



# OFFLU SIV GROUP TECHNICAL MEETING

## ACTIVITY IN VIETNAM

**FAO HEAD QUARTER**

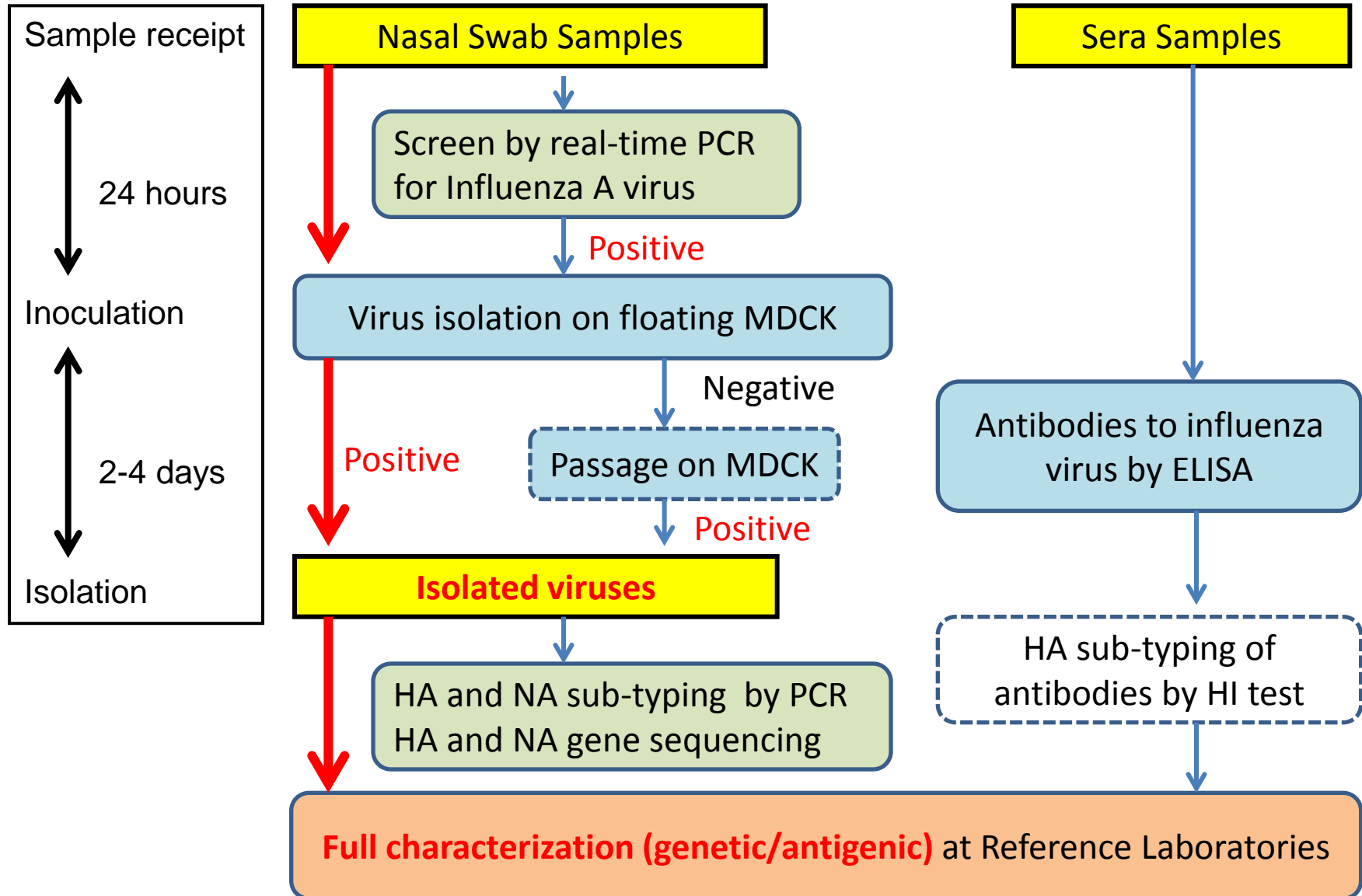
Rome, 16-17 April 2013

Nguyen Tung

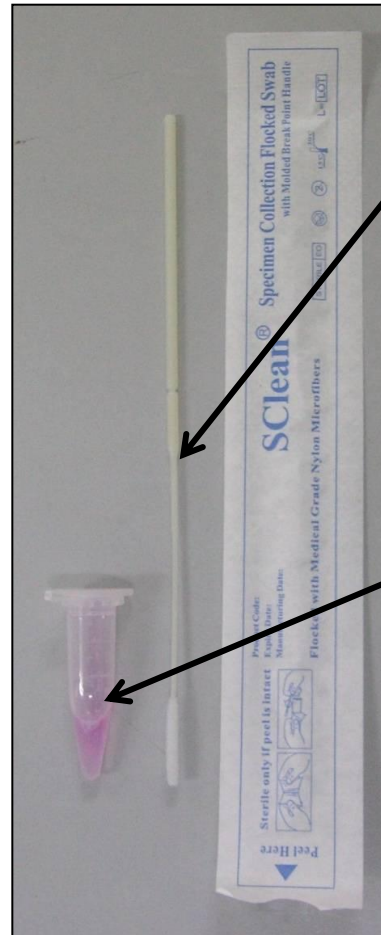
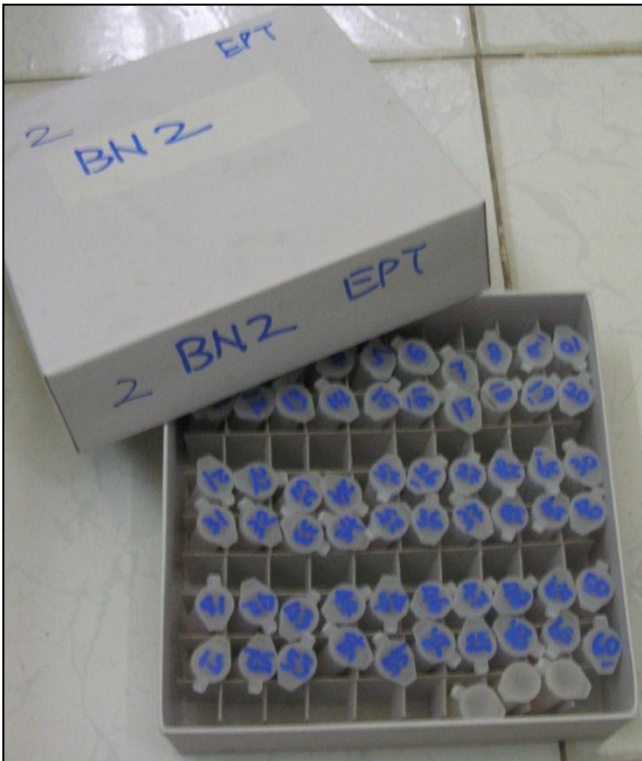
# Swine Influenza surveillance activities in Vietnam

- DAH(Vietnam)-NIAH(Japan) cooperation in 2009-2012
- EPT plus (DAH-FAO/USAID)
- DAH-CDC Co-ag

# Flowchart of Laboratory Testing



# Preparation for sampling



**Flocked nylon swab**

**Virus transport medium**  
(500ul / Eppentube)

EMEM with

- gentamycin 250ug/ml
- penicillin 500units/ml
- streptomycin 500ug/ml
- fungizon 25ug/ml
- BSA 0.5%
- HEPES 10mM

# Collection of Swab Samples



Swab tip is released to the tube by

- twisting the swab shaft, or
- cutting it by scissors

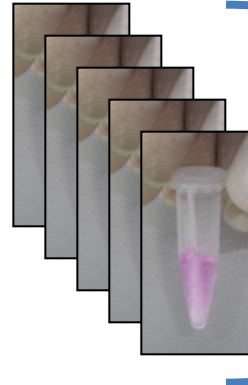
# Processing of Swab Samples for qRT-PCR



1. sample



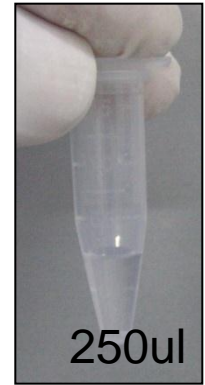
2. Vortex well



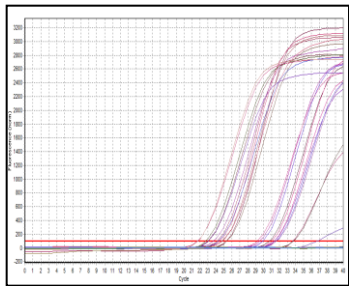
3. Pool 5 samples



50ul  
each



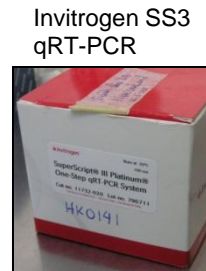
4. Add to lysis buffer



6. Real-time PCR



1 reaction (15ul)	
Reagent	Volume (ul)
DW	4.3
2x RM	7.5
PPP	0.9
Enzyme	0.3
RNA	2.0



Invitrogen SS3  
qRT-PCR



5. RNA extraction by TACO

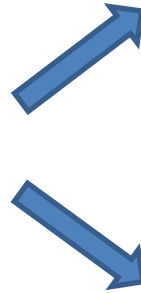
# Processing of Swab Samples for Virus Isolation



1. sample



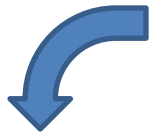
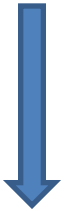
2. Vortex well



3.1 Centrifuge at 15000g for 7 min, or



3.2 Filter with Spin-X column



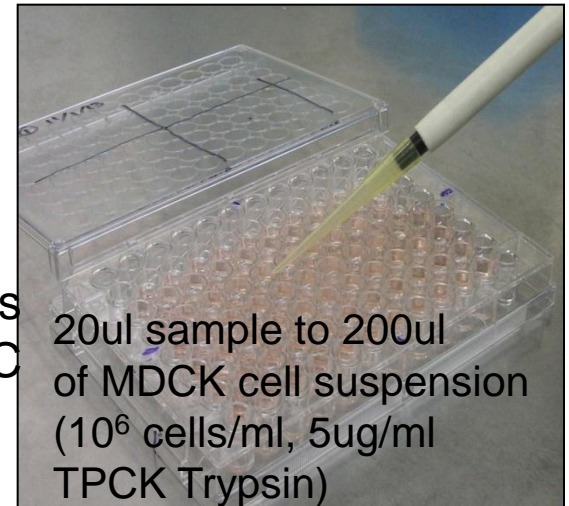
Cell control  
Live cells  
aggregate



CPE+  
Dead cells shrink  
and disperse



3 days  
at 37C



20ul sample to 200ul  
of MDCK cell suspension  
(10<sup>6</sup> cells/ml, 5ug/ml  
TPCK Trypsin)

4. Inoculation to MDCK

5. CPE on floating MDCK

6. Screen by  
HA test

7. Confirm by  
Real-time  
PCR

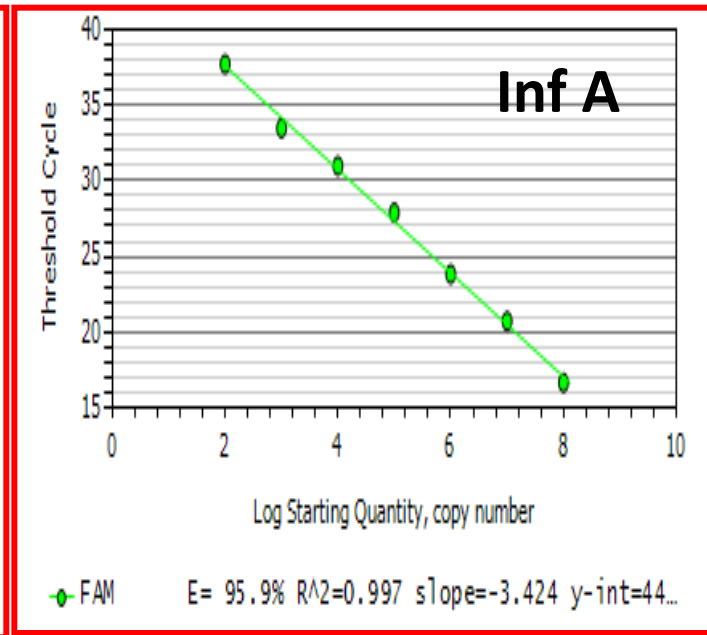
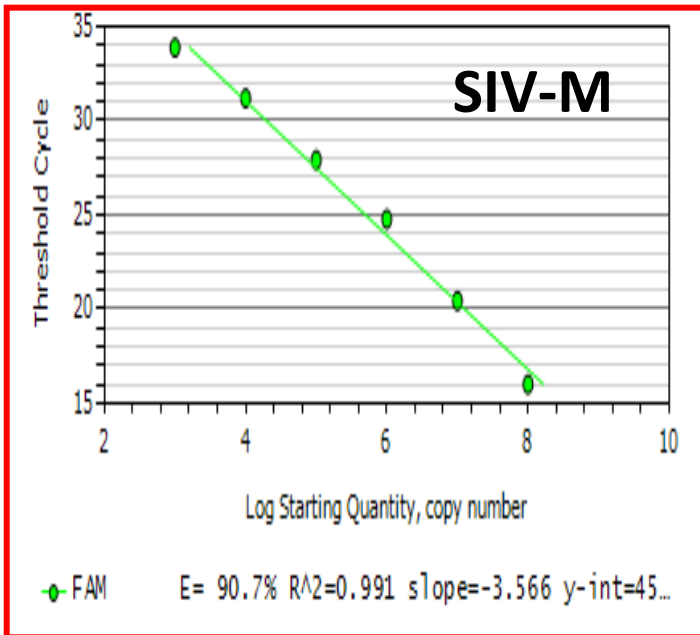




## Sensitivity comparison of qPCR and virus isolation with samples from 2 farms (BN-04&05) in Bac Ninh

		Real-time PCR					Total
		Positive			P/N	Negative	
		<25	25-30	30-35	35<		
Virus isolation on floating MDCK	Pos	7	12	5	1	1	26
	Neg	0	0	4	3	8	15
Total		7	12	9	4	9	41
Total		28			13		41

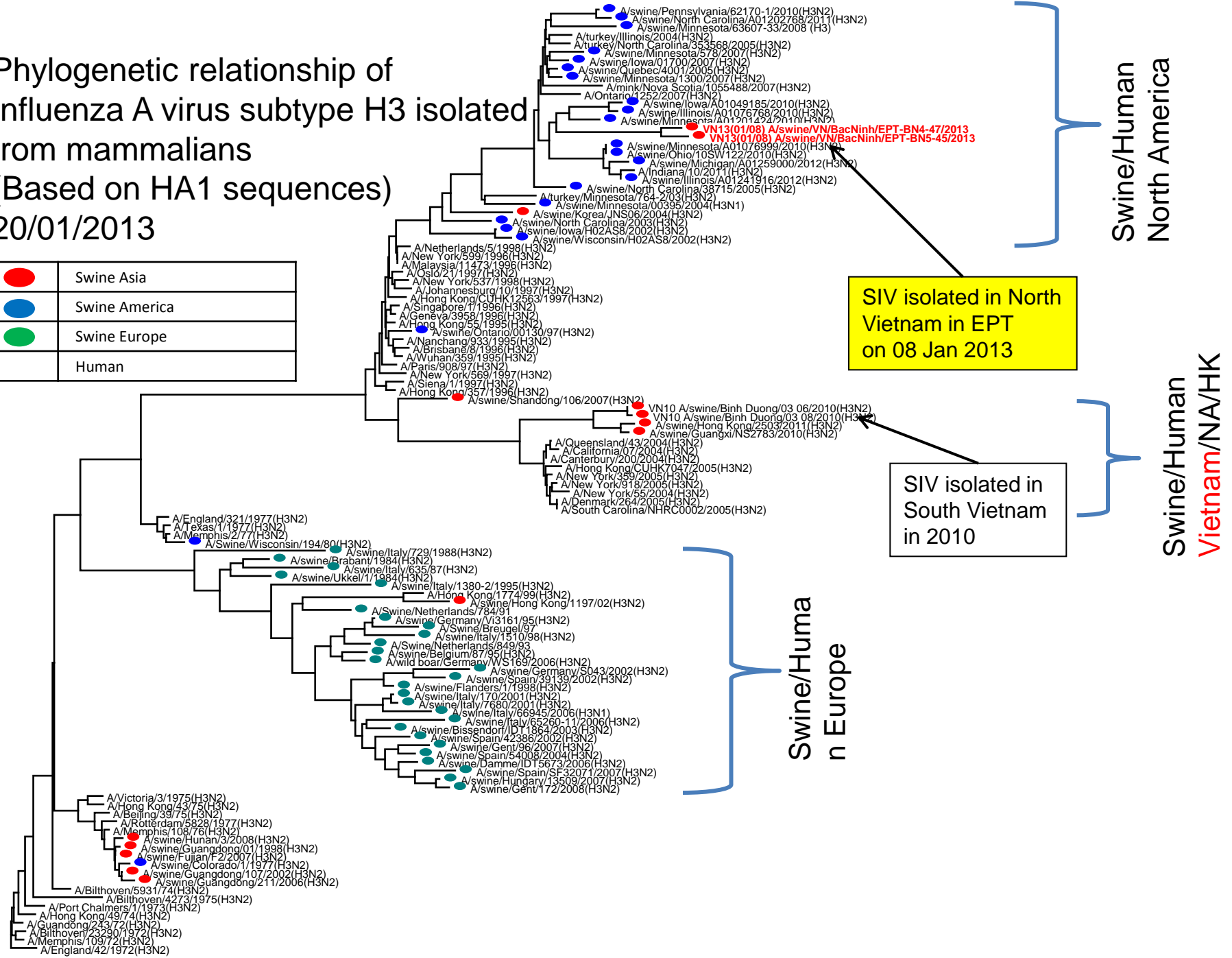
# Efficiency comparison of PPPs with H3N2 virus isolated from a pig farm at Bac Ninh in EPT+ project



	SIV-M (USDA)	Inf A (CDC)
Efficiency	90.7%	95.9%
R <sup>2</sup>	0.991	0.997
Slope	-3.566	-3.424
y intercept	45	44

# Phylogenetic relationship of Influenza A virus subtype H3 isolated from mammals (Based on HA1 sequences) 20/01/2013

<span style="color: red;">●</span>	Swine Asia
<span style="color: blue;">●</span>	Swine America
<span style="color: green;">●</span>	Swine Europe
	Human



SIV isolated in North Vietnam in EPT on 08 Jan 2013

SIV isolated in South Vietnam in 2010

Swine/Human North America

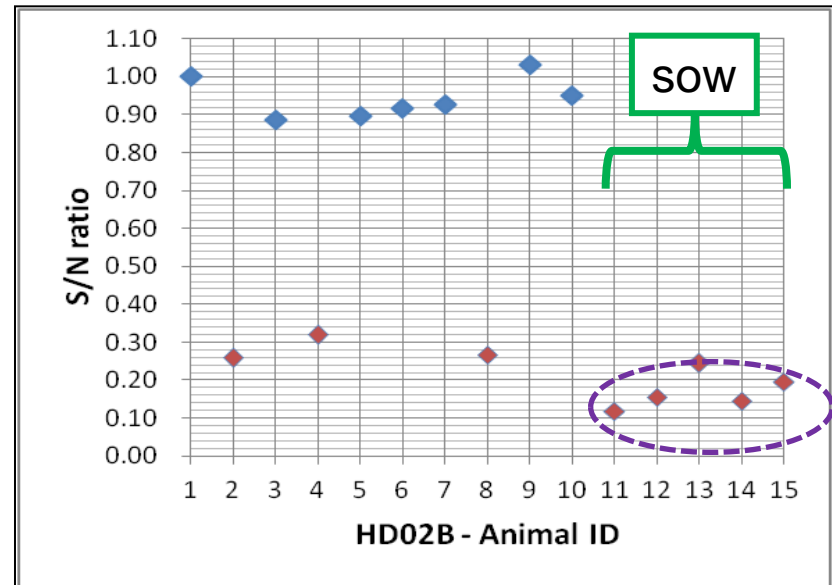
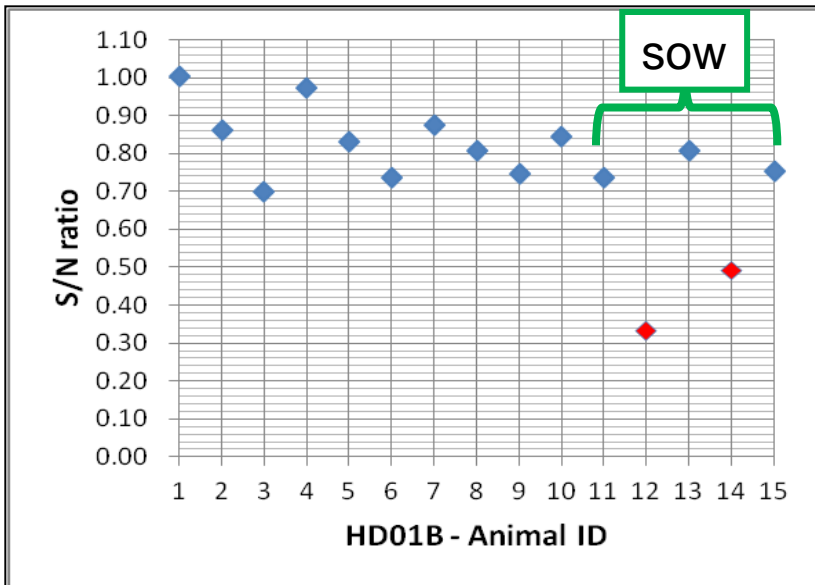
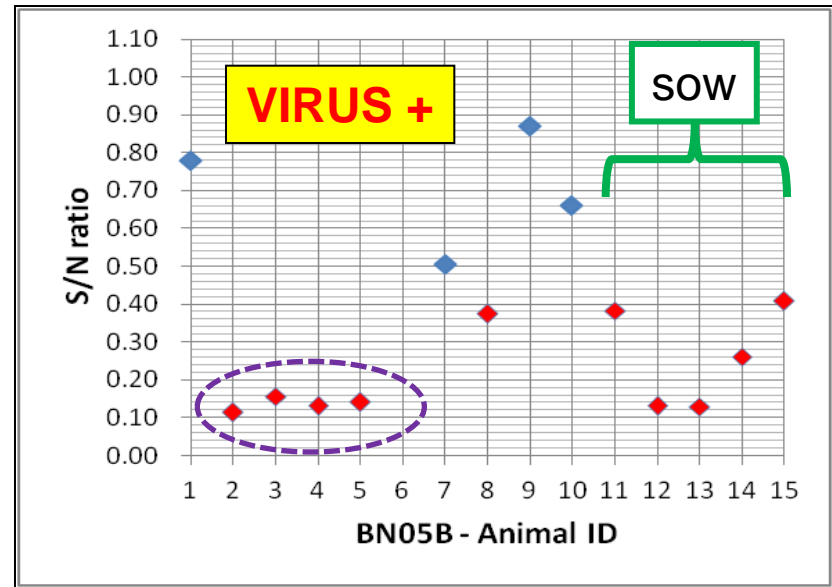
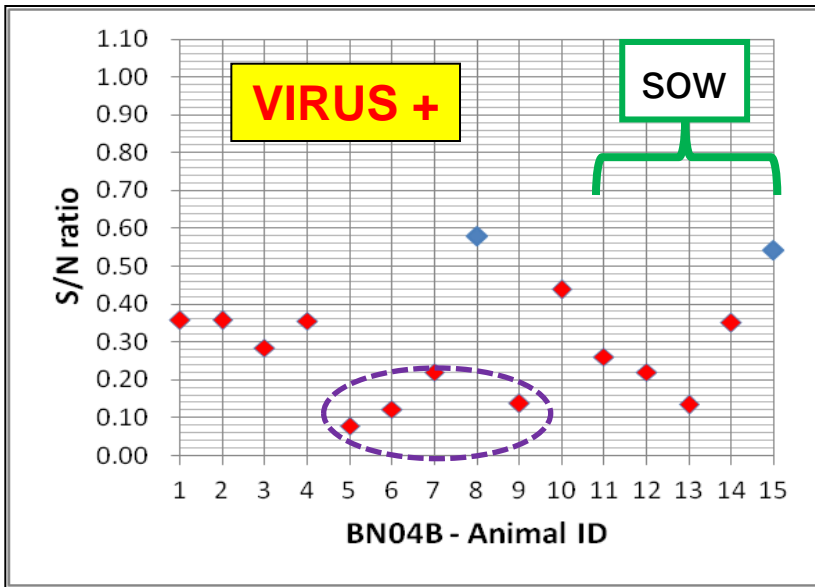
Swine/Human Vietnam/NA/HK

Swine/Human Europe

0.02

# Serology in breeding farms

## ELISA antibodies to Influenza A virus



**Thanks for your attention!**

