2018 WORLD INFLUENZA CONFERENCE
2018世界流感大会
7th - 10th September 2018, Beijing, China
2018年9月7日-10日
Beijing Conference Center
中国·北京·北京会议中心

Influenza vaccine immunogenicity and correlates of protection

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What is a correlate of protection?

Correlate of protection

- An immune response that is responsible for and statistically correlated with protection

Clinical and Vaccine Immunology, July 2010, p. 1055-1065, Vol. 17, No. 7
1071-412X/10/$12.00+0 doi:10.1128/CVI.00131-10
Correlates of Protection Induced by Vaccination Stanley A. Plotkin*

The European Agency for the Evaluation of Medicinal Products
Human Medicines Evaluation Unit

12 March 1997
CPMP/BWP/214/96

COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS (CPMP)

2.5 Antibody titration

All sera shall be assayed for anti-hemagglutinin antibody against the prototype strains by HI (Palmer et al., 1975) or SRH (Schild et al., 1975, Aymard et al., 1980) tests.

Positive and negative sera as well as reference preparations may be obtained from a reference laboratory.

2.6 Interpretation of results and statistics

Antibody titrations shall be done in duplicate; pre- and post-vaccination sera shall be titrated simultaneously.

The titre assigned to each sample shall be the geometric mean of two independent determinations;

European Medicines Agency

London, 24 January 2007
Doc. Ref. EMEA/CHMP/VWP/263499/2006

COMMITTEE FOR HUMAN MEDICINAL PRODUCTS (CHMP)

GUIDELINE ON INFLUENZA VACCINES PREPARED FROM VIRUSES WITH THE POTENTIAL TO CAUSE A PANDEMIC AND INTENDED FOR USE OUTSIDE OF THE CORE DOSSIER CONTEXT.

Immunological assessment and criteria

The comprehensive results from the HI, SRH and microneutralisation assays will form the basis for the assessment of immunogenicity. The choice of methodology and the standardisation of the assays should be addressed by the applicant. Applicants should predefine in the protocol which immunogical parameter(s) will be used in the primary analysis of immunogenicity.

In order to support the dose and regimen that are proposed in the SPC, studies should evaluate immune responses after single and multiple doses. Anti-HA antibodies should be assessed by means of HI and/or SRH assays. Virus neutralisation should also be assessed after single and multiple doses in at least a subset of vaccinees (see above under immunological assessment and criteria).

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New EMA guideliness

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

21 July 2016
EMA/CHMP/VWP/457259/2014
Committee for Medicinal Products for Human Use

Guideline on Influenza Vaccines

Non-clinical and Clinical Module

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New EMA guideliness

6.1.1. Immunological assays and parameters to be assessed

The assessment of the immunogenicity of influenza vaccines is traditionally based on two tests, the haemagglutination inhibition assay [HI] that detect antibody directed against the HA antigen, and the single radial haemolysis assay [SRH]. Neither the HI nor the SRH assays are standardised. It has been shown that they are both subject to considerable inter-laboratory variability. In any one clinical

The Virus Neutralisation [VN] assay quantifies functional antibody. The assay is usually based on detecting the ability of human serum at various dilutions to prevent viral replication in microplates (i.e. using a microneutralisation technique [MN]). It is essential that neutralizing antibody titres are determined in all studies, at least in a representative subset of the study population and preferably in

It is recommended that studies should monitor the quantity and quality of T-cell responses. For example, antigen-specific T-cell frequencies should be estimated (e.g. including Th1, Th2, T regulator cells, memory T cells and relevant cytokines). In addition, a thorough analysis of CD4+ and CD8+ responses, as well as the activation of memory B cells, would allow for a better characterisation of the effect of vaccination on antibody responses and clinical protection.

Applicants may consider evaluating anti-neuraminidase NA antibodies at least in randomly selected subsets. If conducted, the assay used should be validated and should be performed in appropriately experienced laboratories.

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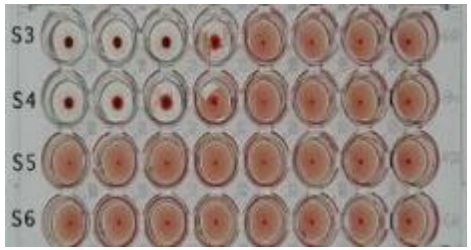
Actual Serological Assays for Abs detection

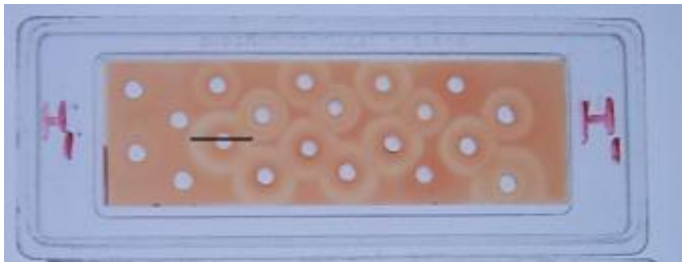
HAI – Haemagglutination Inhibition

- Suitable for screening a large number of samples
- Detects Ab that bind around receptor-binding site in globular head and block agglutination
- Good correlation with MN for seasonal strains
- BSL2 lab need also for pandemic strains
- EMA and FDA Approved



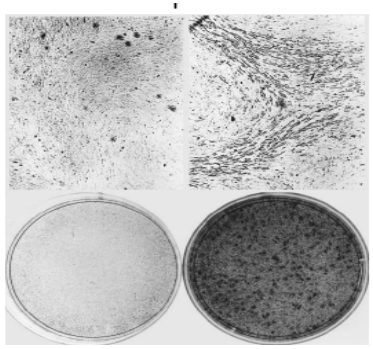
SRH – Single Radial Haemolysis

- Suitable for screening a large number of samples
- Detects Ab that bind around the virus and fix the complement (not IgG2)
- Good correlation with MN for pandemic strains
- BSL2 lab need also for pandemic strains
- EMA Approved



MN – Virus Neutralization

- Titration of functional antibody only
- Gold Standard for confirmation
- Not easy for screening a large number of samples
- High containment (BSL3plus) needed in case of pandemic strains
- Detects Ab that bind around globular head and block virus attachment/entry
- Detects cross-reactive Ab that bind to stem region
- No correlate of protection established



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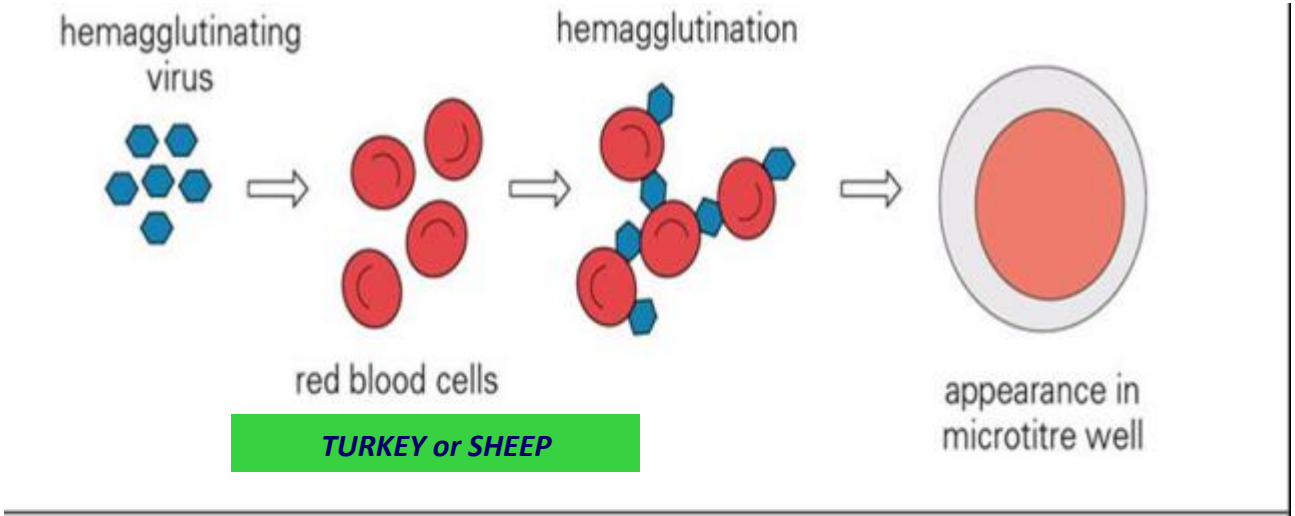
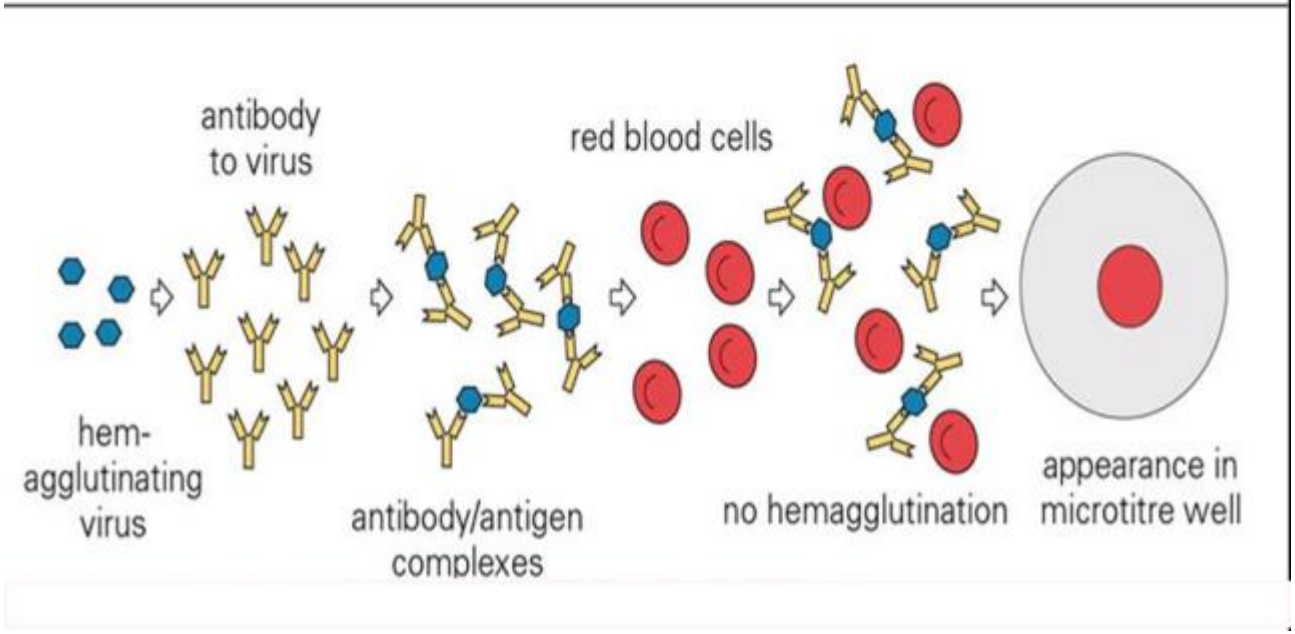
HAI – Haemagglutinin Inhibition





The HAI titer is the serum dilution at which induces 50% of hemagglutination, the reciprocal of this serum dilution is the HAI titre

-Correlate of protection:

HAI titre ≥ 40 for seasonal vaccines

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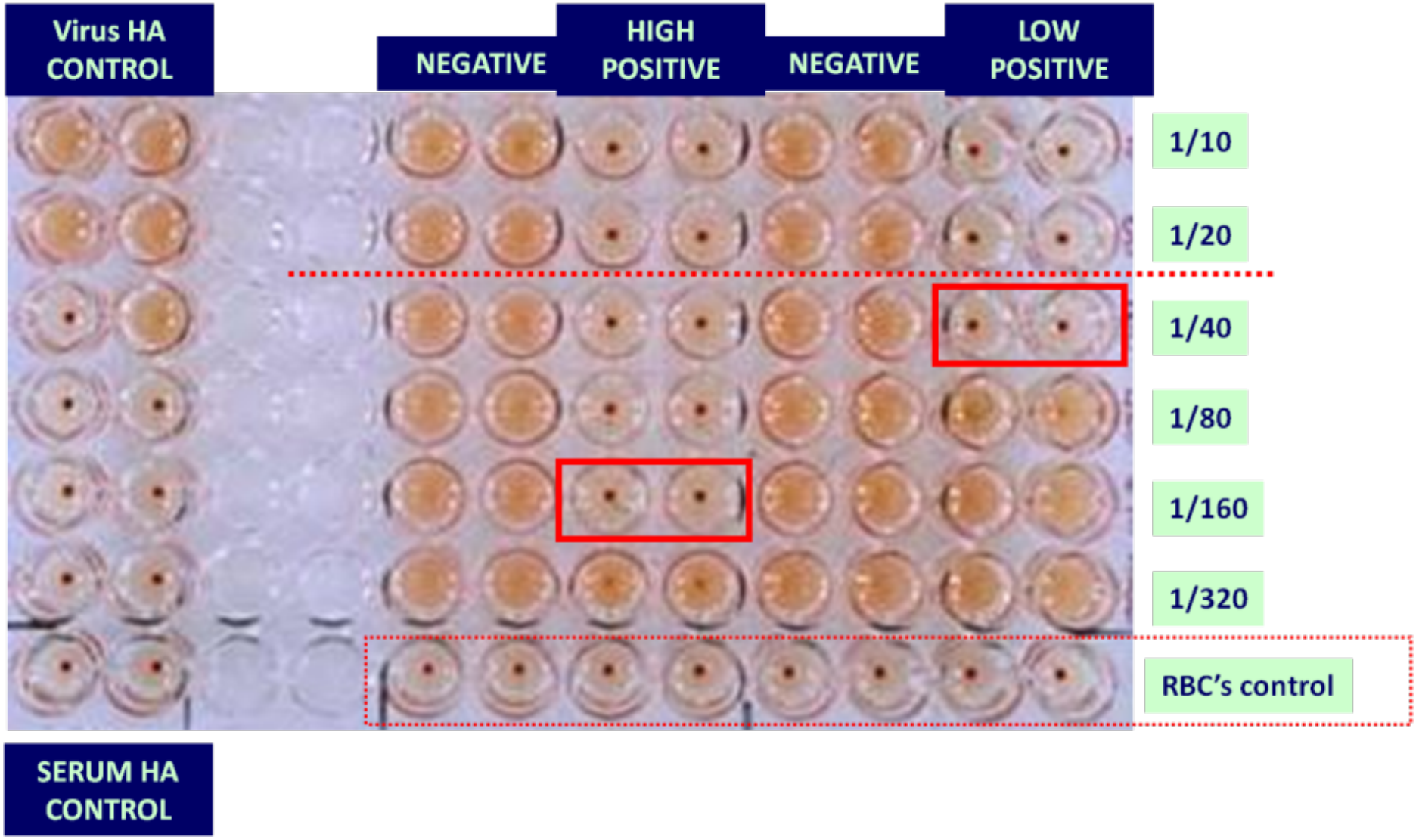
HAI – Haemagglutinin Inhibition



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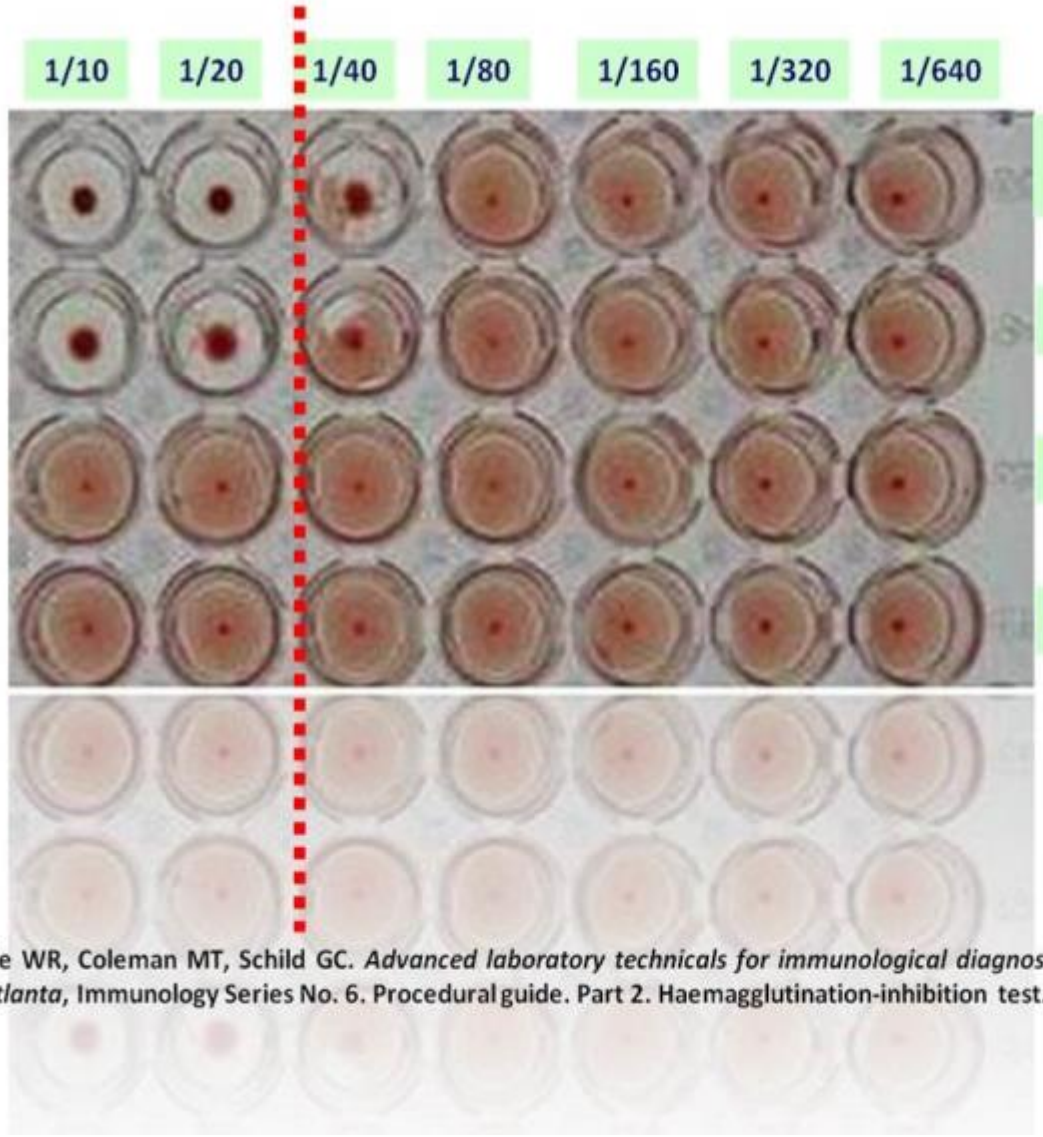
HAI – Haemagglutinin Inhibition



Palmer DF, Dowle WR, Coleman MT, Schild GC. *Advanced laboratory technicals for immunological diagnostic. US Department of Health Education and Welfare, P.H.S. Atlanta,* Immunology Series No. 6. Procedural guide. Part 2. Haemagglutination-inhibition test. 1975. p. 25–62.

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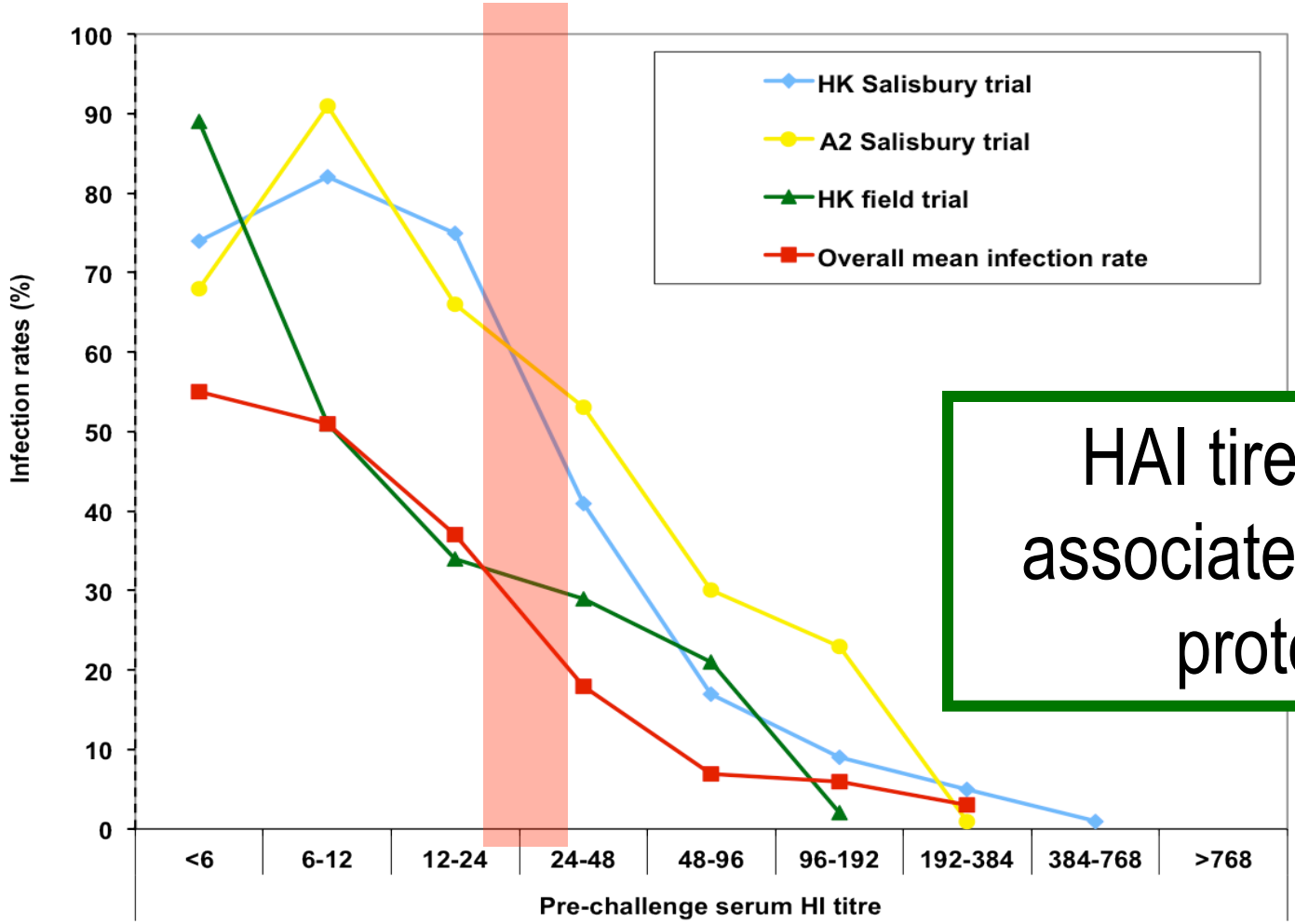
HAI – as a "correlate" established in efficacy trials long time ago



HAI tire of 18-36 associated with 50% protection

Hobson et al, 1972

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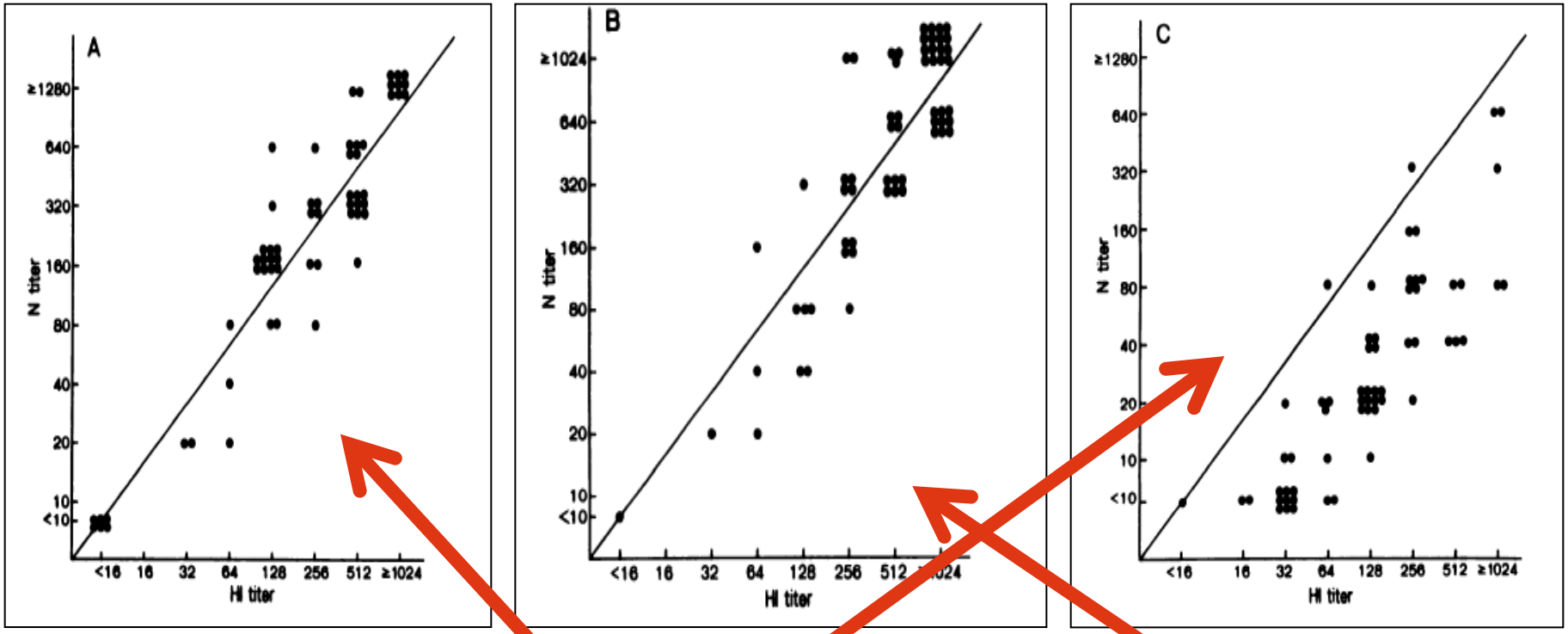
HAI and MN correlation for seasonal strains



Relationship between HAI and neutralizing-antibody (N) titers against A/Yamagata/120/86 (H1N1) (A), A/Fukuoka/C29/85 (H3N2) (B), and A/Shisen/2/87 (H3N2) (C).

Okuno, *J Clin Microbiol.* 1990

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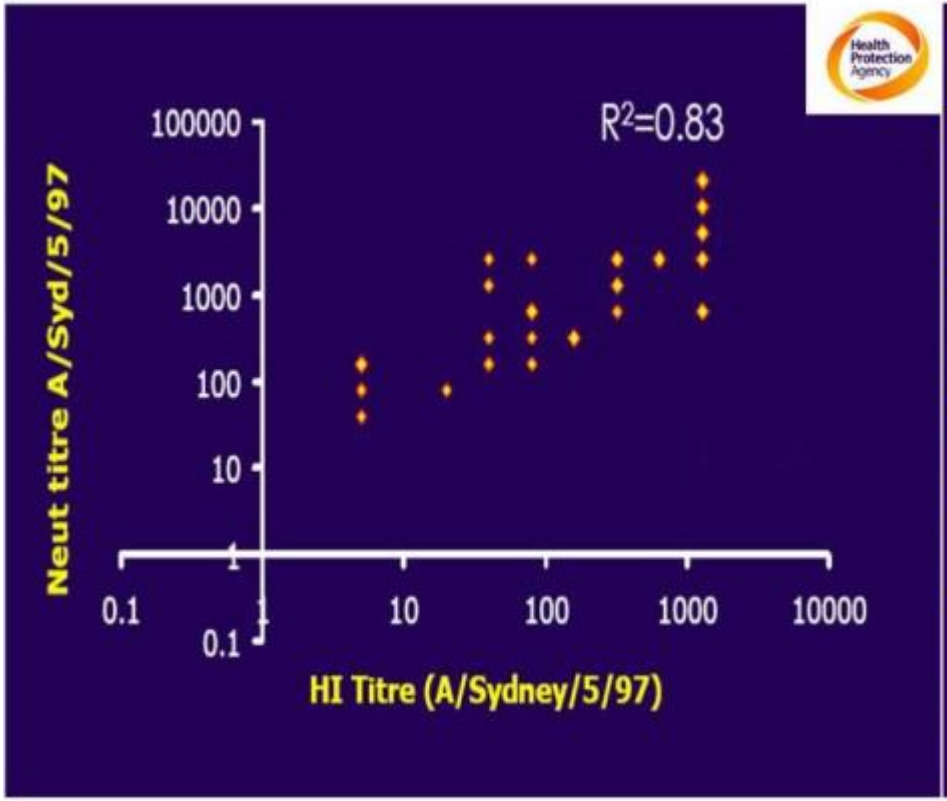
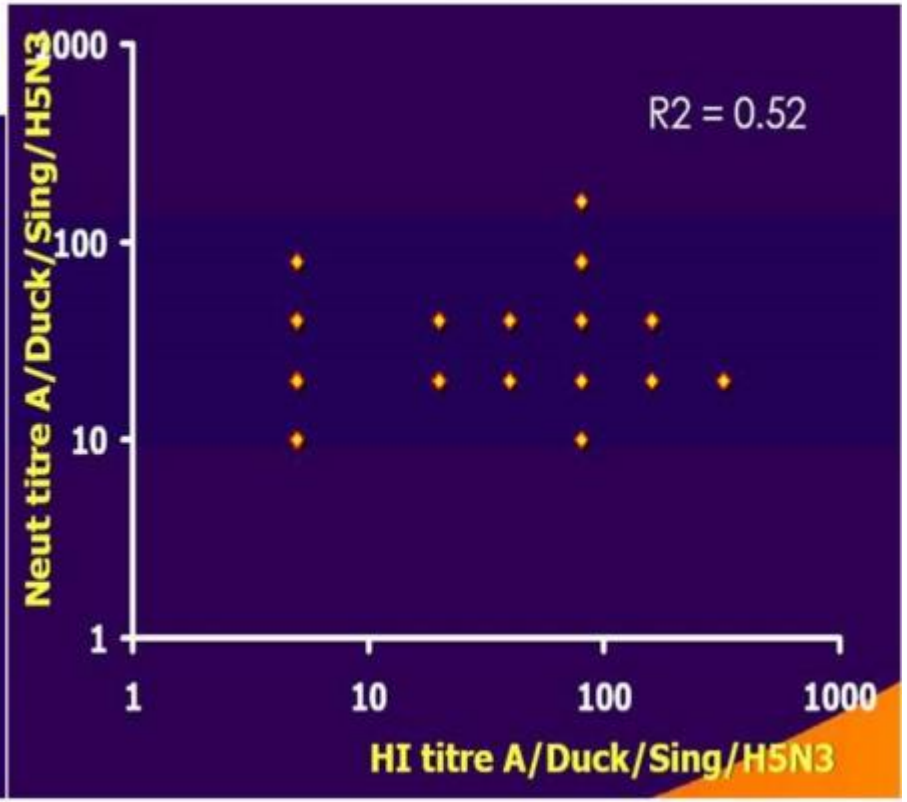
HAI and MN correlation for H5 strains

Comparison Neut / HI H3N2 vaccine sera



Comparison Neut / HI H5N3 vaccine sera



From: M. Zambon, HPA London

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HAI for children

TABLE 3. Estimated Antibody Titers at Day 50 Needed to Provide Various Levels of Protection

Probability of Protection	H3N2 Antibody Titer Level
50%	1:110
60%	1:151
70%	1:215
80%	1:330
90%	1:629

The HAI titer of 1:40, which has been recognized as an immunologic correlate corresponding to a 50% reduction in the risk of contracting influenza, is based on studies in adults.

Neither seasonal nor challenge-based correlates have been evaluated in children.

A recent meta-analysis of the relationship of HI and clinical protection in adults supported the use of a 1:40 titer as a correlate of 50% protection in adults. However, data in this paper do not support the use of a 1:40 titer as a correlate of 50% protection against influenza infection in children less than 6 years of age. A cut-off of 1:110 measured 21 days after the second vaccine dose may be used to predict a 50% clinical protection rate in this age group.

The reason that more antibody was required for protection in children is not known, several factors might contribute to the need for more antibody to provide protection in children.

The most important is probably the fact that the younger the age of the individuals the lower the probability that a child has had immunologic experience (induction of cell-mediated immunity, specific memory) with influenza either through vaccination or infection.

Black S. PIDJ 2011

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Why does HAI underestimate antibodies for Pandemic Strains?

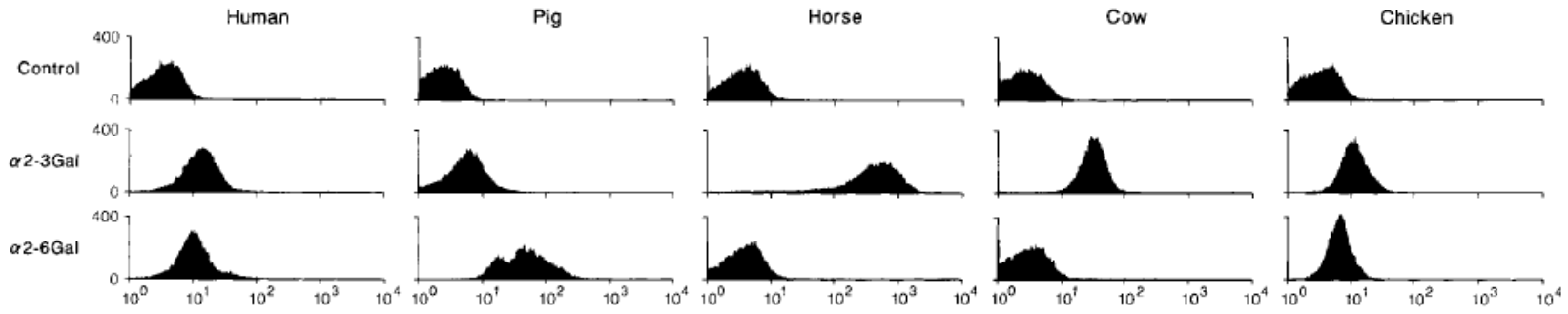
The efficiency of binding of influenza virus is dependent on the specificity of sialic acid (SA) in the cellular receptor

- **Humans: *NeuAc-α2,6,Gal***
- **Birds: *NeuAc-α2,3,Gal***

Receptor specificity of influenza viruses correlates with ability to agglutinate RBCs from different species

- **Human viruses agglutinate chicken/ turkey, human and guinea pig, but not horse or cow RBCs**
- **Avian viruses agglutinate RBCs from all these species**

Comparison of the relative amounts of SAa2,3Gal and SAa2,6Gal linkages on the surface of animal erythrocytes



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H1N1 - HAI different RBCs

		HI titers avian RBCs (~50% α2,3 - 50% α2,6)						HI titers mammals RBCs (~100% α2,3)			HI Titer
Samples	MN study (NT mean)	Turkey 0.5%	Chicken 0.35%	Mallard 0.35%	Guineafowl 0.35%	Phaesant 0.35%	Pigeon 0.35%	M-Sheep 0.5%	Horse (IT) 0.5%	Horse (AR) 0.5%	Humans 0.50%
21	5	5	5	5	5	5	5	5	5	5	5
22	5	5	5	5	5	5	5	5	20	5	5
23	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	5	5	5	5	5	5	5
34	14	5	5	5	5	5	5	5	160	160	5
35	14	5	20	20	20	20	40	40	320	160	40
27	**20**	**5**	**40**	**5**	**5**	**5**	**5**	**80**	**1280**	**1280**	**80**
33	20	5	5	5	5	5	5	20	160	160	5
40	20	5	20	5	5	5	5	20	5	5	20
28	28	160	80	160	5	160	320	160	1280	1280	640
29	28	160	80	160	80	80	320	160	1280	1280	640
36	28	5	10	5	5	10	5	5	320	160	40
30	40	80	80	80	80	80	320	160	1280	1280	160
38	**56**	**40**	**20**	**5**	**5**	**5**	**5**	**40**	**1280**	**5**	**160**
39	56	5	5	5	5	5	5	5	160	160	20
26	80	5	5	5	5	5	5	5	320	160	5
37	80	5	5	5	5	5	5	20	1280	160	160
32	95	40	40	5	5	5	80	40	1280	1280	160
25	160	40	40	5	5	40	80	160	1280	640	80
31	226	5	5	5	5	5	80	5	640	640	40

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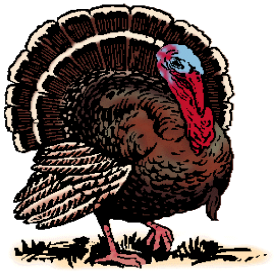
HAI does not work for pandemic

- It has been recognised that the haemagglutination–inhibition (HAI) test is not sufficiently sensitive to detect human antibody to A/H5N1 influenza virus.
- A modified single radial haemolysis (SRH) test has been described, the SRH immunoplates were prepared as described by Schild using turkey erythrocytes.
- The SRH test recognised the antibody in a specific antiserum to A/H5N1/Hong Kong/489/97 virus.
- H5 SRH antibody induced by human A/H5N1 virus infection and A/H5N3/Duck/Singapore/97 vaccination correlated with antibody detected by MN techniques.
- The modified SRH test offers a good serological technique for detecting antibody to H5 haemagglutinins.

A single radial haemolysis assay for antibody to H5 haemagglutinin

J.M. Wood[a,*], D. Melzack[a], R.W. Newman[a], D.L. Major[a], M. Zambon[b], K.G. Nicholson[c], A. Podda[d]



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SINGLE RADIAL HAEMOLYSIS (SRH)

Single Radial Haemolysis (SRH) is routinely used for the detection of influenza-specific (and rubella) IgG antibody.

- SRH has been shown to be sensitive, specific, and reliable.
- SRH plates are usually prepared in the laboratory using commercially available reagents.
- Test sera are placed in wells on a plate containing agar with influenza antigen-coated RBC and guinea-pig complement.
- The presence of influenza-specific IgG is detected by the lysis of influenza antigen-coated RBC mediated from **GUINEA-PIG complement**.
- The zone of lysis around the well is dependent on the level of specific antibody present.

Schild GC, Pereira MS, Chakraverty P. ***Single-radial-hemolysis: a new method for the assay of antibody to influenza haemagglutinin. Applications for diagnosis and seroepidemiologic surveillance of influenza.*** Bull World Health Organ. **1975**; 52(1):43-50.

Russell SM, McCahon D, Beare AS. ***A single radial haemolysis technique for the measurement of influenza antibody.*** J Gen Virol. **1975** Apr;27(1): 1-10.

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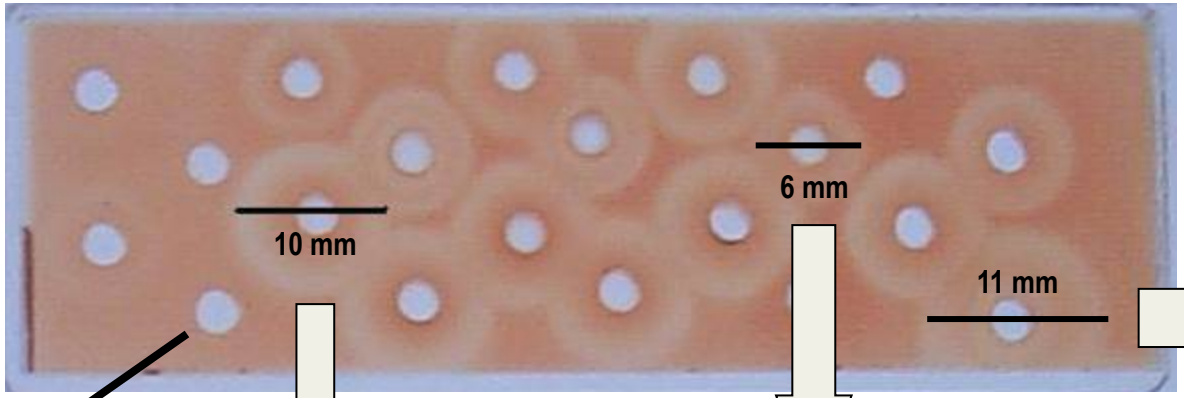
SINGLE RADIAL HAEMOLYSIS (SRH)

- The size of the haemolysis zone around a well containing serum is measured in mm. The diam is then transformed in area.
- If the area size is greater than 25 mm^2, then the subject is considered to be seroprotected.
- If the area size is <= 4 mm^2, then the subject is considered negative according to EMEA guidelines.



Diam = 2.256 mm
Area = 3.997 mm^2
Negative

Area = 28.274 mm^2
Low Positive - Seroprotection

Area = 78,540 mm^2
High Positive - Seroprotection

Area = 95.033 mm^2
High Positive – Seroprotection

Positive control

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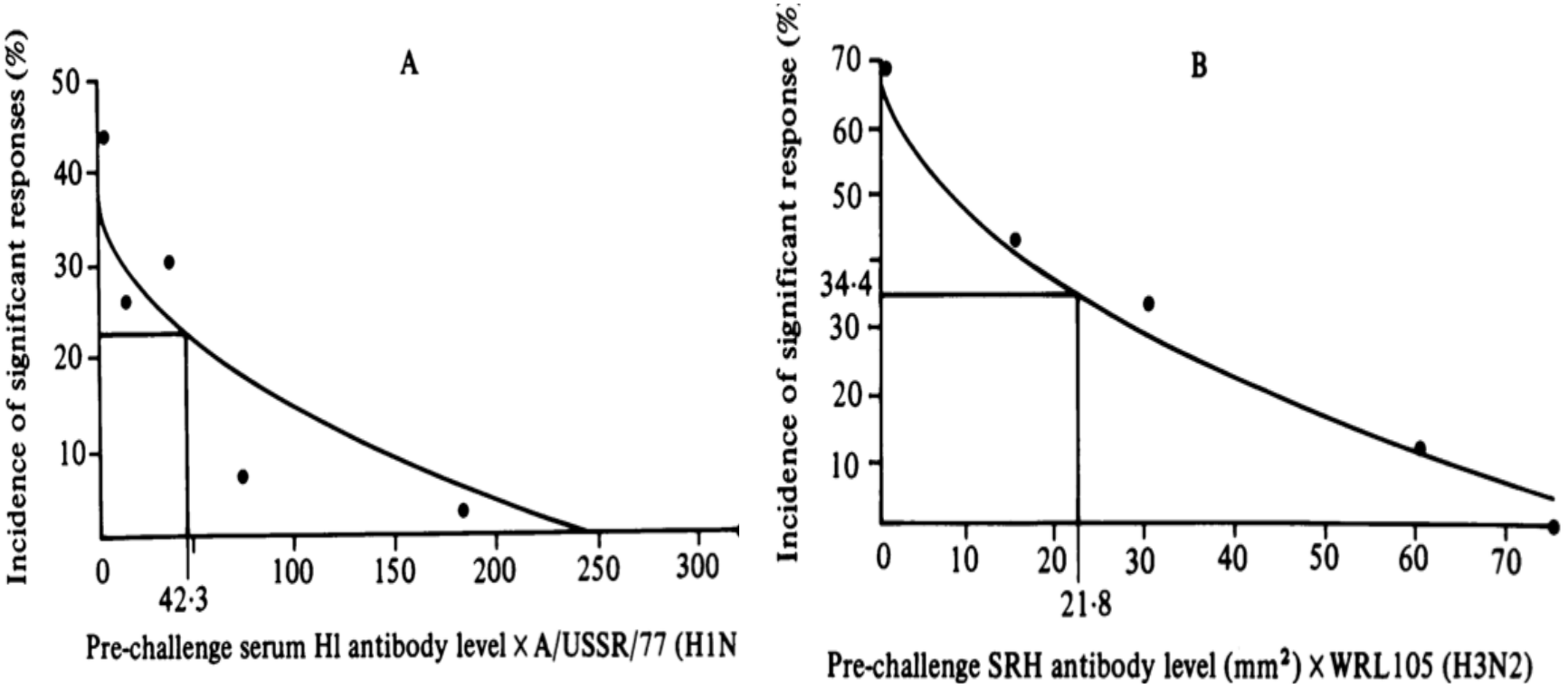
SINGLE RADIAL HAEMOLYSIS (SRH)

- 50% protection was associated with SRH zone areas of 20.0-25.0 mm^2
- H1N1 (HI 42) and SRH zone area 21mm^2 .



Al-Khayatt et al. J Hyg 1984

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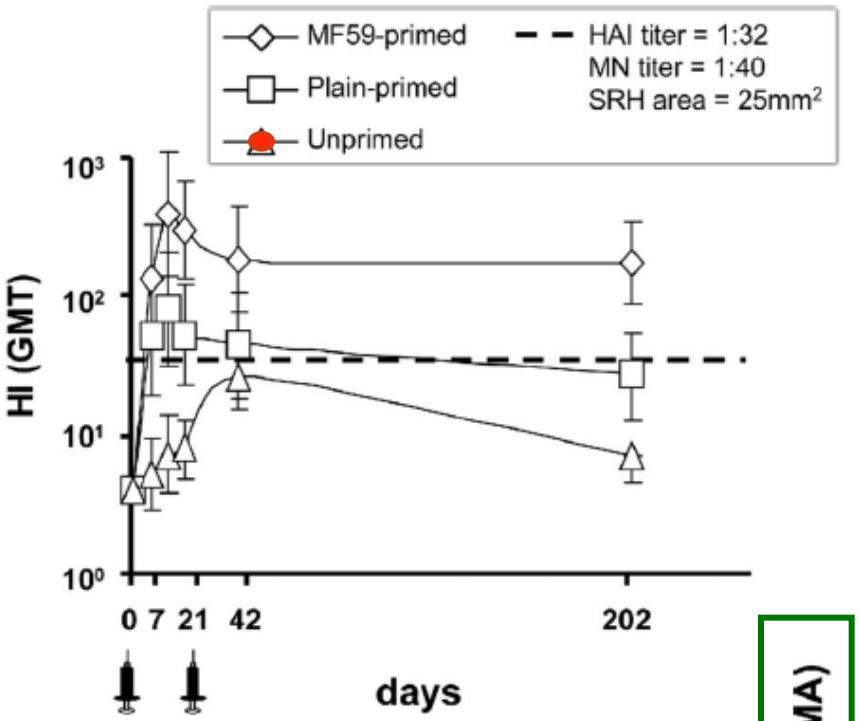
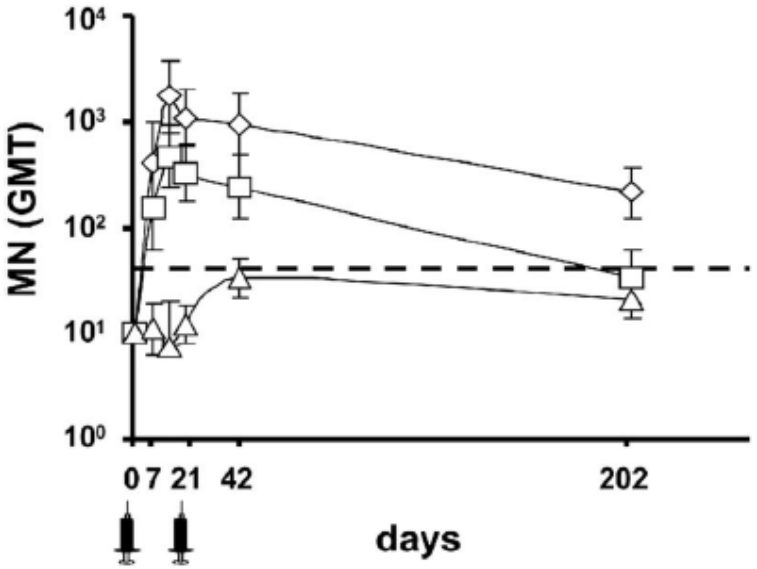
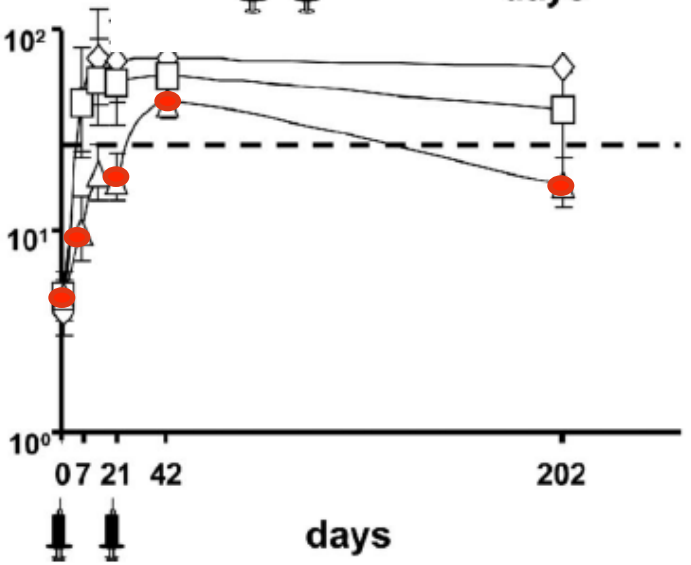
H5N1 - SRH - sensitivity





SRH (GMA)



Galli et all. PNAS 2009

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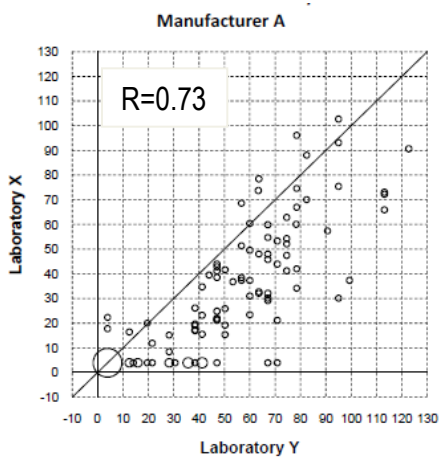
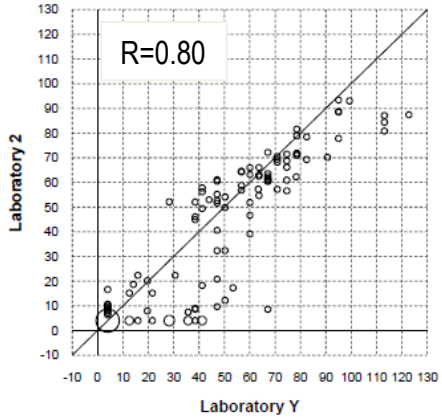
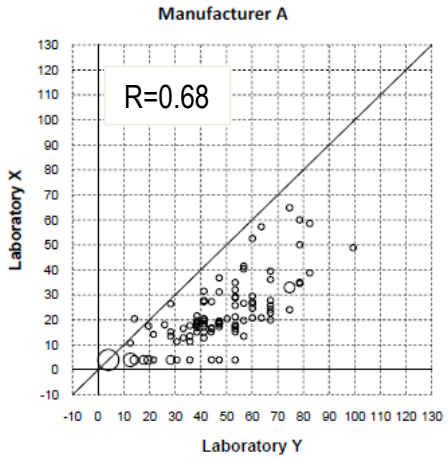
SRH – correlation interlab, seasonal strains

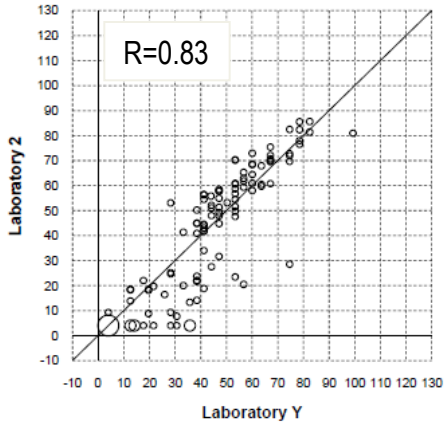
A/H1N1/Solomon Islands/3/2006



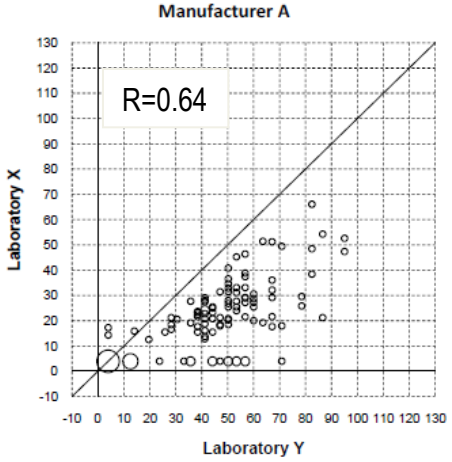


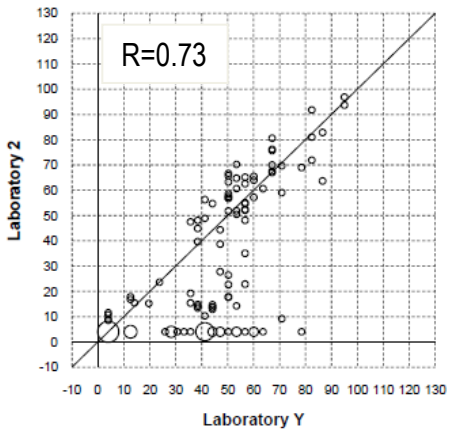
A/H3N2/Wisconsin/67/2005





B/Malaysia/2506/2004





Laboratory Y is Reference Lab for SRH

BSP063-Influenza - Collaborative Study - Biological Standardisation Programme - Council of Europe, EDQM

CDC Neutralisation Test

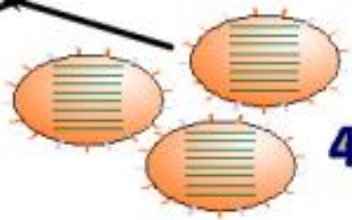
1. Treat sera

56°C 30 min/RDE

2. Add Sera

3. Dilute sera

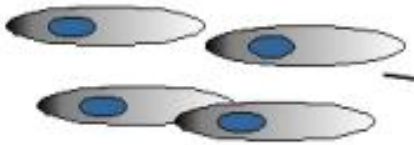
1h @37°C



4. Add Virus

100 $TCID_{50}$/well

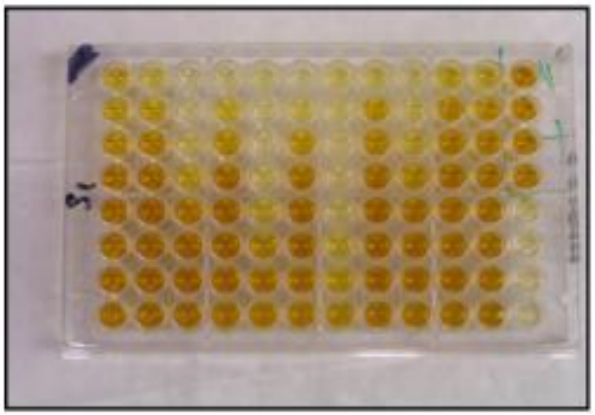
5. MDCK Cells



$1.5x10^4$ cells/well

16-22 hr @37°C

6. Fix cells and run NP ELISA



From: Rowe T. *J Clin Microb* 1999

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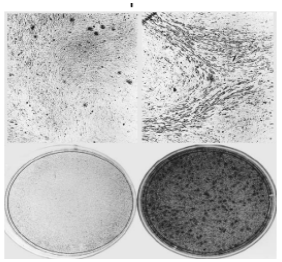
MN assay – Read out is a critical point

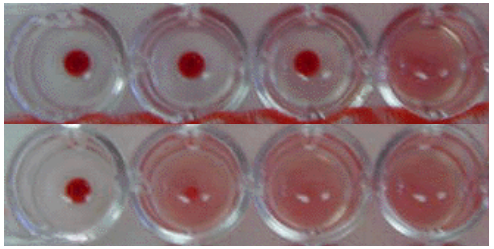
•by ELISA-NP in each well:

1.Rowe T, Abernathy RA, Hu-Primmer J, Thompson WW, Lu X, Lim W, Fukuda K, Cox NJ, Katz JM. (1999) Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. *J Clin Microbiol* 37:937-943.

•by CPE for each well:



•by HI in surnatant of each well:



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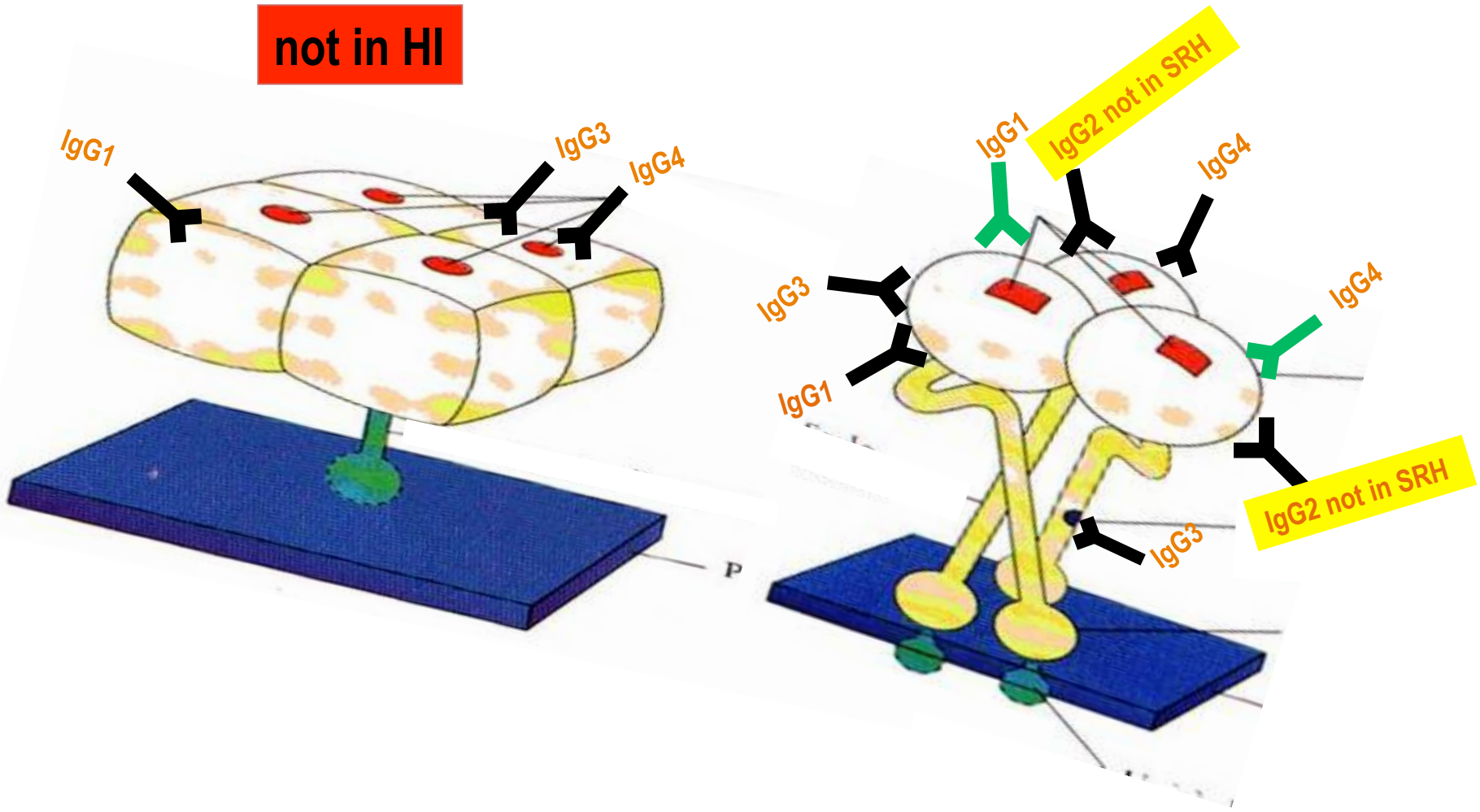
HAI / MN / SRH different Ab detection

functional, only in MN

not in HI



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ELISA, not new assay, different approach

HA

HA	VN		
ELISA	Neg	Pos	Total
Neg	161	8	169
Pos	29	19	48
Total	190	27	217

Sens. = 0.70
Spec. = 0.85

HA	SRH		
ELISA	Neg	Pos	Total
Neg	149	20	169
Pos	28	20	48
Total	177	40	217

Sens. = 0.50
Spec. = 0.84

HA	HI		
ELISA	Neg	Pos	Total
Neg	167	2	169
Pos	37	11	48
Total	204	13	217

Sens. = 0.81
Spec. = 0.85

HA1

HA1	VN		
ELISA	Neg	Pos	Total
Neg	183	8	191
Pos	7	19	26
Total	190	27	217

Sens. = 0.70
Spec. = 0.96

HA1	SRH		
ELISA	Neg	Pos	Total
Neg	171	20	191
Pos	6	20	26
Total	190	27	217

Sens. = 0.50
Spec. = 0.97

HA1	HI		
ELISA	Neg	Pos	Total
Neg	190	1	191
Pos	14	12	26
Total	204	13	217

Sens. = 0.93
Spec. = 0.92

From: M. Zambon, HPA London

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HA1

HA2

Less cross-reactivity when only HA1 and not HA2 is used in ELISA?

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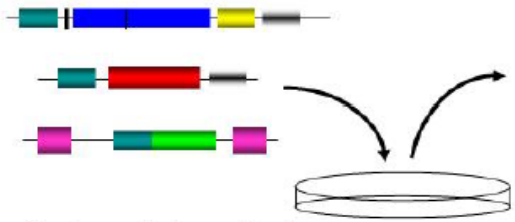
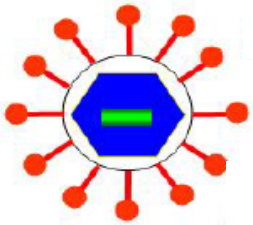
MN for Pandemic Assay *using PP*



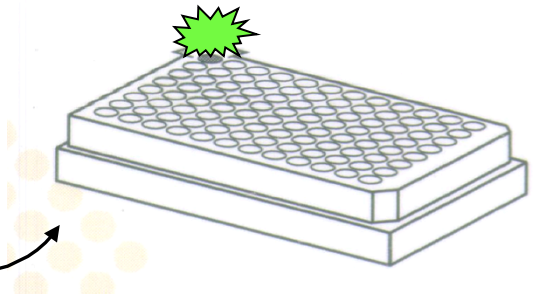
3 plasmid transient transfection into 293T cells



Haemagglutin (HA)

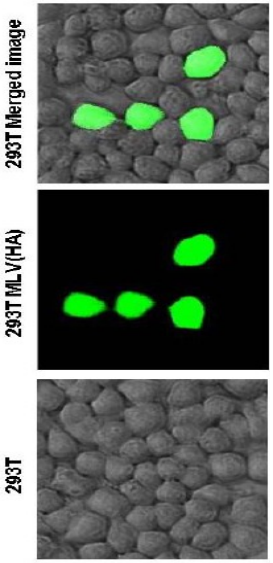
Lentiviral Gag-Pol

Vector encoding luciferase



Measure N-Ab titre of serum using a luciferase readout

Vaccine 27 (2009) 5998–6003



Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Pseudoparticle neutralization is a reliable assay to measure immunity and cross-reactivity to H5N1 influenza viruses

Isabella Alberini[a], Elena Del Tordello[a], Alba Fasolo[a], Nigel J. Temperton[b], Grazia Galli[a], Chiara Gentile[c], Emanuele Montomoli[c], Anne K. Hilbert[d], Angelika Banzhoff[d], Giuseppe Del Giudice[a], John J. Donnelly[a], Rino Rappuoli[a,*], Barbara Capecchi[a]

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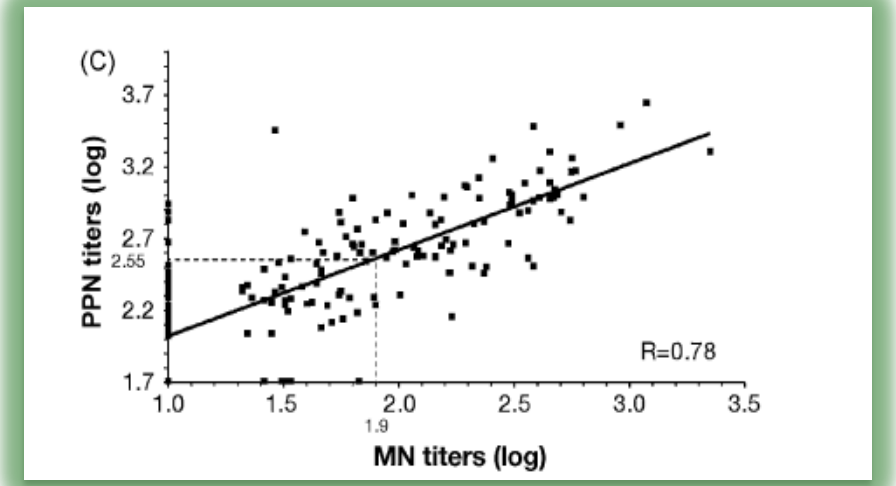
H5N1/VIET MN PP-based correlation

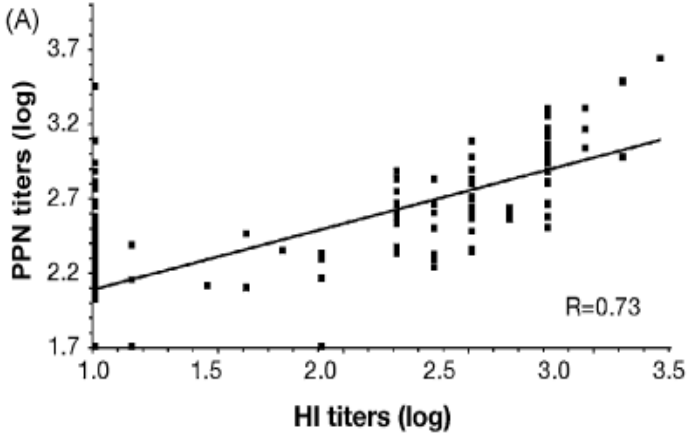
http://www.ClinicalTrials.gov NCT00382187

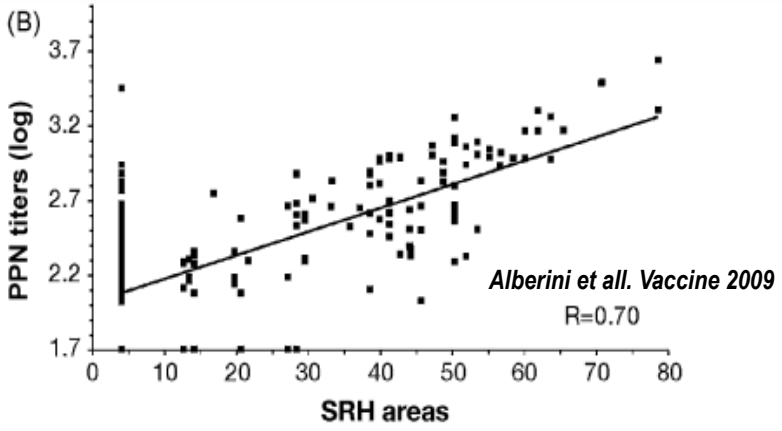
The administered vaccine was a monovalent H5N1 subunit influenza vaccine derived from the /Vietnam/1194/2004

40 adults were enrolled in the study:

- ***one group received 15g of plain H5N1(Non-Adj-15; N= 13);***
- ***second group received 7.5g of H5N1 adjuvanted with MF59 (MF59-7.5; N= 14);***
- ***third group received 15g of H5N1 adjuvanted with MF59 (MF59-15; N= 13).***







In the picture vertical dashed line indicate the value of MNlog10 titer = 1.9 (corresponding to a titer of 1:80), the proposed threshold of protective antibodies, horizontal dashed line indicate the corresponding value of PPN log10 titer = 2.55 (corresponding to a titer of 1:357).

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the role of Neuraminidase

TBA - **Traditional Thiobarbituric acid method** (Warren -1959; Aminoff -1961)

- **Miniaturized TBA method** (Sandbulte et al. 2009)

BASED ON THE CHEMICAL CONVERSION OF SIALIC ACID TO CHROMOPHORE

Impractical for large numbers of samples and employs hazardous chemicals

ELLA - **Enzyme-linked lectin assay** (Lambre et al. 1990 and Cate et al. 2010)

BASED ON PEANUT AGGLUTININ (PNA) THAT BINDS TO RESIDUAL TERMINAL GALACTOSE AFTER NA CLEAVAGE

Practical method suitable for determination of NI titers in human sera

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ELLA: source of antigen

- ✓ *Reassortant virus*

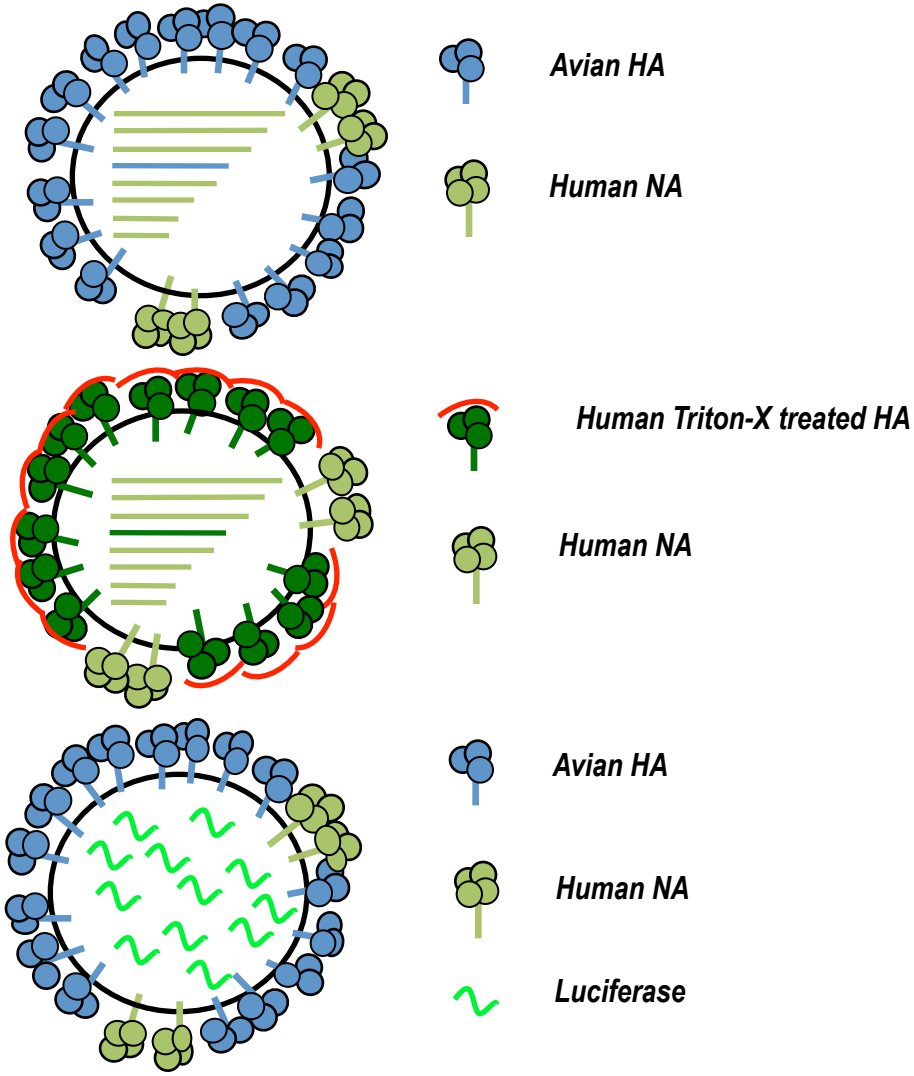
(Couzens et al., 2014)

- ✓ *Triton-X inactivated virus*

(Jonges et al., 2010)

- ✓ *NA-bearing PV*

(Prevato et al., 2015)



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MUCOSAL - IgA

STATE OF ART ABOUT IgA ASSAYs FOR INFLUENZA

- NO standardized protocols
- NO positive controls available
- NO standardized KITs

Many different approaches for IgA detection and sample standardization

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ELISA - ASSAY

The E.L.I.S.A. assays are the best method to detect IgA antibodies in different samples (Nasal, Saliva or Sera)
but <u>WAHT IS THE BEST PROTOCOL?</u>

Human Vaccines & Immunotherapeutics 9:9, 1962–1970; September 2013; © 2013 Landes Bioscience

Intranasal vaccination with an inactivated whole influenza virus vaccine induces strong antibody responses in serum and nasal mucus of healthy adults

Akira Ainai,[1,2] Shin-ichi Tamura,[2] Tadaki Suzuki,[2] Elly van Riet,[1] Ryo Ito,[2,3] Takato Odagiri,[1] Masato Tashiro,[1] Takeshi Kurata[2], and Hideki Hasegawa[2,*]

[1]Influenza Virus Research Centre; National Institute of Infectious Diseases; Tokyo, Japan; [2]Department of Pathology; National Insti
[3]Biological Science and Technology; Tokyo University of Science; Chiba, Japan

Keywords: influenza virus, intranasal vaccination, neutralizing antibody, haemagglutinati
healthy adult volunteer

Abbreviations: S-IgA, ...eutralization titer; HI assay
assay; HI titer, haemagg... haemagglutination-inhib
...nked immunosorbent assay

«SHORT assay»

Haemagglutination inhibition (HI) and neutralization (NT) titers as well as haemagglutini
responses were examined in 50 healthy adults aged between 22 and 69 y old after two intran
inactivated whole virus vaccine derived from A/Victoria/210/2009 virus (45 μg HA per dose) a
HI titers after two-doses of the nasal vaccine showed >2.5-fold rise in the ratio of geometric me
>40% of subjects with a ≥4-fold increase in titer and >70% of subjects with a titer of ≥1:40,
with an effective outcome of vaccination in the criteria defined by the European Medicines Ag
antibody responses correlated with HI antibody responses, although NT titers were about 2-

DOI:10.1111/j.1750-2659.2011.00330.x
www.influenzajournal.com
Original Article

Induction and maintenance of anti-influenza antigen-specific nasal secretory IgA levels and serum IgG levels after influenza infection in adults

Chisa Fujimoto,[a] Noriaki Takeda,[a] Atsushi Matsunaga,[b] Ayako Sawada,[b] Takeshi Tanaka,[c] Takashi Kimoto,[d] Wakako Shinahara,[d] Takako Sawabuchi,[d] Miyoko Yamaguchi,[d] Masaki Hayama,[e] Hiroaki Yanagawa,[f] Mihiro Yano,[d] Hiroshi Kido[d]

[a]Department of Otolaryngology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan. [b]Ohkita Medical Clinic, Osaka, Japan. [c]Mino Tanaka Hospital, Tokushima, Japan. [d]Division of Enzyme Chemistry, Institute for Enzyme Research, The University of Tokushima, Tokushima, Japan. [e]Department of Otolaryngology, NTT West Osaka Hospital, Osaka, Japan. [f]Center of IRB, Tokushima University Hospital, Tokushima, Japan.
Correspondence: Hiroshi Kido, MD, PhD, Division of Enzyme Chemistry, Institute for Enzyme Research, The University of Tokushima, 3-18-15 Kuramoto, Tokushima 770-8503, Japan. E-mail: kido@ier.tokushima-u.ac.jp

Accepted 2 December 2011. Published Onli

«LONG assay»

Objectives To determine the induction and changes in anti-influenza virus secretory IgA (s-IgA) levels in nasal washes and serum IgG levels in patients with influenza.

Methods The study recruited 16 patients with influenza aged 35·6 ± 9·6 years in 2007/2008 and 2008/2009 seasons. Nasal washes and serum were obtained throughout the first year. Anti-

toward the initial levels within 300 days, although the levels at day 143 ± 70 were 3·03-fold of the initial. Individual serum IgG levels also returned toward the initial levels within 300 days, although the mean levels remained high probably because of re-infection in a subgroup of patients. Although influenza A (H3N2) was a minor epidemic subtype in both flu seasons, a significant rise in

THANKS A LOT for your attention

montomoli@vismederi.com

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BACK UP SLIDEs

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HAI for B strains

WHO Global Influenza Surveillance Network

Manual for the laboratory diagnosis and virological surveillance of influenza

The HI test modified by ether treatment in the sero-epidemiological surveillance of influenza B

By R. PYHÄLÄ, M. KLEEMOLA and R. VISAKORPI

National Public Health Institute, Mannerheimintie 166, *SF*-00280 *Helsinki* 28, *Finland*

2.E). Influenza A antigens contained in the kit are suitable for the serological diagnosis of influenza A(H1) or A(H3) infections. Due to the decreased sensitivity to antibody rises of

influenza B whole viral antigens, ether-treated antigens are required for use in the serological diagnosis of influenza B infections – these are also provided in the kit.

CORRELATES OF PROTECTION ?????

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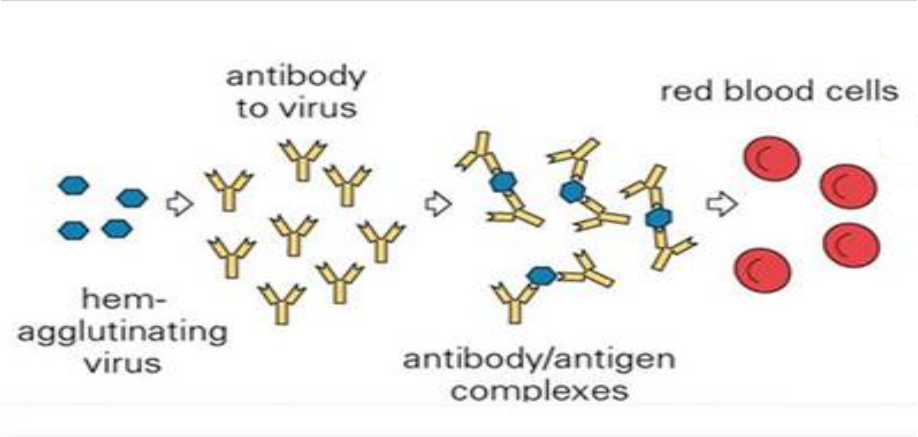
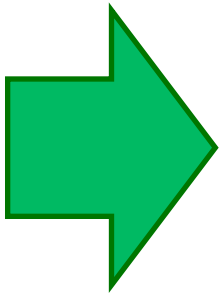
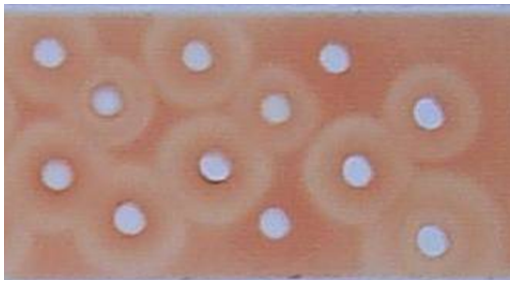
SINGLE RADIAL HAEMOLYSIS (SRH)

The interaction of antigen with receptors expressed on RBCs surface, mediated by guinea pig complement produces the haemolysis of red blood cells item.
RBCs and guinea pig complement are the KEY reagents of SRH.
In order to improve the performance of SRH the mixture of antigen combined to RBCs and guinea pig complement able to give a more clear and readable haemolysis should be chosen.







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VALIDATION

Journal of Clinical Virology 56 (2013) 323–330

Contents lists available at SciVerse ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Validation of the modified hemagglutination inhibition assay (mHAI), a robust and sensitive serological test for analysis of influenza virus-specific immune response

A. Morokutti [a,c], M. Redlberger-Fritz [b], S. Nakowitsch [a], B.M. Krenn [a], N. Wressnigg [a], A. Jungbauer [c], J. Romanova [a], T. Muster [a], T. Popow-Kraupp [b], B. Ferko [a,*]

Journal of Immunological Methods 422 (2015) 95–101

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Validation of Single Radial Haemolysis assay: A reliable method to measure antibodies against influenza viruses

Claudia Maria Trombetta [a,1], Daniele Perini [b,1], Licia Vitale [b], Rebecca Jane Cox [c,d,e], Valerio Stanzani [b], Simona Piccirella [b], Emanuele Montomoli [a,b,*]

[a] Department of Molecular and Developmental Medicine, University of Siena, Via Aldo Moro, 53100 Siena, Italy
[b] VisMederi srl, Enterprise in Life Science, Via Fiorentina 1, 53100 Siena, Italy
[c] The Influenza Centre, Department of Clinical Sciences, University of Bergen, Bergen, Norway
[d] Jebsen Centre for Influenza Vaccine Research, University of Bergen, Bergen, Norway
[e] Department of Research and Development, Haukeland University Hospital, Bergen, Norway

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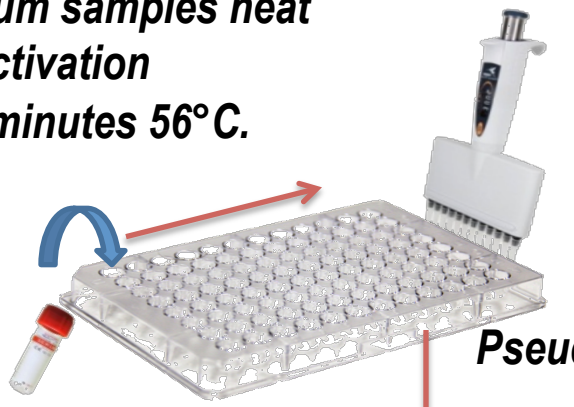
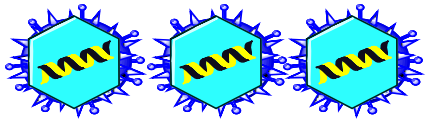
Pseudotype cell-based MN procedure

serum samples heat inactivation
30 minutes 56°C.



Pseudotype titration

Pseudotype

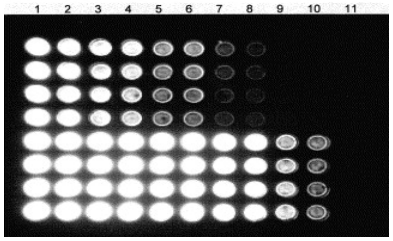


*10k to 50k RLU pp **

1 hour @ 37°C ; 5%CO2

HEK-293T cells (1x10⁴ cells/50µl).

48h ; @ 37°C ; 5%CO2



Firefly Luciferase RLU – (luminomenter)

** a pilot study with positive standard is required to optimize the amount of pp to use*

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Challenges for correlates of protection

- *Need for HAI, MN and SRH titres to be associated with laboratory confirmed influenza (culture and PCR)*
- *Need for well defined correlates of protection (H5, H7 and pediatric population)*
- *More information concerning the NA role in protection*
- *Validated, standardized assays to reduce laboratory variation*
- *Protocols, reagents and international antibody standard*
- *Development of novel standardized assays for novel vaccines*

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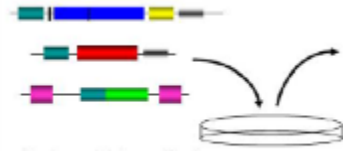
Other and future assays for Abs detection

ELISA – Enzyme Linked ImmunoSorbent Assay

- Suitable for screening a much larger number of samples
- Automation is possible
- No correlate of protection established
- Use of HA is preferable ….. HA1 is better
- Suitable to detect IgG, IgM, IgA in serum and nasal washes

ppMN – Pseudoparticle MN

- Suitable for screening a large number of samples
- Good correlation with MN for seasonal and pandemic strains
- BSL2 lab need also for pandemic strains
- It is not easy to set up and validate



Neuraminidase Assays

- Several assays available
- Should be better define role on NA-Ab in protection
- BSL2 lab need also for pandemic strains