

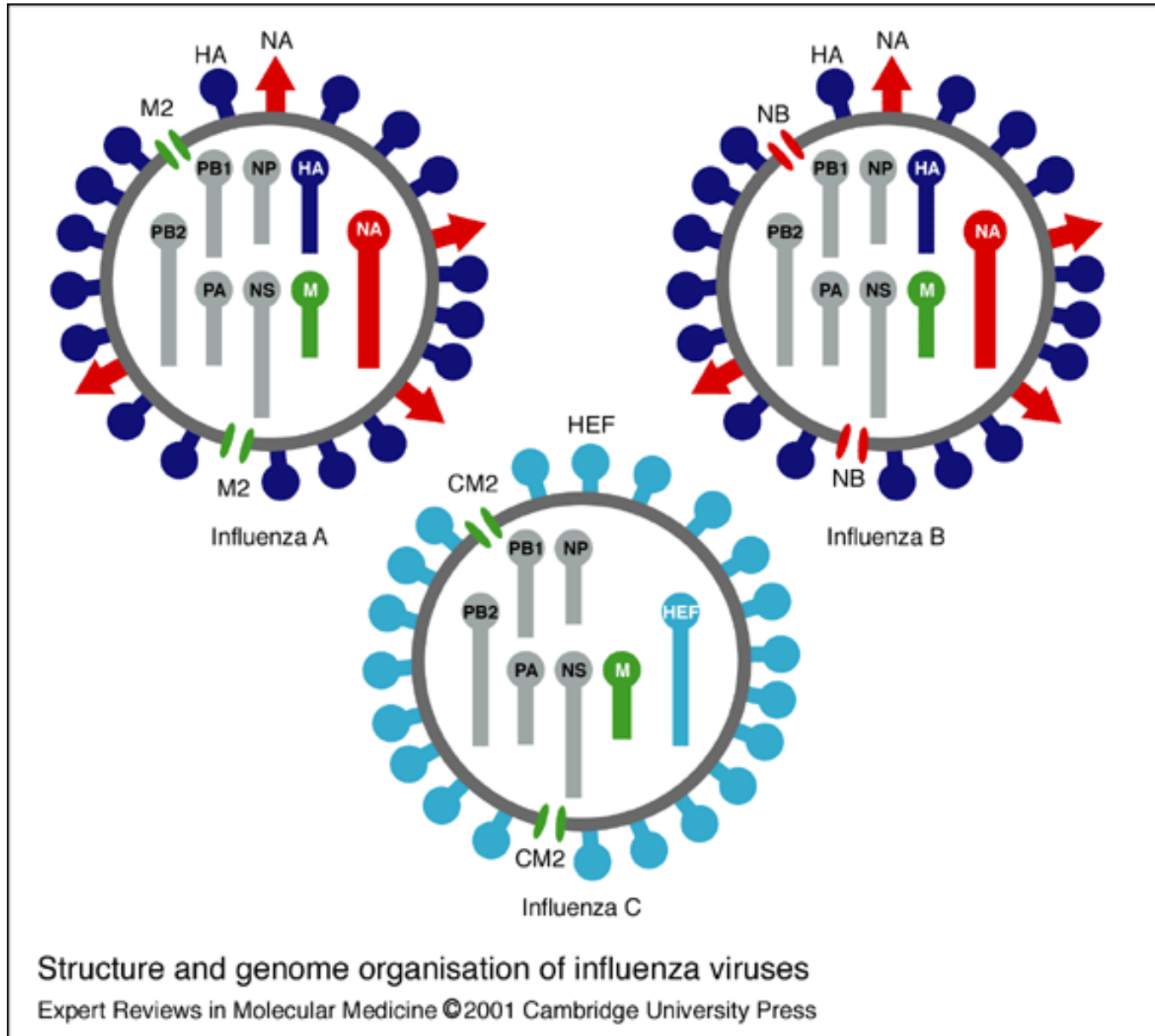


Influenza viremia in blood donors

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Influenza genome



History of influenza A pandemics

- 1889 H2N2
 - 1900 H3N2
 - 1918 H1N1
 - 1957 H2N2
 - 1968 H3N2
 - 1977 H1N1
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- The diagram consists of two vertical curly braces on the right side of the list. The first brace spans from the 1918 H1N1 entry to the 1957 H2N2 entry. The second brace spans from the 1957 H2N2 entry to the 1977 H1N1 entry. Small black arrows point from the right side of each brace to the corresponding year and strain name in the list.
- “Spanish”
“Asian”
“Hong Kong”
“Russian”

Does viremia occur in influenza A?

- Viremia is rarely detected during symptomatic influenza infection
- Viremia may occur during pre-symptomatic or asymptomatic influenza

Viremia in influenza A

- 15 subjects infected by nasal challenge with Bethesda 10/63 strain (Asian flu, H2N2)
- Virus detected in only one sample (nasal) using rhesus monkey kidney tissue culture
- 6 subjects seroconverted
- 4 seroconverting subjects tested with more sensitive egg inoculation of amniotic sac

Viremia in influenza A (H2N2)

Day after challenge	% detectable viremia
1	100
2	50
3	25

- Viremia detected in 4 of 4 patients tested at day 1
- One subject with 3 days' viremia was asymptomatic but did seroconvert

Primary Outcome

- Determine the prevalence of influenza A viremia or antigenemia during periods of outbreak among healthy blood donors

Secondary outcome

- Measure the relative sensitivity of RNA and antigen detection assays in different blood compartments

Study design -- Validation

- Validate available detection assays for use on different blood fractions (e.g. plasma, RBC, PBMC)
- Use virus spiking experiments for H3N2 influenza A into whole blood followed by separation of compartments
- Infect ferrets with H5N1, then longitudinally measure virus in each of the separated blood components

Detection of influenza A

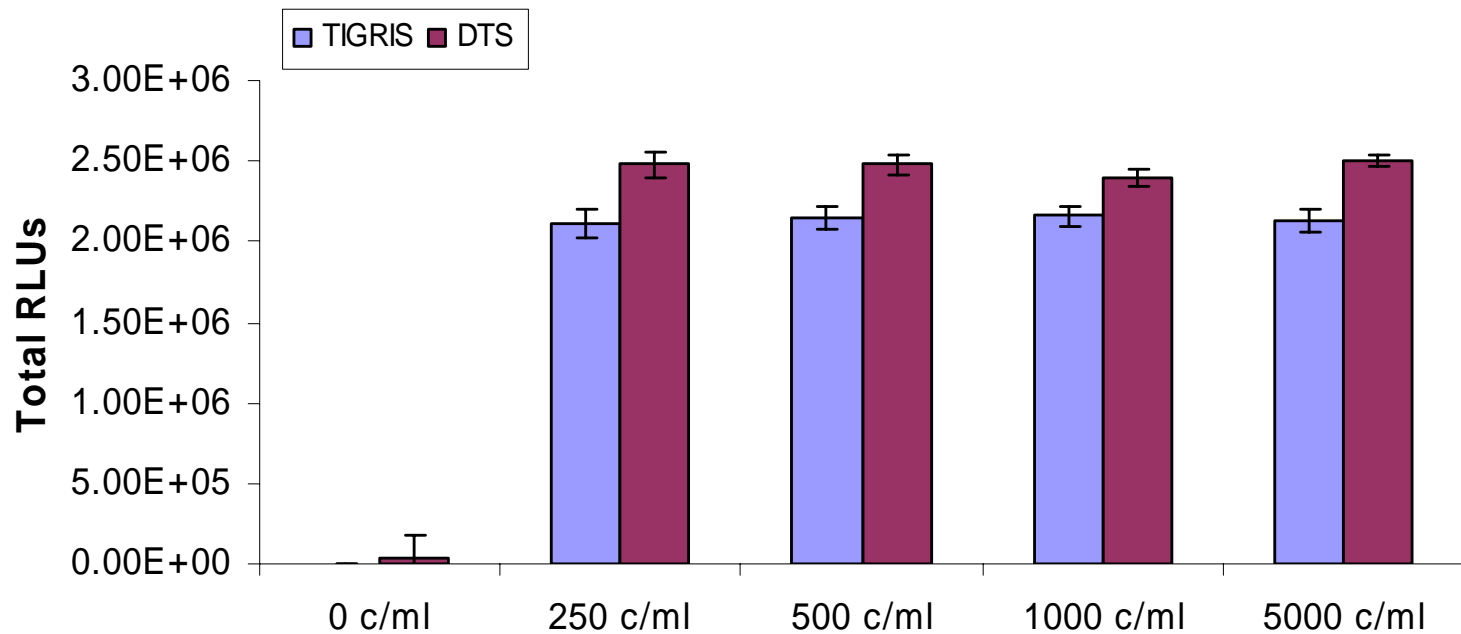
- Techniques have improved since the 1960's
- PCR and TMA technologies are highly sensitive and specific
- Direct antigen detection also possible

Gen-Probe Influenza A TMA specificity

Isolate	ATCC Number	Target	Flu A Ttime	IC	Target	Flu B Ttime	IC
Adeno 4	RI-67 (ATCC VR-4)	Flu A	-	+	Flu B	-	+
Adeno 7	Gomen (ATCC VR-7)	Flu A	-	+	Flu B	-	+
Adeno 11	Slobitski (ATCC 12-VR)	Flu A	-	+	Flu B	-	+
Adeno 18	DC strain (ATCC VR-19)	Flu A	-	+	Flu B	-	+
Adeno 29	Strain BP-6 (ATCC VR-272)	Flu A	-	+	Flu B	-	+
Human coronavirus CoV	Human coronavirus 229E, Group 1 (ATCC VR-740) Human coronavirus OC43, Group 2 (ATCC VR-759)	Flu A	-	+	Flu B	-	+
Parainfluenza virus 1	Clinical Isolate	Flu A	-	+	Flu B	-	+
Para 1	Parainfluenza type1 (ATCC VR-1380)	Flu A	-	+	Flu B	-	+
Para 2	Clinical Isolate	Flu A	-	+	Flu B	-	+
Para 2	Parainfluenza 2, Greer strain (ATCC VR-92)	Flu A	-	+	Flu B	-	+
Para3	Clinical Isolate	Flu A	-	+	Flu B	-	+
Para 4a	Parainfluenza 4a, strain M-25 (ATCC VR-1378)	Flu A	-	+	Flu B	-	+
Rhinovirus	Clinical Isolate	Flu A	-	+	Flu B	-	+
Respiratory Syncyntial Virus	Clinical Isolate	Flu A	-	+	Flu B	-	+
RSV	RSV strain B WV/14617/85 [B-1 wildtype] (VR-1400)	Flu A	-	+	Flu B	-	+
RSV	RSV strain A-2 (VR-1540)	Flu A	-	+	Flu B	-	+
Flu A control RNA	IVT of Flu A/Beijing (H1N1) (CDC isolate)	Flu A	17.7	n/a	Flu B	-	+
Flu A	A/Port Chalmers/1/73 (H3N2) (ATCC VR-810)	Flu A	14.2	n/a	Flu B	-	+
Flu A	A/Mal/302/54 (H1N1) (ATCC VR-98)	Flu A	12.3	n/a	Flu B	-	+
Flu A	A/Hong Kong/8/68 (H3N2) (ATCC VR-544)	Flu A	11.3	n/a	Flu B	-	+
Flu B Control RNA	IVT of Influenza B/Maryland/1/59 (ATCC VR-296)	Flu A	-	+	Flu B	23.2	n/a
Flu B	B/Lee/40 (ATCC VR-101)	Flu A	-	+	FluB	20.5	n/a

Gen-Probe TMA sensitivity

Influenza A



BD Directigen EZ -- Sensitivity

Influenza Viral Strain	Limit of detection (CEID50/mL*)
A A/PR/8/34 (H1N1)	1.75E+04
A A/FM/1/47 (H1N1)	1.98E+03
A A/NWS/33 (H1N1)	1.00E+04
A A1/Denver/1/57 (H1N1)	5.56E+03
A A/New Jersey/8/76 (H1N1)	4.45E+03
A A/Port Chalmers/1/73 (H3N2)	1.00E+03
A A/Hong Kong/8/68 (H3N2)	2.78E+02
A A2/Aichi2/68 (H3N2)	3.50E+03
A A/Victoria/3/75 (H3N2)	2.78E+04

* CEID50/mL = Chick Embryo Infectious Dose at which 50% of the embryos perish

BD Directigen EZ -- Animal Strains

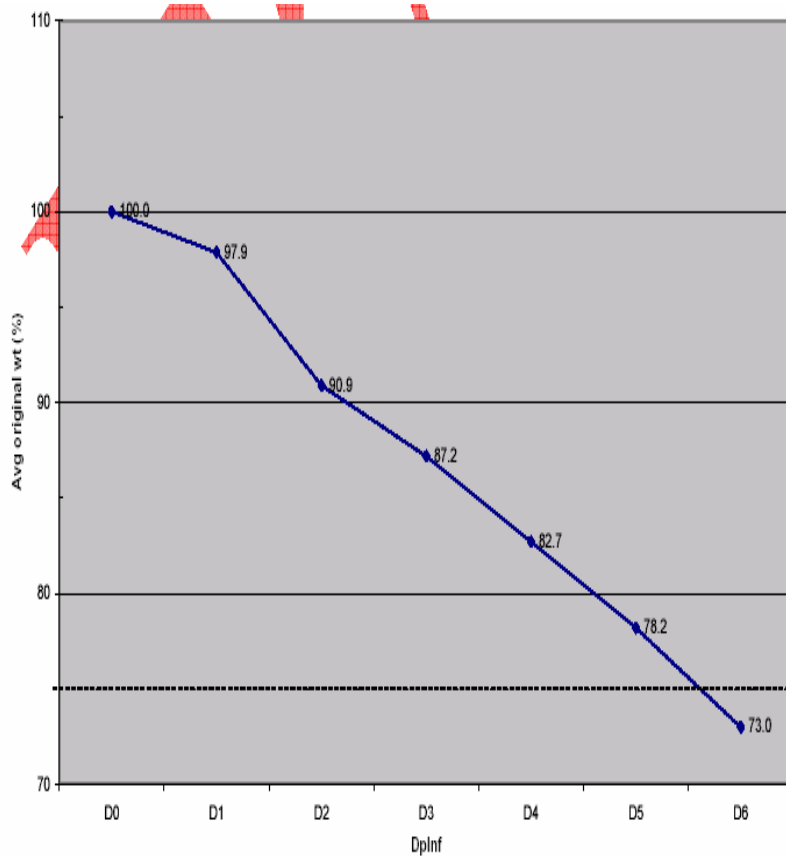
Influenza Virus Animal Isolates	Sub Type	Tested Concentration (CEID50/mL)	Flu A Result	Flu B Result
A/Turkey/Kansas/4880/80	A (H1N1)	2.00E+08	P	N
A/Mallard/New York/6750/78	A (H2N2)	3.16E+07	P	N
A/Turkey/England/69	A (H3N2)	1.26E+07	P	N
A/Chicken/Alabama/75	A (H4N8)	2.00E+06	P	N
A/Turkey/Wisconsin/68	A (H5N9)	7.94E+07	P	N
A/Turkey/Canada/63	A (H6N8)	7.94E+05	P	N
A/Turkey/Oregon/71	A (H7N3)	1.26E+07	P	N
A/Turkey/Ontario/6118/67	A (H8N4)	1.26E+07	P	N
A/Turkey/Wisconsin/66	A (H9N2)	2.00E+06	P	N
A/Chicken/Germany/N/49	A (H10N7)	5.01E+07	P	N
A/Duck/Memphis/546/74	A (H11N9)	7.94E+07	P	N
A/Duck/Alberta/60/76	A (H12N5)	3.16E+07	P	N
A/Gull/MD/704/77	A (H13N6)	3.16E+07	P	N
A/Mallard/Gurjev/263/82	A (H14N5)	3.16E+07	P	N
A/Shearwater/WA/2576/79	A (H15N6)	2.00E+06	P	N

Ferret model of avian influenza

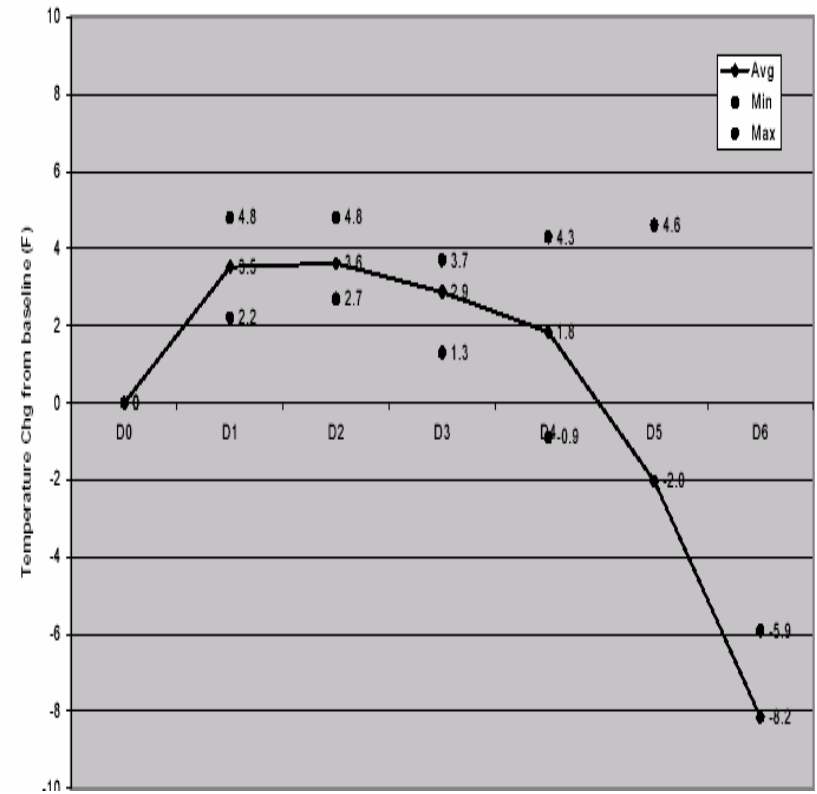
- HPAI H5N1 1203, Vietnam isolate
- Widely dispersed virus in tissues
- 100% mortality at 7 days

Clinical course following infection

Weight loss



Temperature change



Gross pathology of ferret lung

-Ctrl



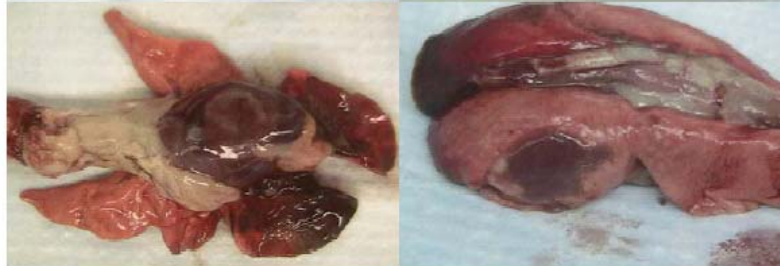
D2 pInf



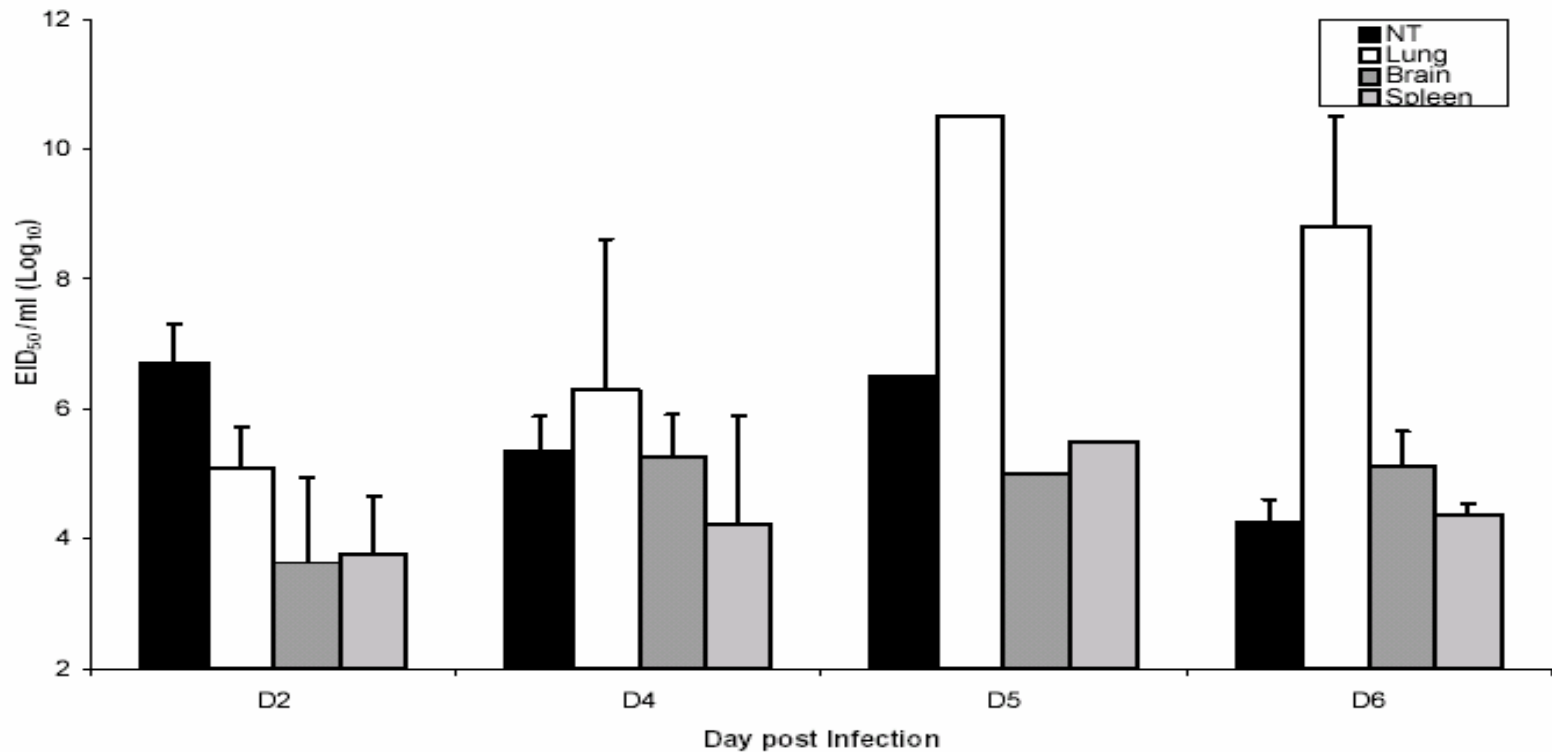
D4 pInf



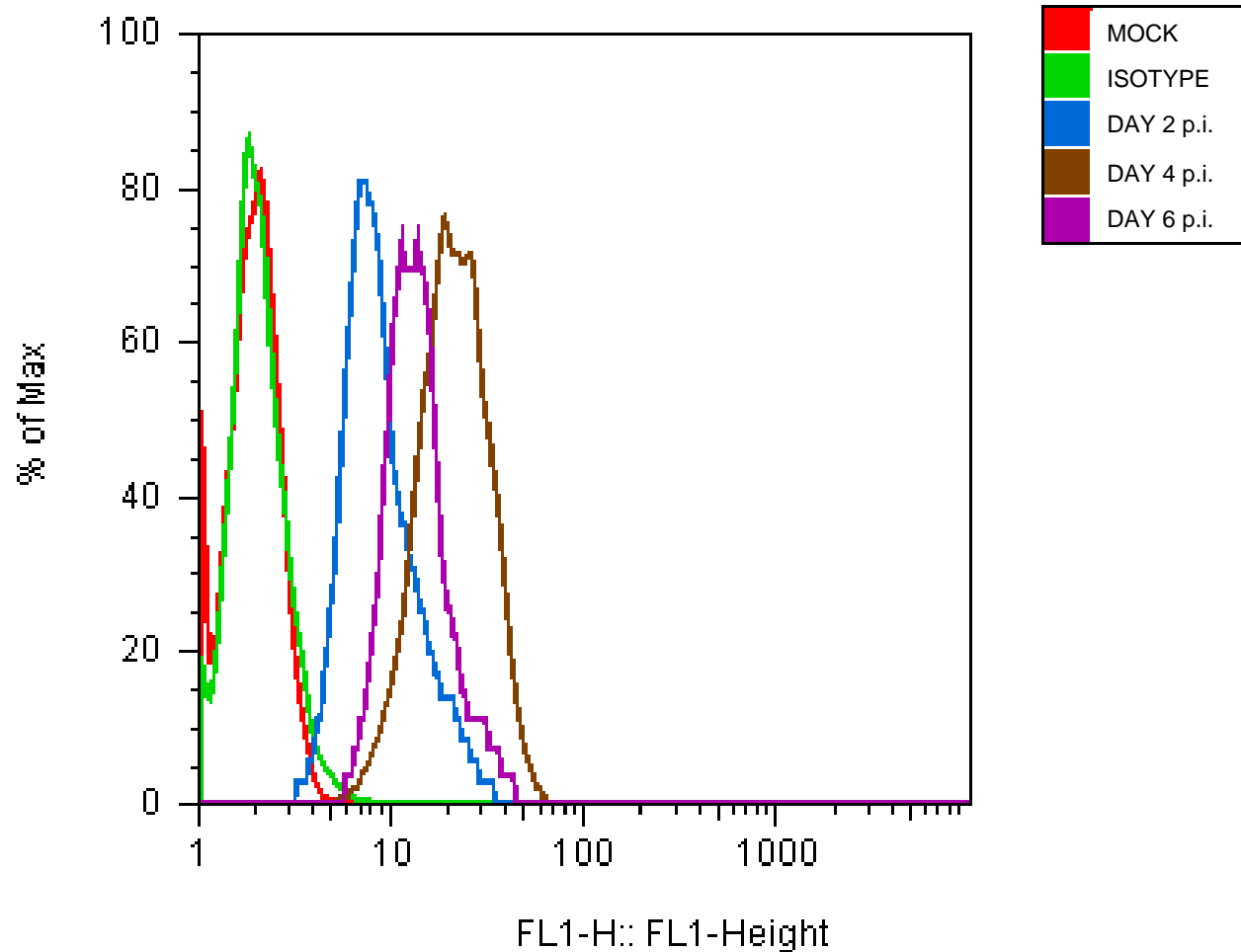
D6 pInf



Viral load from various tissues following infection with H5N1



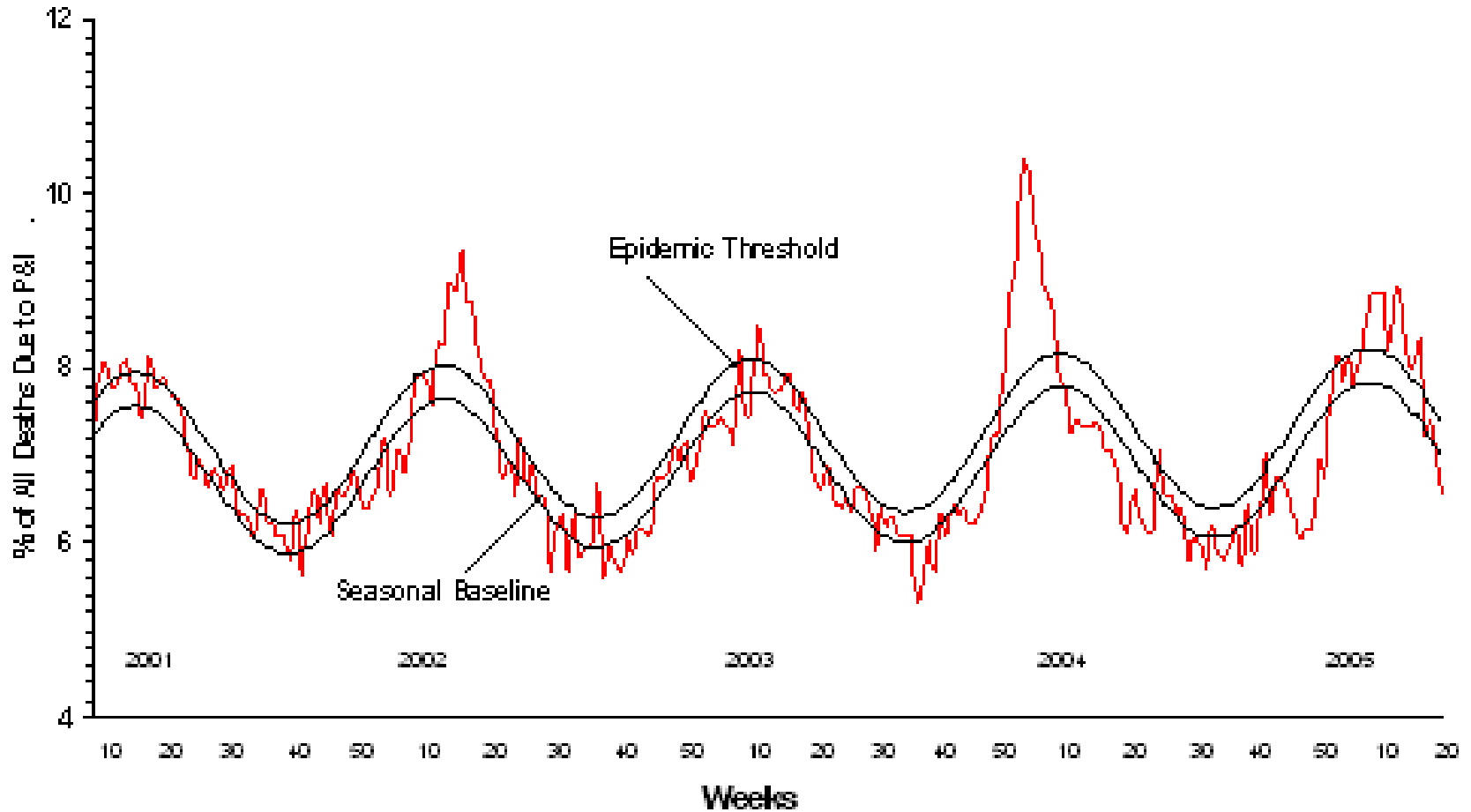
Phosphorylation state of a lymphocyte activation protein in H5N1-infected ferrets



Study design – viremia prevalence

- Once antigen and RNA detection assays validated, measure frequency of both in blood donor population
- Target donors most likely to have viremia using existing repositories (e.g. RADAR)

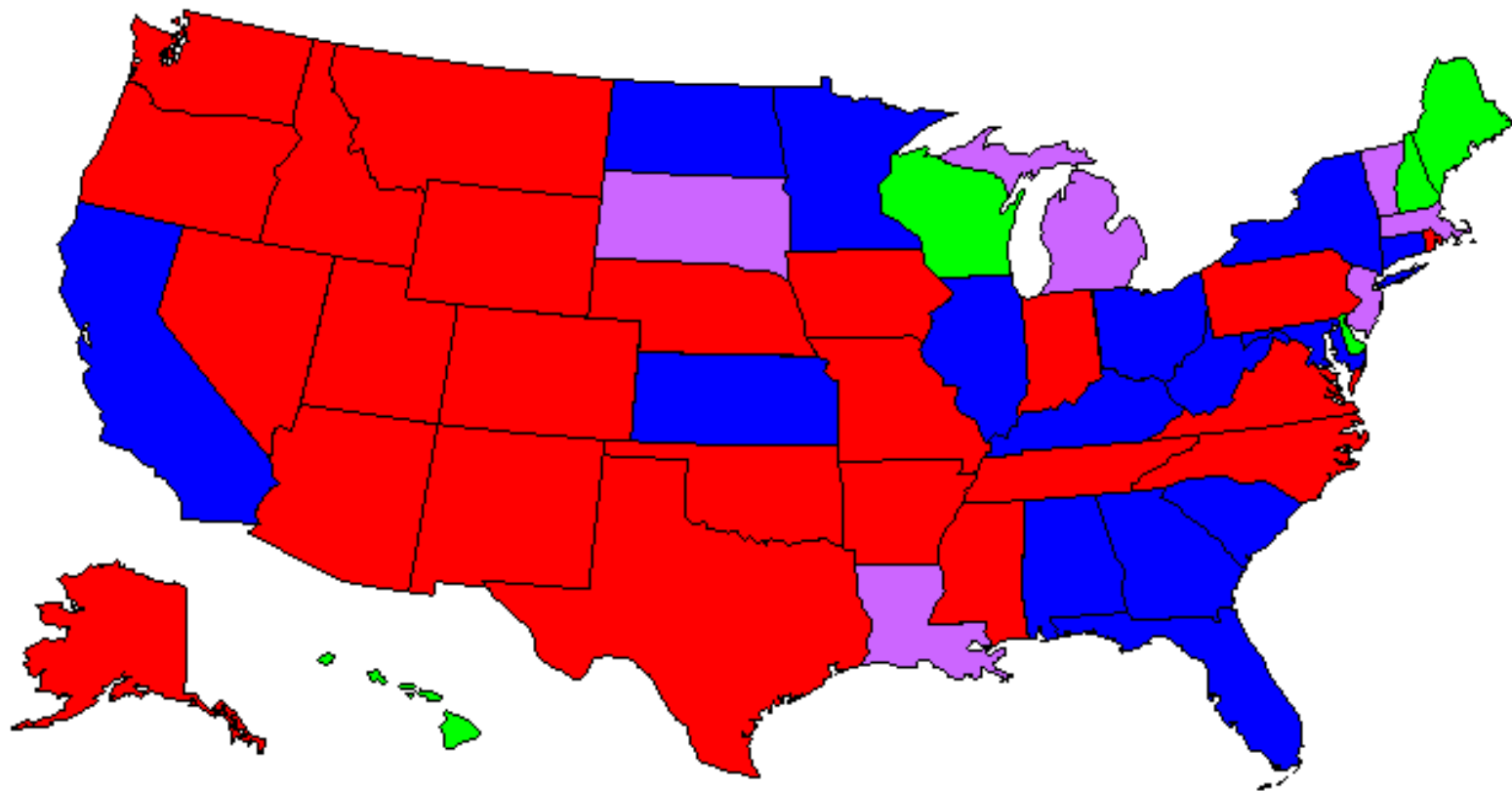
Pneumonia and Influenza Mortality for 122 U.S. Cities Week Ending 5/21/2005



from <http://www.cdc.gov/flu/weekly/fluactivity.htm>

Weekly Influenza Activity Estimates Reported by State & Territorial Epidemiologists

Week ending December 6, 2003 - Week 49



No Report



No Activity



Sporadic



Local Activity



Regional



Widespread



from <http://www.cdc.gov/flu/weekly/fluactivity.htm>

Regional US influenza activity

	Available date range		Widespread activity			
			00-01	01-02	02-03	03-04
Chesapeake-ARC	4/12/2000	12/20/2002	1/14-1/20	1/20-1/26	X	
SouthEastern MI - ARC	4/18/2000	12/11/2003	X	X	2/23-3/8	X
Southern CA – ARC	4/11/2000	11/14/2002	X	X	X	
Blood Centers of the Pacific	4/11/2000	12/5/2002	X	X	X	
Oklahoma Blood Institute	5/2/2000	12/10/2003	1/28-2/17	X	X	11/30-12/10
Institute for Transfusion Medicine	9/12/2000	12/1/2003	X	X	2/2-2/8	11/16-12/1
Florida Blood Services	9/26/2000	6/12/2002	X	X	X	

x = No widespread activity during the year.

Power calculations

Sample size	Observed prevalence	95% CI	
		Lower limit	Upper limit
1000	0%	0.00%	0.30%
	1%	0.48%	1.83%
	2%	1.23%	3.07%
	3%	2.03%	4.26%
	4%	2.87%	5.41%
	5%	3.73%	6.54%
	6%	4.61%	7.66%

Conclusions

- Viremia likely occurs during pre-symptomatic influenza A infection
- The incidence of viremia during influenza outbreaks is unknown
- We can now address the frequency of influenza A viremia amongst blood donors using new, sensitive assays

Conclusions

- Detecting influenza A viremia will have implications for blood safety, and detection will allow studies of transmission
- Detection of viremic donors will allow detailed immunology and natural history studies of asymptotically infected donors
- The ability to detect H5N1 influenza A in blood donors could present a valuable public health monitoring tool