

# International standards for H5N1 antibodies



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# Problems in comparing H5N1 vaccine trials

- Alum adsorption is not uniform between antigens and Al salts
  - Cannot extrapolate from one vaccine to another
- Vaccine standardisation
  - How to test low dose vaccines?
  - How to test adjuvanted vaccines?
- Serology tests affected by technical variability
  - HI test
    - Erythrocytes, RDE etc
  - VN test
    - Cells, test protocol, starting dilution etc

# H5N1 serology tests

# Haemagglutination Inhibition test

## Use for seasonal influenza

- Technically easy and long history of use for antigenic analysis and serology
  - ‘Gold standard’
- Accepted correlates of immunity
  - Regulatory approval
- High level of variability between labs
  - 2005 EU collaborative study on seasonal flu vaccines (BSP063) showed more than 16 fold variation in HI titres between laboratories which influenced compliance with licensing criteria for serology
  - 2005/6 WHO collaborative study with H3N2 viruses showed 16-128 fold variation in HI titres between laboratories (*Stephenson et al, in press*)

# Haemagglutination Inhibition test

## Use for H5N1 influenza

- HI test with turkey cells insensitive for antibody to H5N1 viruses
  - Not all turkeys are the same
  - Use of horse cells improves sensitivity to levels associated with virus neutralisation test
  - Horse HI test affected by source/age of horse cells – reproducibility?

# Single Radial Haemolysis test

## Use for seasonal influenza

- Technically more demanding than HI
  - Only 2-3 labs using this test
- Accepted correlates of immunity
  - Regulatory approval in EU
- Lower level of variability between labs than HI
  - In *Wood et al, 1994*, variability between labs was 3.8 fold

# Single Radial Haemolysis test

## Use for pandemic influenza

- Sensitive test for 97 H5N1 antibody but needed extra steps to remove non-specific zones
  - Good correlation with VN test (EU data)
- U of Siena has adapted SRH test for A/Vietnam/1194/04 virus

# Virus Neutralisation test

## Use for seasonal influenza

- VN tests have been used in research for many years but techniques have evolved separately in different labs
- There are no accepted correlates of immunity
- Technical differences between labs affect reproducibility (H3N2 flu vaccines, WHO study)

# Virus Neutralisation test

## Use for pandemic influenza

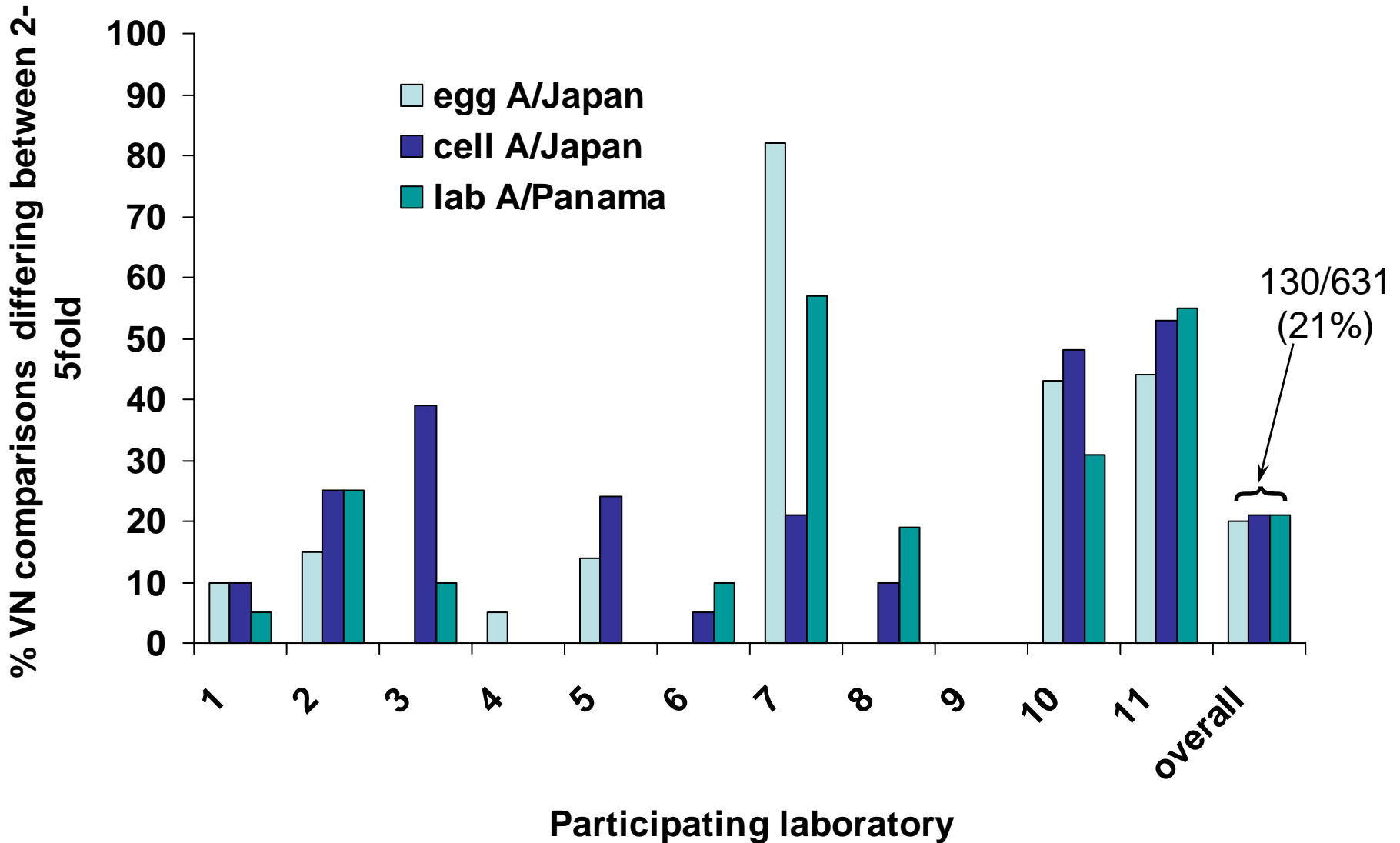
- **Sensitive for H5N1 antibody in convalescent and post vaccination sera**
  - 97 H5N3 vaccine good correlation with SRH test and horse HI test (EU data)
  - 04 H5N1 vaccine good correlation with horse HI test (EU and USA data)
- **Technical differences between labs will probably affect reproducibility**
- **Unsure about correlates of immunity**

# Virus neutralisation collaborative study (WHO, CDC, NIBSC, HPA)

- **Eleven laboratories**
  - VN and HI tests of 21 sera for H3N2 antibody
  - Three different H3N2 viruses used for tests
- **Some laboratories could not obtain consistent VN results in repeat tests (6% of repeat VN tests differed by >5 fold)**

*Stephenson et al – in press*

# Results: *comparison of VN replicate assays differing >2 fold within laboratories*



# Virus neutralisation collaborative study (WHO, CDC, NIBSC, HPA)

## Reproducibility of HI and VN titres between laboratories

Serum N

	GMT	Range	Fold variation	GCV (%)
HI	39	10-128	128	278
VN	139	80-2560	724	529

# Virus neutralisation collaborative study (WHO, CDC, NIBSC, HPA)

Reproducibility between laboratories is improved by  
use of a standard antibody

All sera

	Absolute titres		Relative titres	
	GCV (%)	Range	GCV (%)	Range
HI	138	98-720	64	53-231
VN	359	252-1012	115	51-312

# Standardisation of serology tests

## Options

- All tests in one laboratory
- Standardise protocols
  - Difficult to enforce
  - Despite standardisation, local differences in cells, erythrocytes etc
  - Can advise on important aspects of tests
- Use of standard serum

# Preparation of an H5N1 antibody International Standard

- WHO International Standards have been successfully used to standardise antibody tests for other infectious agents (Measles, B19, Polio, HBV, HAV, Rabies, Rubella, Smallpox, VZ)
- The next VN/HI collaborative study will be an H5N1 study and used to establish an H5N1 International Standard (WHO meeting, Copenhagen 2006)
  - Study protocols provided if needed; will advise on important aspects of tests
- WHO has approved the project at ECBS 2006
  - Initially for A/Vietnam/2004 antibody

# Source of material for IS

- **Need 2-3 litre pool of serum or plasma**
  - 2-3000 vials of IS
- **In addition, need several serum samples to use as test serum panel in collaborative study**
  - Useful to include national/regional H5N1 antibody standards

# Progress

- **NIBSC requested post-H5N1 vaccine sera from three vaccine companies**
  - All agreed to help
  - Trial sera arrived for testing in early 2007
- **Sheep anti A/Vietnam/1194/2004 serum will be evaluated as candidate IS**

## Next steps

- **Agree collaborative study protocol**
- **Test and freeze dry sera for study**
- **Conduct collaborative study**
- **Assign unitage to IS**
- **Acceptance by ECBS**

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