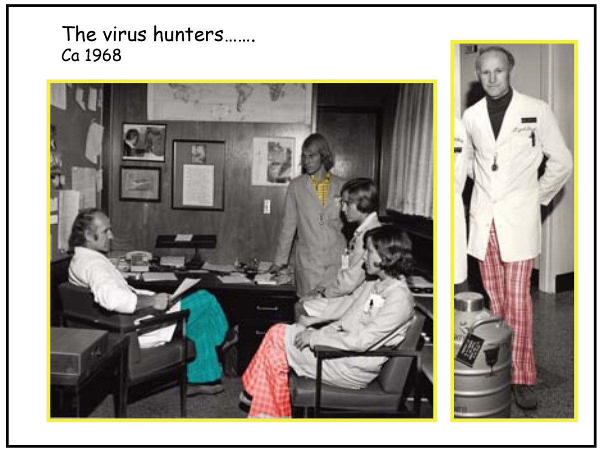
St. Jude Children's Research Hospital

The virus hunters…….
Ca 1968

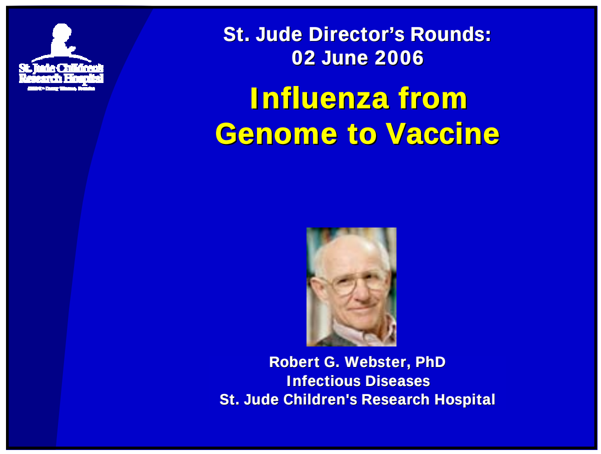
The CPU hunter…….
Ca 1984

EVOLUTION


News Focus
Changes on the farm may be fostering the evolution of the swine flu virus—and if a dangerous new strain crosses back into humans, it could have deadly consequences
Chasing the Fickle Swine Flu
Rapid response. Robert Webster (left) and Richard Webby work to trace the dramatic evolution of swine flu in North America.
SCIENCE VOL 299 7 MARCH 2003


The virus hunters…….
Ca 1968


St. Jude Children's Research Hospital
HARTWELLCENTER
for BIOINFORMATICS & BIOTECHNOLOGY
Large-Scale Sequence Analysis of Avian Influenza Isolates
John C. Obenauer,[1] Jackie Denson,[1] Perdeep K. Mehta,[1] Xiaoping Su,[1] Suraj Mukatira,[1] David B. Finkelstein,[1] Xiequn Xu,[1] Jinhua Wang,[1] Jing Ma,[1] Yiping Fan,[1] Karen M. Rakestraw,[1] Robert G. Webster,[2,4] Erich Hoffmann,[2] Scott Krauss,[2] Jie Zheng,[3] Ziwei Zhang,[3] Clayton W. Naeve[1,4*]
Science
Detection Technologies
AAAS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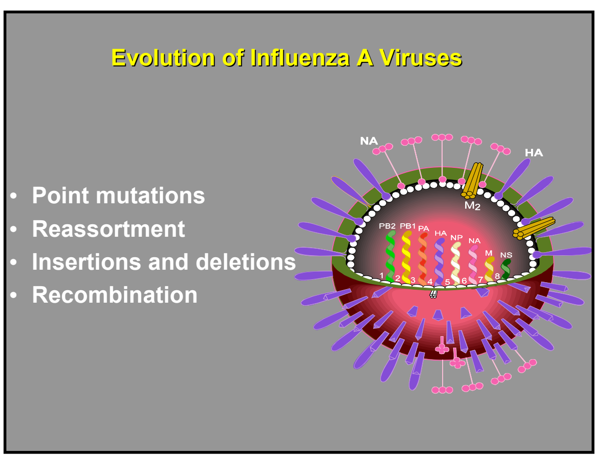
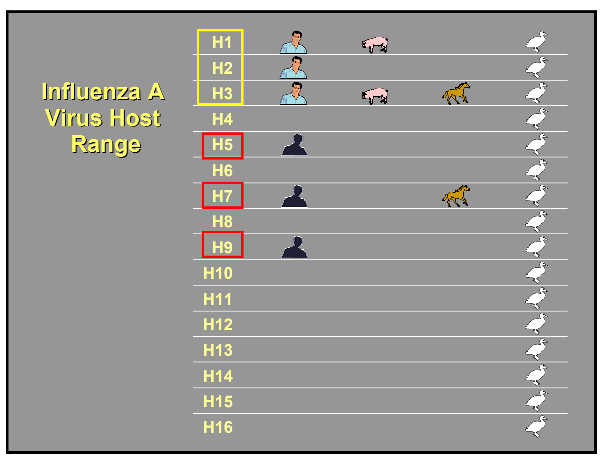
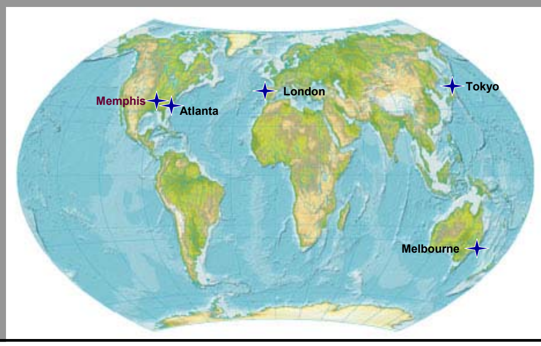
Influenza from
Genome to Vaccine
Robert G. Webster, PhD
Infectious Diseases
St. Jude Children's Research Hospital


Evolution of Influenza A Viruses
• Point mutations
• Reassortment
• Insertions and deletions
• Recombination
NA
HA
M2
PB2 PB1 PA HA NP NA M NS
1 2 3 4 5 6 7 8


Influenza A
Virus Host
Range
H1
H2
H3
H4
H5
H6
H7
H8
H9
H10
H11
H12
H13
H14
H15
H16

WHO Collaborating Centers for Reference & Research on Influenza

4 Human/1 Animal



Influenza Global Organizations

Global:	WHO	►	Geneva
	FAO	►	Rome
	OIE	►	Paris

Humans: ***Annual Epidemics***

➢Vaccines H1N1, H3N2, B

Pandemics

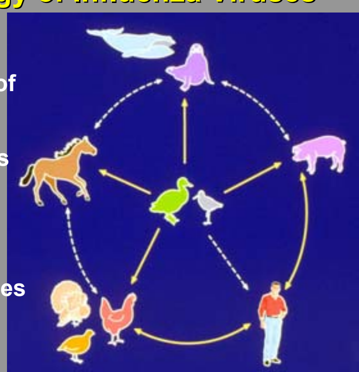
➢2 to 3 per century

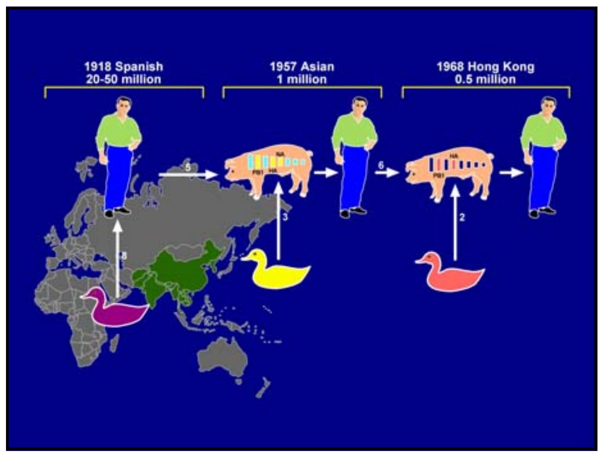
Lower Animals: ➢Origins and Spread in Wild Birds

➢Disease in domestic poultry, pigs, horses

The Ecology of Influenza Viruses

- That there are a limited number of host specific lineages of influenza viruses
- There is geographical separation into Eurasian and American lineages





Spread of H5N1 Influenza in Asia 2004 2006

➢ 1996 H5N1 in geese in Guangdong

➢1997 6 of 18 humans die of H5N1

➢2002/3 Re-emergence and spread in Asia

➢2005 – May – H5N1 kills Barheaded geese in Qinghai China

Current status:
Human Cases: 224
Human Deaths: 127

Spread of H5N1

How Did the H5N1 Virus Spread Westwards So Quickly

- Migratory birds/globalized poultry industry
- Cold winter, Baltic Sea froze
- Europe – 700 wild bird outbreaks
  - –4 commercial bird outbreaks
- Africa
  - –Introduction to Nigeria
  - –Not in wild birds
  - –Will it burn out?

"The Trojan Horses"

How Pathogenic Is This Virus?

A/Vietnam/1203/04 (H5N1)

- Kills chickens in less than one day
- Kills ducks in 1 2days
- High risk of death in humans
  - Diarrhea
  - Respiratory symptoms
- High Risk of death in ferrets
  - Respiratory symptoms
  - Diarrhea
  - Hind Leg paralysis

Does H5N1/04 Replicate and Transmit in Pigs?

	Infected	Contact
Vietnam/1203/04	+++	0
Ck/Vietnam/C-58/04	+++	0
DK/TH/D4AT/04	+++	0
GS/TH/G7CS/04	+++	0

Choi et al 2005

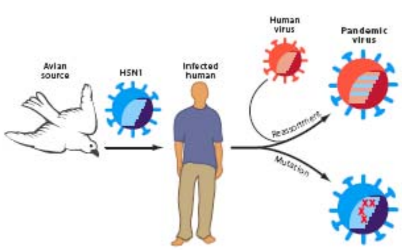
Expanding Host Range for Influenza

H5N1 in Thailand

Experimental transmission in domestic cats

Kuiken et al, Science 2004

Will H5N1 Acquire Transmissibility?



HAPPY NEW YEAR OF THE DOG

Acknowledgements

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St. Jude Children's Research Hospital
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Influenza Research Group

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Vietnamese Ministry of Agriculture and Rural Health Development
Dr. TD Nguyen

Thailand Bureau of Disease Control and Veterinary Services
Dr. Chantanee Buranathai

Kasetsart University, Kamphaengsaen Campus
Dr. Thaweesak Songserm

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Influenza from Genome to Vaccine

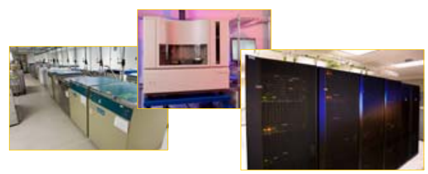
Clayton W. Naeve, PhD
Hartwell Center
St. Jude Children's Research Hospital

The St. Jude Influenza Genome Project

Clayton W. Naeve, Ph.D.
Director's Rounds
June 2, 2006

The St. Jude Influenza Genome Project

- The first large-scale bird flu genomics effort - launched 11/1/2005
  - Dr. Robert Webster's avian influenza virus collection
  - Hartwell Center biotechnology/bioinformatics expertise/resources
  - Computational Horsepower



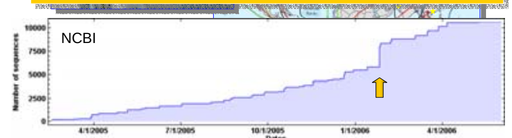
The St. Jude Influenza Genome Project

www.stjudeflugene.org

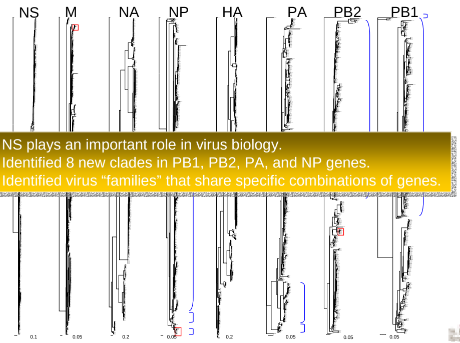
The St. Jude Influenza Genome Project

SJ Influenza Virus Repository

- Sampled 417 viruses (all known serotypes)
- Synthesized 534 segment-specific primers
- Sequenced 124,000 templates
- Produced 58,550,436 bp raw data
- Assembled 2,823 complete genes (4,719,005 bp finished sequence)
- Assembled 250 complete genomes

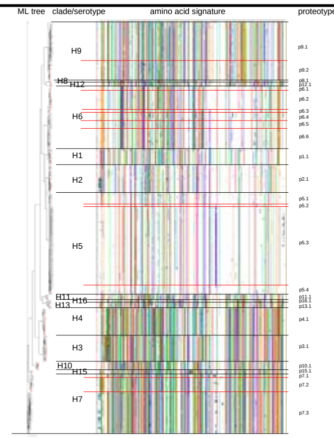


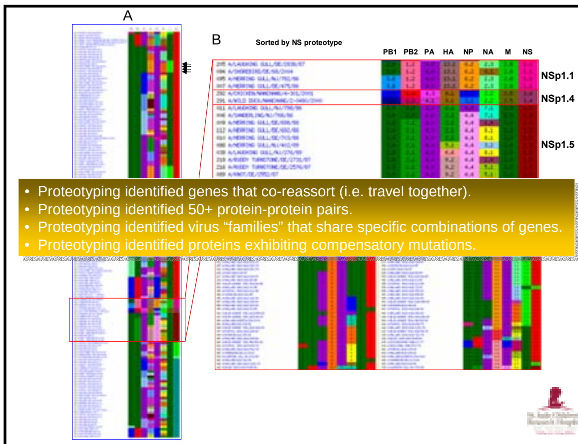
The St. Jude Influenza Genome Project



- NS plays an important role in virus biology.
- Identified 8 new clades in PB1, PB2, PA, and NP genes.
- Identified virus “families” that share specific combinations of genes.

HA All Avian
ML tree
692 sequences
57.7% conserved





- Proteotyping identified genes that co-reassort (i.e. travel together).
- Proteotyping identified 50+ protein-protein pairs.
- Proteotyping identified virus “families” that share specific combinations of genes.
- Proteotyping identified proteins exhibiting compensatory mutations.

The St. Jude Influenza Genome Project

NS1 contains a PDZ ligand

- High level of variability relative to other core proteins.
- Many examples of specific proteotype pairs (non-random reassortment and/or compensatory mutations) among core proteins.
- Surprising conservation of core gene combinations revealed by specific proteotype pairs.
- Normal function: abrogate the host cell antiviral response (IFN, NF-κB, PKR).

```
>002NS
MDSNTVSSFQVDCFLWHVRKRFADQELGDAPFLDRLRRDQKSLRGRGSTLGLDIETATRAGKQIVERILE
EESDEALKMTIASVPASRYLTDMTLEEMSRDWFMLMPKQKVAGSLCIRMDQAIMDKNIILKANFSVIFDR
LETLILLRAFTEEGAIVGEISPLPSLPGHTDEDVKNAIGVLIGGLEWNDNTVRVSETLQRFAWRSSNEDG
RPPLPPKQKRKMARTIESEV
```

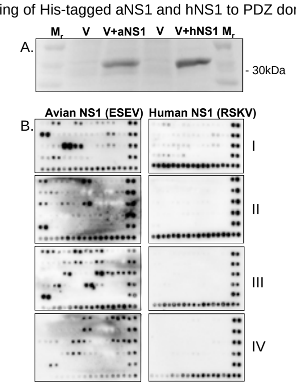
The St. Jude Influenza Genome Project

Distribution of PL in 1,196 NS1 proteins

PL	Avian	Human	Swine	Equine	-3
	5	2	4		
EPEV	23	21	1	14	
EPKV				1	
ESEI	48		4		
ESEV	**483**	12	7	10	
ESKV	55				E/G
GPEV	2	1	19	2	
GPKV			6		
GSEA	1				
GSEI			1		
GSEV	4		4		
GSKV	1	2			
KSEV		1			
RPKV		1			
RSEA			1		
RSEV		22	4		R/K
RSKI		9			
RSKV		**403**	21		
TSEV			1		
Totals	**622**	**474**	**73**	**27**	

The St. Jude Influenza Genome Project

Binding of His-tagged aNS1 and hNS1 to PDZ domains



The St. Jude Influenza Genome Project

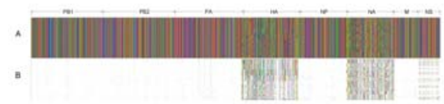
- A completely novel mechanism by which avian influenza interacts with cells.
- aNS1 in combination with HA, and perhaps other genes, contributes to high virulence of H5N1 strains.

Name	Spanish Flu	Asian Flu	Hong Kong Flu	"Bird Flu"	"Bird Flu"	"Bird Flu"	"Bird Flu"	"Bird Flu"	Seasonal Flu
Distribution	Pandemic	Pandemic	Pandemic	Hong Kong	Hong Kong	Netherlands	Hong Kong	SE Asia	
Serotype	H1N1	H2N2	H3N2	H5N1	H9N2	H7N7	H5N1	H5N1	
Year	1918	1957	1968	1997	1999	2003	2003	2004	
Excess Deaths - USA	500,000	70,000	34,000	6 of 18	0 of 2	1 of 83	1 of 2	100 of 200	10-30000
Mortality Rate	2.50%	<2.0%	<2.0%	33%	0%	0.01%	50%	51%	0.01%
PL	KSEV	RSKV	RSKV	EPEV	no PL	EPEV GPEV	ESEV ESEV	ESEV	RSKV
PDZ binding of 123 tested	12	0	0	20	?	20	30	30	0
PDZ binding Dsh by NMR	++	-	-	++		++	++	++	-

Obenauer et al., Large Scale Sequence Analysis of Avian Influenza Isolates
Science 311:1576-1580, 2006

The St. Jude Influenza Genome Project

- Bioinformatics analysis
  - Comparative genomics of avian and human influenza
  - Co-evolution analyses
  - SNP analyses
- Role of PDZ proteins in influenza infection
  - Characterization of PDZ ligand by NS1 mutagenesis
  - Identification of cell NS1 binding partners using proteomics methods.
  - Effect of avian vs human NS proteins on cell pathways (alone and in combination with other viral proteins)



The St. Jude Influenza Genome Project

- **Hartwell Center for Bioinformatics & Biotechnology**
  - John Obenauer, Ph.D.
  - Jackie Denson
  - Perdeep Mehta, Ph.D.
  - Xiaoping Su, Ph.D.
  - Suraj Mukatira, Ph.D.
  - David Finkelstein, Ph.D.
  - Tony Xu, Ph.D.
  - Jinhua Wang, Ph.D.
  - Jing Ma, Ph.D.
  - Yiping Fan, Ph.D.
  - Karen Rakestraw
  - Jianling Armstrong
  - Stephanie Tate
  - Caroline Aldridge
  - Momodou Sanyang
  - Scott Olsen
  - Patrick Rodriquez
  - Bob Cassell
- **Robert G. Webster, Ph.D., Infectious Diseases Virology Division**
  - Erich Hoffman, Ph.D.
  - Scott Krauss
- **Jie Zheng, Ph.D., Structural Biology**
  - Ziwei Zhang
- **Funded by ALSAC, CCSG, US Public Health Service, and the Hartwell Foundation**

St. Jude Director's Rounds:
02 June 2006

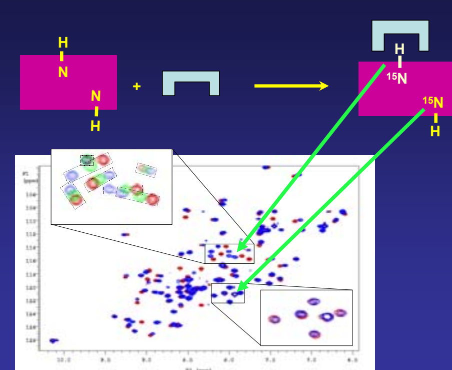
Influenza from Genome to Vaccine

Jie Zheng, PhD
Structural Biology
St. Jude Children's Research Hospital

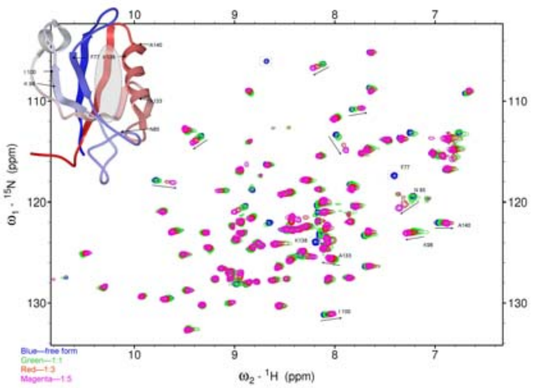
PDZ domains

- PDZ domains are one of the most commonly found protein-protein interaction domains in organisms from bacteria to humans. Typically, PDZ domains play important roles assembling signal pathway components into large supramolecular complexes.
- The PDZ domain is a small protein interaction module that specifically recognizes the carboxyl terminus of proteins.
- In our laboratory, we have studied the PDZ domain of Dishevelled, the first PDZ domain of PSD95 and the seventh PDZ domain of GRIP1.

Chemical Shift Perturbation



PSD95 PDZ1 / GSESEV



NMR studies showed that the C-terminal peptides of NS1 proteins interact with PDZ domains at the conversional peptide binding site with different binding affinities.

	GSESEV (2003)	GSEPEV (1997)	GSKSEV (1918)	GSRSKV (low-pathogenic)
Dsh	++	++	++	-
PSD-95	+++	±	++	±
GRIP1	-	-	-	-

Future studies

- *Functional studies* - In humans, there are about 800 PDZ domains within about 400 proteins. Using computational, protein array, NMR and other biophysical approaches, we want to identify the “true” binding partner(s) of NS1 proteins.
- *Diagnostic tool* - Combining mutagenesis with our structural biology studies, we would like to design ‘super PDZ domains’ to recognize specific NS1 proteins with high binding affinity.

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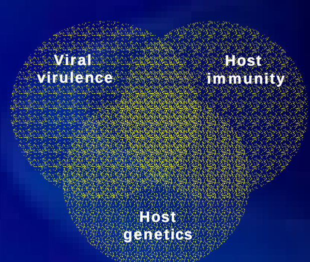
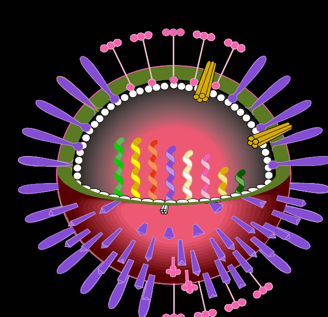
Influenza from Genome to Vaccine

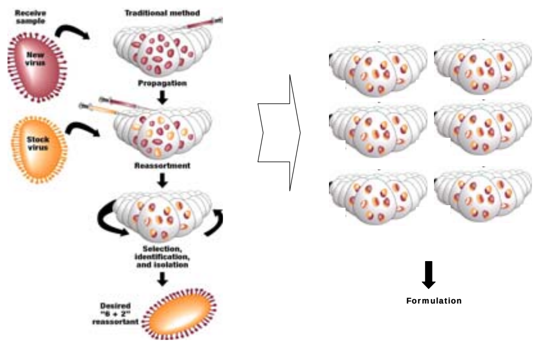
Richard Webby, PhD
Virology/Infectious Diseases
St. Jude Children’s Research Hospital

H5N1 pathogenicity



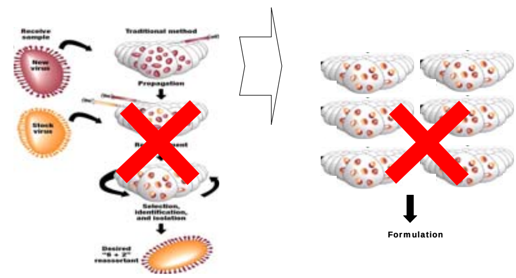


Inactivated flu vaccines



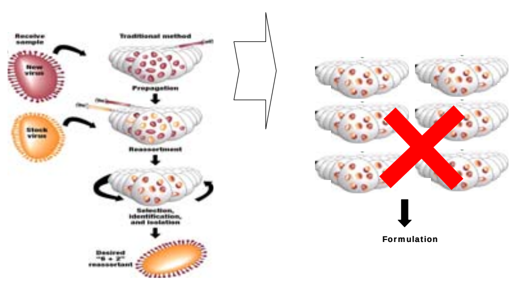
Problems with H5N1

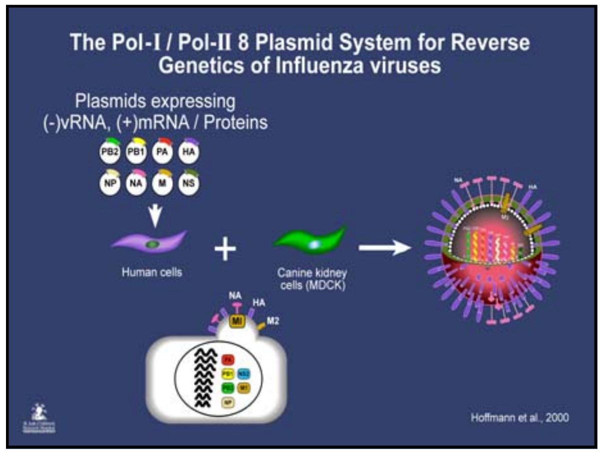
1) kills embryonated eggs



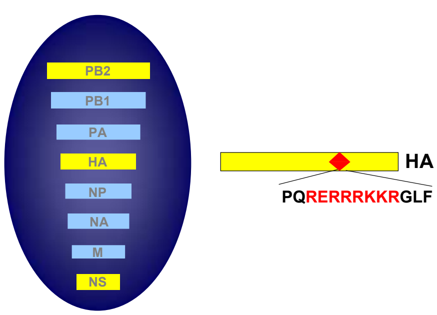
Problems with H5N1

1) kills embryonated eggs
2) highly pathogenic to humans

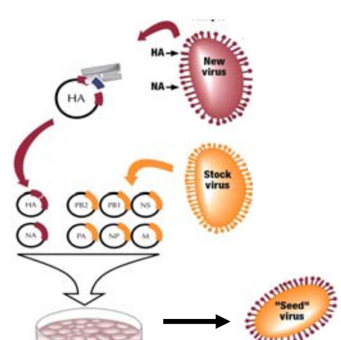




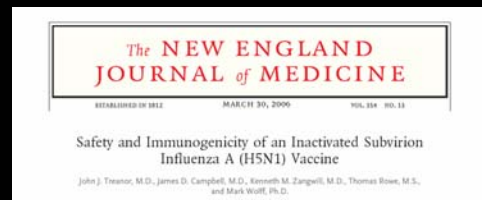
H5N1 Pathogenicity



Seed strains to H5N1



GMP Processing Rooms at St Jude

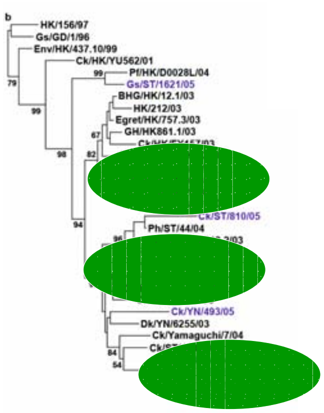


The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MARCH 30, 2006 VOL. 354 NO. 13

Safety and Immunogenicity of an Inactivated Subvirion Influenza A (H5N1) Vaccine

John J. Treanor, M.D., James D. Campbell, M.D., Kenneth M. Zangwill, M.D., Thomas Rowe, M.S., and Mark Wolff, Ph.D.



Summary

- Can respond rapidly to influenza threats with reverse genetics
  Requires -containment facilities
  -access to validated cell bank
  -access to virus
  -production facilities
- Need to determine best use of vaccines and antivirals
- Influenza is not going away

Acknowledgments

Infectious Diseases

GMP folks (St Jude, LLC, and St Jude/LLC)

Hartwell Center

Environmental Health and Safety

Biostatistics

ARC

Pathology

Legal Counsel

Technology Licensing

St. Jude Children's Research Hospital

End

Richard Webster, PhD
Clayton Naeve, PhD
Jie Zheng, PhD
Richard Webby, PhD

More medical education materials are available at:

www.Cure4Kids.org