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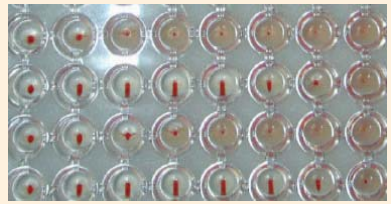
Limitations of current serologic assays to detect antibody responses to HA and NA

FDA/NIH/WHO workshop: Immune Correlates of Protection, December 10-11, 2007

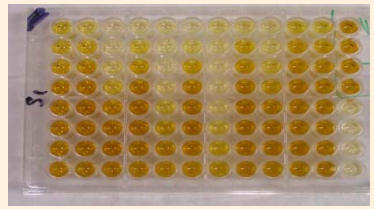
John Wood
NIBSC

Assays to be discussed

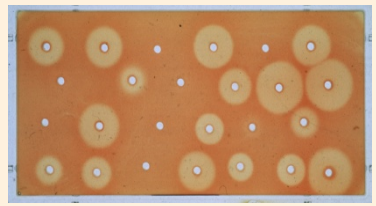
HA Haemagglutination-inhibition



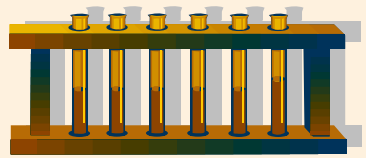
HA Virus neutralisation –
(microneutralisation)

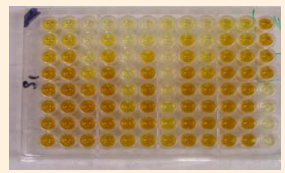


HA Single Radial Haemolysis



Neuraminidase assays





Haemagglutination-Inhibition assay

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Advantages

- Technically simple- easy to automate
- Considerable experience evaluating antibody responses to infection/vaccination
- Convenient for antigenic analysis – years of experience
- Correlates of immunity well documented for seasonal flu
- Good correlation with VN titres

Limitations

- Insensitive for antibody to flu B, H5 and H7 viruses
- Technical aspects of test (erythrocytes, RDE) affect HI titres
- Poor reproducibility between labs

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Haemagglutination-Inhibition assay

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Advantages

- Correlates of immunity well documented for seasonal flu
  - De Jong (2003) review of 24 studies in healthy children and adults involving natural infection and challenge infection with H2N2, H3N2, H1N1 and B viruses
    - Median HI titre of 1:28 associated with 50% protection;
    - Median HI titre of 1:192 associated with 90% protection.
  - Conclusion that HI of 1:40 is justified for seasonal flu
- Good correlation with VN titres
  - Vaccine studies - only where strains in vaccine and assay are homologous
  - VN is more strain specific than HI *(De Jong, 2003; Stephenson et al, 2007; M. Zambon pers. com.)*

Haemagglutination-Inhibition assay

Limitations

- Insensitive for antibody to flu B, H5 and H7 viruses
  - B assay, use of split antigens with decreased strain specificity (*Monto and Maassab, 1981; Kendal and Kate, 1983)*
  - H5 and H7, use of horse HI *(Stephenson et al, 2003)*
- Poor reproducibility between labs
  - *Wood et al, 1994* – greatest variation in HI titres 32 fold
  - *EDQM study 2005* - HI >16 fold variation; compliance with CHMP licensing varies with from lab to lab
  - *Stephenson et al, 2007* – H3N2 HI 6-128 fold variation

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Advantages and limitations of Horse HI assay

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Advantages

- Test is sensitive for antibody to H5 and H7 haemagglutinins
- Good correlation with VN test *(Confirmed H5N1 cases: J Katz, pers. com. ; H5N1 vaccine trials: J Katz, pers. com.; Treanor et al, 2006; Bresson et al, 2006)*
- Can use inactivated antigen – BSL2

Limitations

- Unsure whether hHI titre of 1:40 relates to 50% protection against an H5N1 virus?
- Agglutination affected by aa changes near HA rbs
  - Evaluate specificity and sensitivity of hHI for new H5N1 strains *(J Katz pers. com.)*
- May not be as robust as turkey HI
  - Affected by age and source of horse erythrocytes
- Reproducibility between labs unknown

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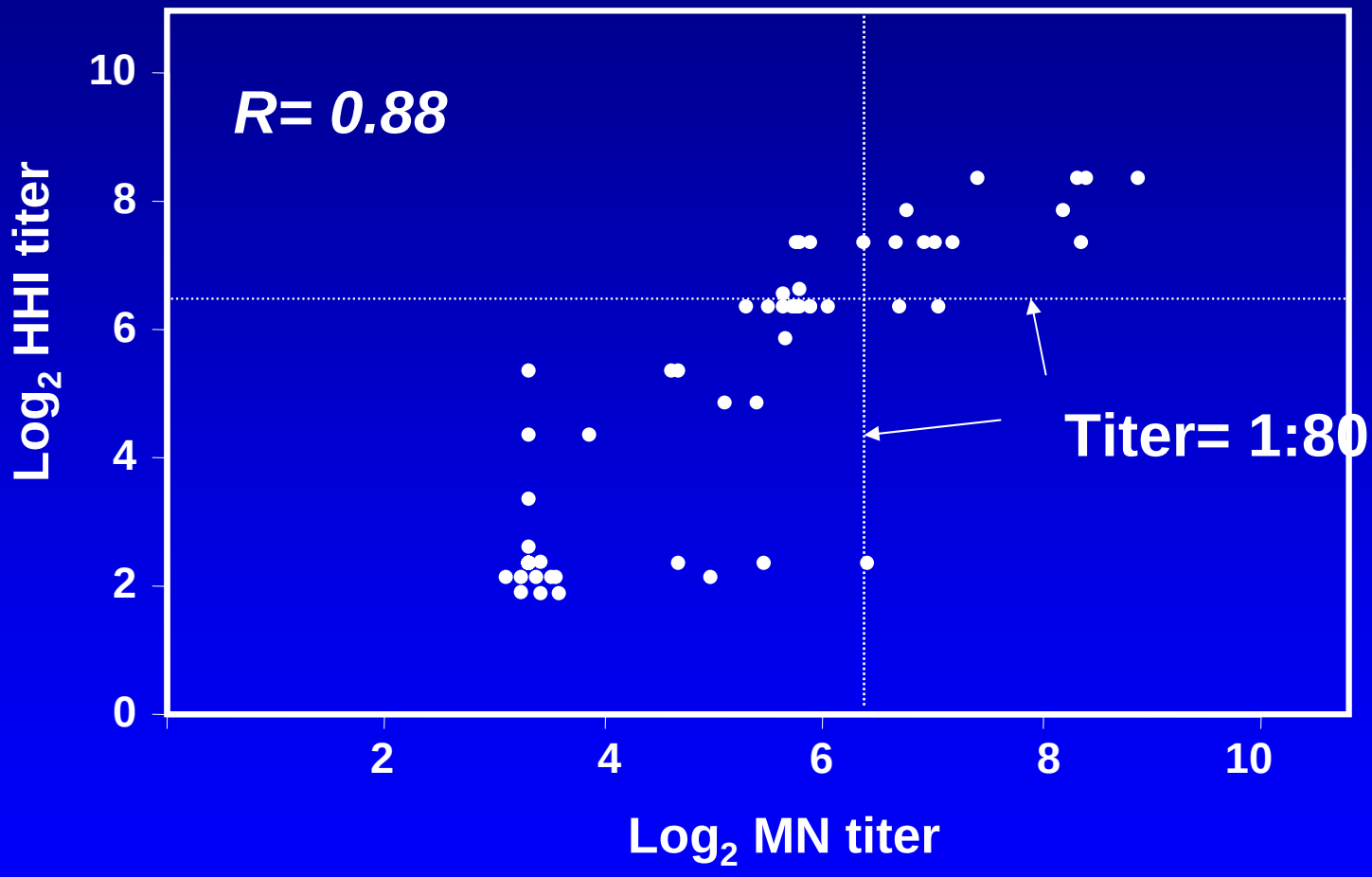
Correlation of Microneutralization (MN) and Horse RBC HI (HHI) titers for Sera from Individuals Vaccinated with VN/1203 H5N1 Vaccine*



** NIAID/NIH supported clinical trial in healthy adults*

CDC

Specificity of Horse RBC HI assay versus MN assay for H5N1 Clade 1 and 2 viruses

Clade 1 viruses		Clade 2 viruses	
Horse HI assay	MN assay	Horse HI assay	MN assay
93.8%	100%	100%	100%

- 80 U.S. control sera from persons aged 20-70 years
- Positive = titer of 1:80 or greater in either assay

CDC

Virus neutralisation assay

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Advantages

- Functional assay
- Suitable for semi-automation
- Equivalent sensitivity to other HA antibody assays for seasonal viruses (HI, SRH)
- More strain specific than HI for seasonal and H5N1 viruses *(De Jong, 2003; Stephenson et al, 2007; M. Zambon pers. com.)*
- Equivalent sensitivity to hHI and SRH for antibody to H5N1 viruses

Virus neutralisation assay

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Limitations

- Correlates of immunity unknown, although VN titre of 1:20-80 used to indicate a seropositive for H5N1 *(J Katz, M Zambon pers. com.)*
- Need for live virus – BSL2+ (rg H5N1virus), BSL3+ (HP H5N1virus)
- Technical aspects of assay can affect titres (Virus growth kinetics; protocol differences for serum treatment and dilution, amount of virus, neutralisation time, diluent)
- Poor reproducibility between labs (Stephenson et al, 2007)

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Variability of Virus Neutralisation assay

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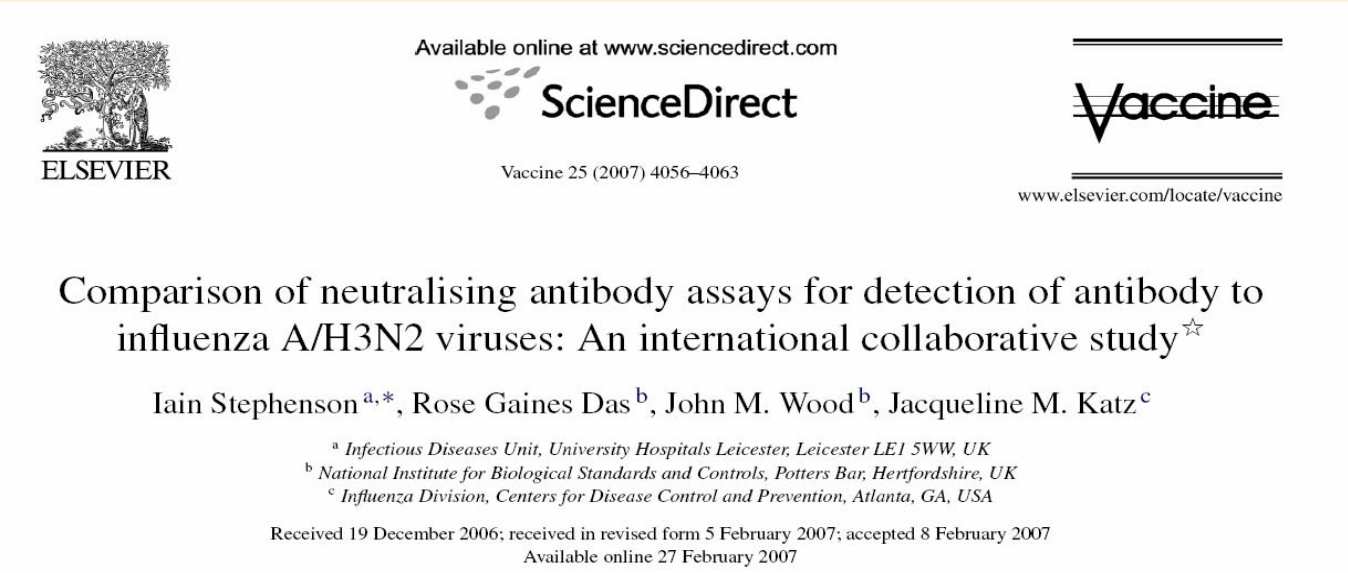

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Comparison of neutralising antibody assays for detection of antibody to influenza A/H3N2 viruses: An international collaborative study☆

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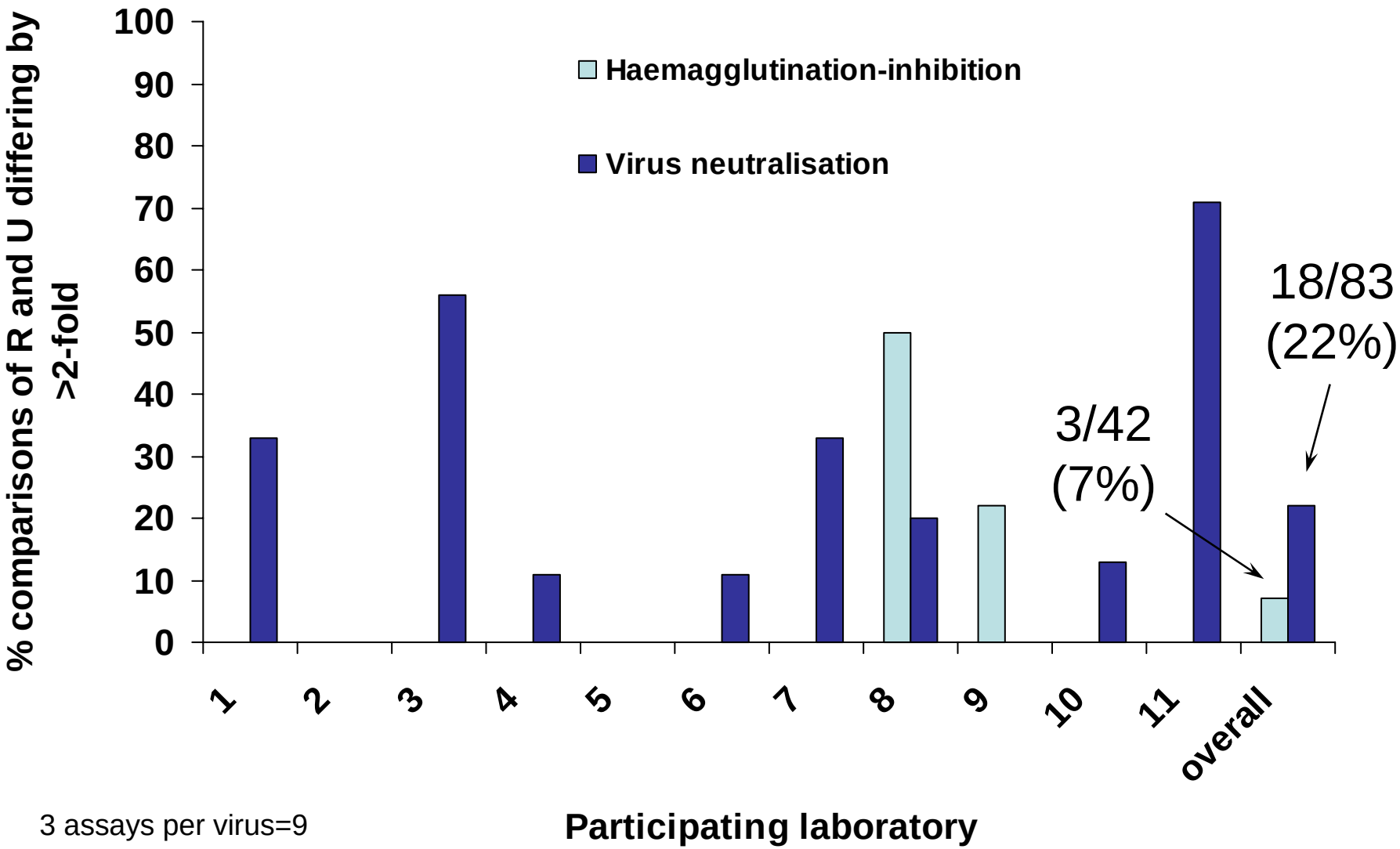
[b] *National Institute for Biological Standards and Controls, Potters Bar, Hertfordshire, UK*
[c] *Influenza Division, Centers for Disease Control and Prevention, Atlanta, GA, USA*

Received 19 December 2006; received in revised form 5 February 2007; accepted 8 February 2007
Available online 27 February 2007

- Comparison of HI and VN for H3N2 virus
- 11 labs from 8 countries
- Panel of 19 sera from vaccine trials

Results: comparison of replicate samples within laboratories (R and U)



3 assays per virus=9

Reproducibility of ‘absolute’ titres between laboratories

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Assay Type	HI		VN	
Laboratory	**Minimum**	**Maximum**	**Minimum**	**Maximum**
001	10	640	10	>2560
002	10	10240	7	12141
003	8	2048	53	81920
004	<10	1280	<10	2560
005	40	>=1920	28	4520
006	<10	2560	<80	2560
007	<10	640	<10	>1280
008	20	1280	<10	160
009	<10	2560	10	5120
010	<10	640	<10	2560
011	20	5120	20	5120

Variability of Virus Neutralisation assay

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Assays of serum N	GMT	Range	Fold difference	GCV
HI	39	10-1280	128	278%
VN	130	80-2560	724	529%

Median GCVs

HI 138-261% VN 256-323%

Use of standard serum

Median GCVs HI 64-108% VN 85-115%

Single Radial Haemolysis assay

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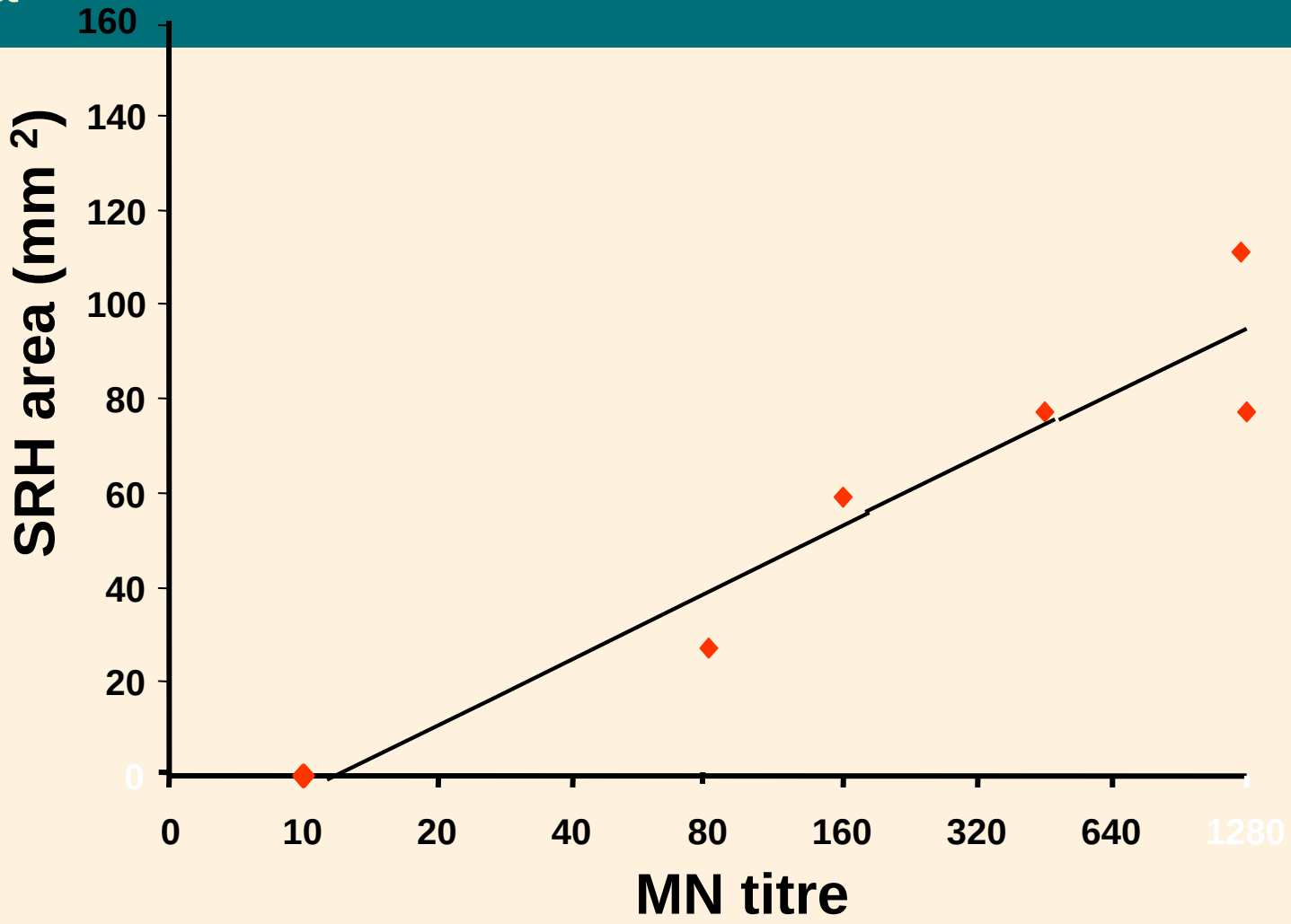
Advantages

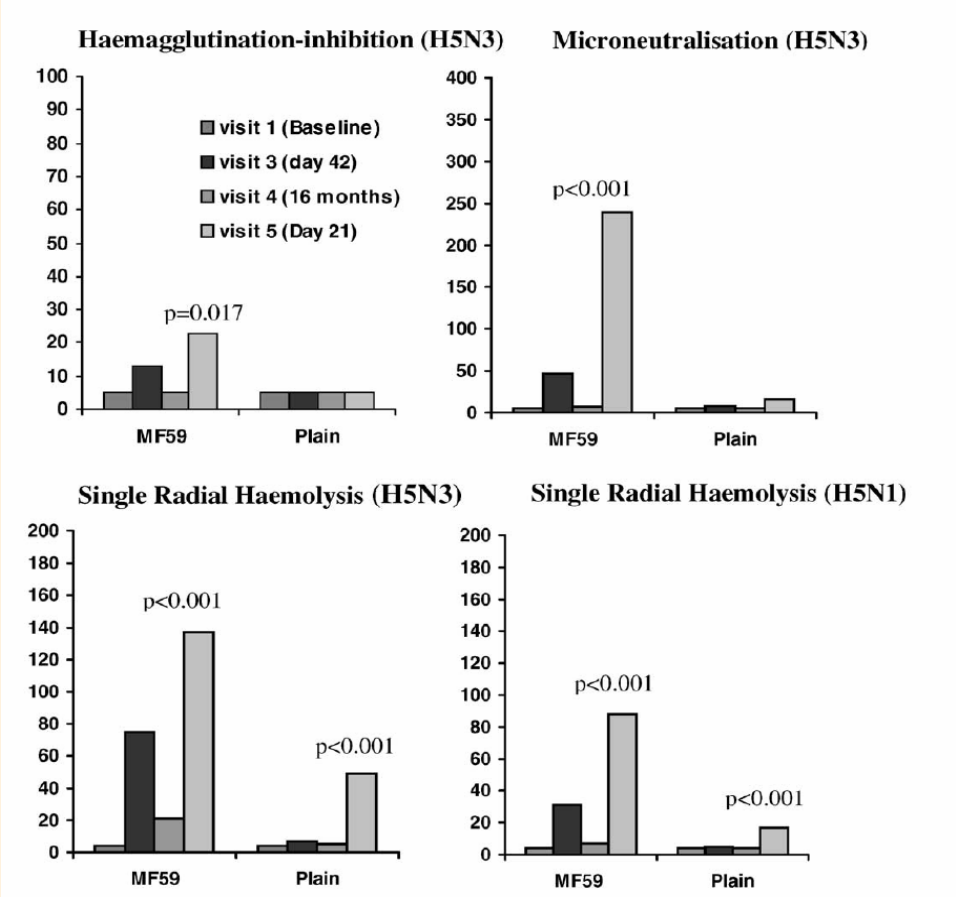
- SRH has equivalent sensitivity to HI (seasonal viruses); greater sensitivity than HI for B viruses
- SRH titre of 25mm² relates to 50% protection – seasonal flu
  - De Jong (2003) summary: three studies of H3N2 and B vaccination/natural infection
- For antibody to 1997 H5 viruses, SRH has greater sensitivity than turkey HI and equivalent sensitivity to VN (Stephenson et al, 2003)
- Better reproducibility between labs
  - Collaborative study with seasonal strains (Wood et al, 1994): HI 32 fold variation, SRH 3.8 fold variation between labs

Correlation between SRH and VN antibody to A/HK/97 for Hong Kong sera



MN data from J. Katz (CDC)

Correlation between SRH and VN for antibody to 1997 H5N1 viruses



Stephenson et al, 2003

Single Radial Haemolysis assay

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Limitations

- Can detect antibody to virus internal proteins
- Unsure about correlates of immunity for H5N1
- Technical details can affect clarity of zones
  - Complement, erythrocytes, source of virus
  - Can be difficult to read zones
  - Assays more demanding for Clade 1 H5N1 viruses

SRH assay of sera from human A/Hong Kong/97 (H5N1) cases

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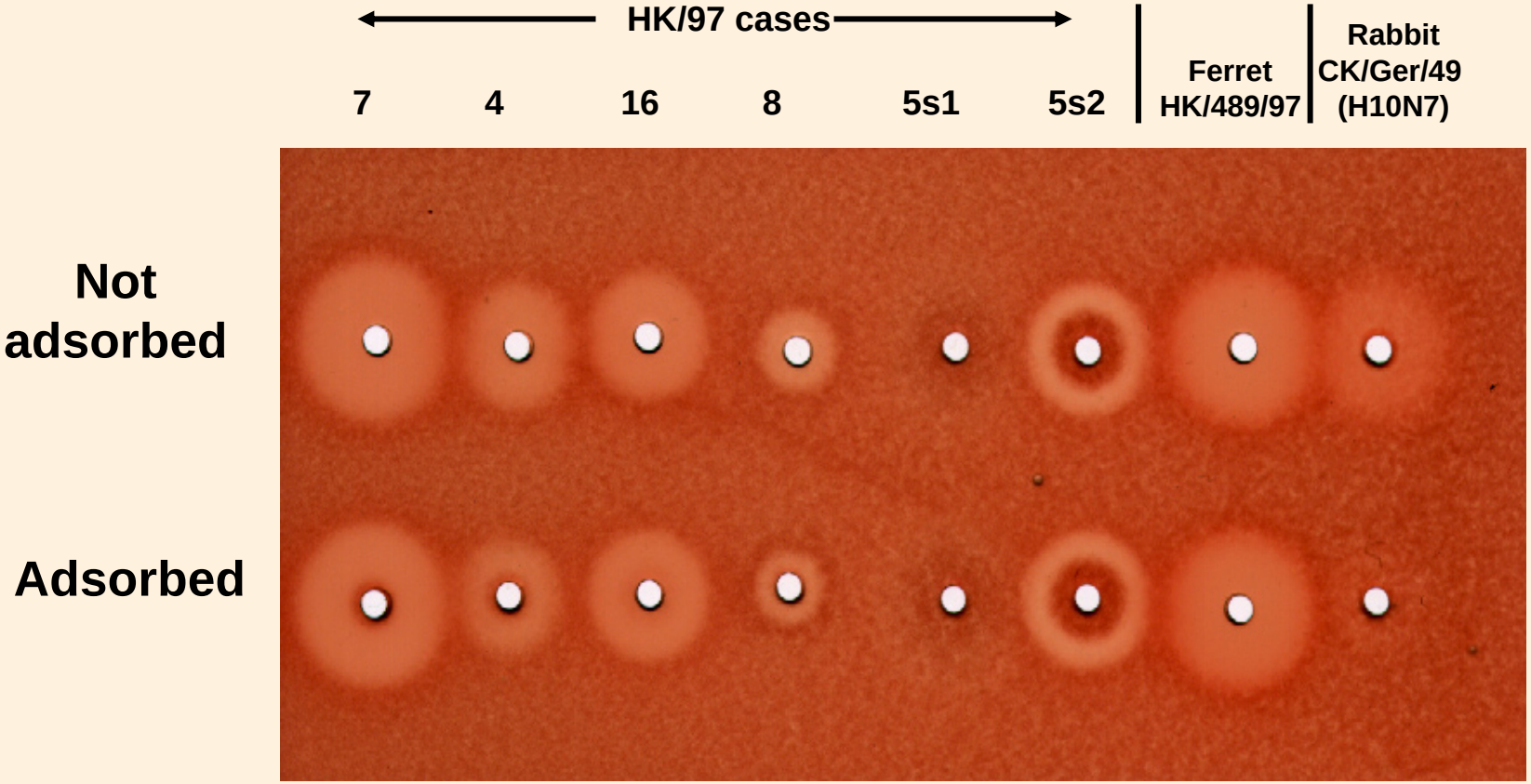

A/Hong Kong/489/97 (H5N1) virus

Assays for antibody to neuraminidase

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Neuraminidase enzyme inhibition

Advantages

- Allowed assays of NA antibody *(Aymard-Henry et al, 1973)*
- NI antibody associated with protection in mice; vaccines stimulate NI antibody in animals and humans (*various authors*)
  - NA has a role in protection yet NA content of vaccines and antibody to NA in vaccines are not regularly assessed

Limitations

- Laborious, use of toxic chemicals, not suitable for automation
- NA enzyme activity is labile
- Not sufficiently sensitive – poor levels of NI antibody in vaccine trials
- Low level of NA enzyme activity in MDCK cell grown viruses *(Aymard, 2003)*
- Antibody to HA can cause ‘steric hindrance’ – need reassortant viruses *(Kilbourne, 1968)*

Assays for antibody to neuraminidase

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ELISA assays

- *Partially pure NA – Murphy et al, 1980; Khan et al, 1982*
- *Capture Mab - Gerentes et al, 1998)*

Advantages

- Technically easier than NI – can automate
- More sensitive than NI
  - Post-vaccine sera: low levels of NI ab, but equivalent levels of ELISA NA ab and HI ab *(Powers et al, 1996)*
- Could be adapted to assay vaccine NA content

Limitations

- Reliance on Mabs, limits use for new variants – only N2 assay developed
- Specificity of antigen NA assay depends on availability of pure NA
- Unsure about reproducibility
- Unsure about correlates of immunity

Key assay limitations – action needed

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Need to standardise assays for antibody to H5 HA

- variability of hHI, VN, SRH titres
- comparability, sensitivity, specificity

- WHO collaborative study to evaluate H5N1 serological techniques and to establish an International Standard for H5N1 antibody
  - Plasma from two NIBRG-14 H5N1 vaccine trials pooled (2L) and freeze dried at NIBSC as candidate International Standard
  - Test sera filled and coded
  - Sheep anti-NIBRG-14 HA also to be evaluated as a standard serum
  - US human serum spiked with sheep H5 antibody to be evaluated
  - Viruses: A/Vietnam/1194/04 NIBRG-14, A/turkey/Turkey/1/05 NIBRG-23, CDC A/Anhui/05 RG6 virus
  - Reagents shipped November 26
  - Investigators: UK - NIBSC, HPA, U Hosp Leicester ; USA - CDC, CBER, NIAID
  - 17 participants agreed

Key assay limitations – action needed

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Need comparative evaluation of assays for antibody to H5 NA

- Novel and existing assays
- Evaluate sensitivity, specificity, reproducibility
  - Standardised assays
  - Standardised vaccines

Assays for antibody to H5 HA and NA

- Need correlates of immunity (especially for VN)

Prepare for the unexpected

- Adequate controls/back-up assays
- Investigations

Acknowledgements

Iain Stephenson, University Hospitals Leicester, UK

Maria Zambon, HPA, UK

Jackie Katz, CDC, USA

Rose Das, Diane Major, Bob Newman, NIBSC, UK