New Serological Assays

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CDC

A Modified HI assay using Stabilized Human RBC (*Morokutti et al., J Clin Micro 2012*)

- Inter-laboratory variability in the HI assay exists for multiple reasons including differences in protocols, differences in RBCs used and subjective read-outs
- The standard HI was optimized to improve robustness and then validated
- Key differences in the modified HI include:
  - Use of 0.08% stabilized human erythrocytes in 1% BSA to facilitate RBC settling and reduce non-specific antibody binding
  - Microscopic plate readout
- Modified HI compared with standard WHO manual HI
- Modified HI was linear, specific and up to 8-fold more sensitive than standard HI
- Modified HI performed equally well with different subtypes
  - H3N2, H1N1pdm09, H5N1

Summary of Standard and Modified HAI Assay Protocols

Parameter	Standard HAI	Modified HAI
Serum dilution	1:8	1:8
RDE-treatment and heat inactivation	Overnight @ 37 °C; 45 min @ 56 °C	Overnight @ 37 °C; 45 min @ 56 °C
Erythrocyte source	Human or animal (e.g. chicken, horse)	Human (stabilised)
Erythrocyte concentration[a]	0.8% (human) or 0.5% (animal)	0.08%
Microtitre plates	U- (human) or V-(animal) bottom	V-bottom
Assay buffer	DPBS	DPBS + 1% BSA
Assay volume[b]	25 µl	25 µl
Virus antigen (HAU/25 µl)	4	4
First incubation (after mixing of serum and virus antigen)	30 min	45 min
Second incubation (after addition of erythrocytes)	60 min	75 min
Assay temperature	RT (or +4 °C, in case of virus antigens with high neuraminidase activity)	RT
Assay plate readout	By eye, 60° tilted plate	Microscope (40-fold magnification)
Endpoint determination for seropositivity	Settled erythrocytes and leakage pattern	Clear to irregular shaped erythrocyte dot
Reference material	Varies with virus antigen and erythrocyte source	NIBSC sheep serum

[a] Diluted in the respective assay buffer (DPBS for HAI or 1% BSA in DPBS for mHAI).
[b] The same amount of 25 µl for serum, assay buffer, assay antigen and RBC solution.

- Quality of RBC is critical; RBC with clear supernatant and defined expiration of one week are optimal
- Stabilized human RBC are commercially available

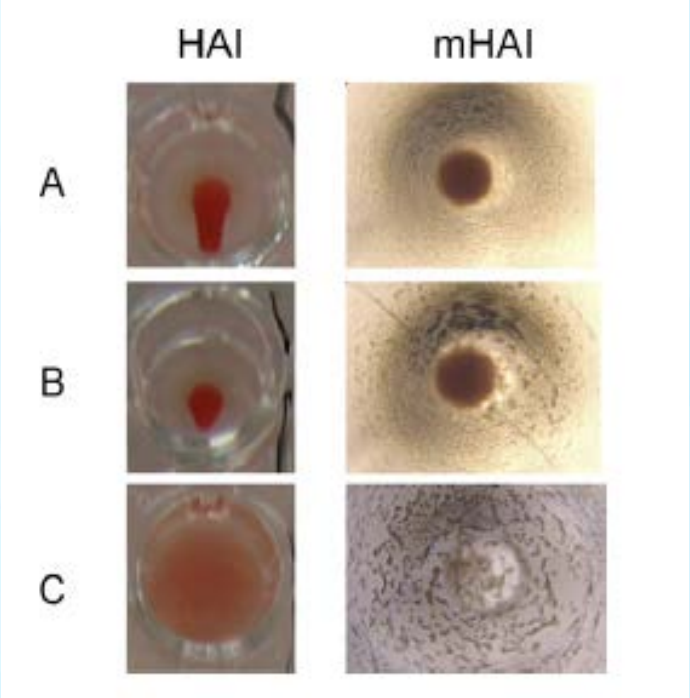
Read-out in Standard and Modified HI Assays

Standard HI

100% hemagglutination
Inhibition: streaming

50% hemagglutination
Inhibition: tear-drop,
incomplete streaming

0% hemagglutination
Inhibition:
cross-linked lattice of RBC
(RBC "shield")



Modified HI
(40X magnification)

Clear RBC dot visible

Irregular shaped dot

Irregular cell
"agglomerates"

Comparison of Titers Obtained by Standard and Modified HI Assays using Egg-grown Vaccine Viruses and Post-vaccination Human and Ferret Antisera

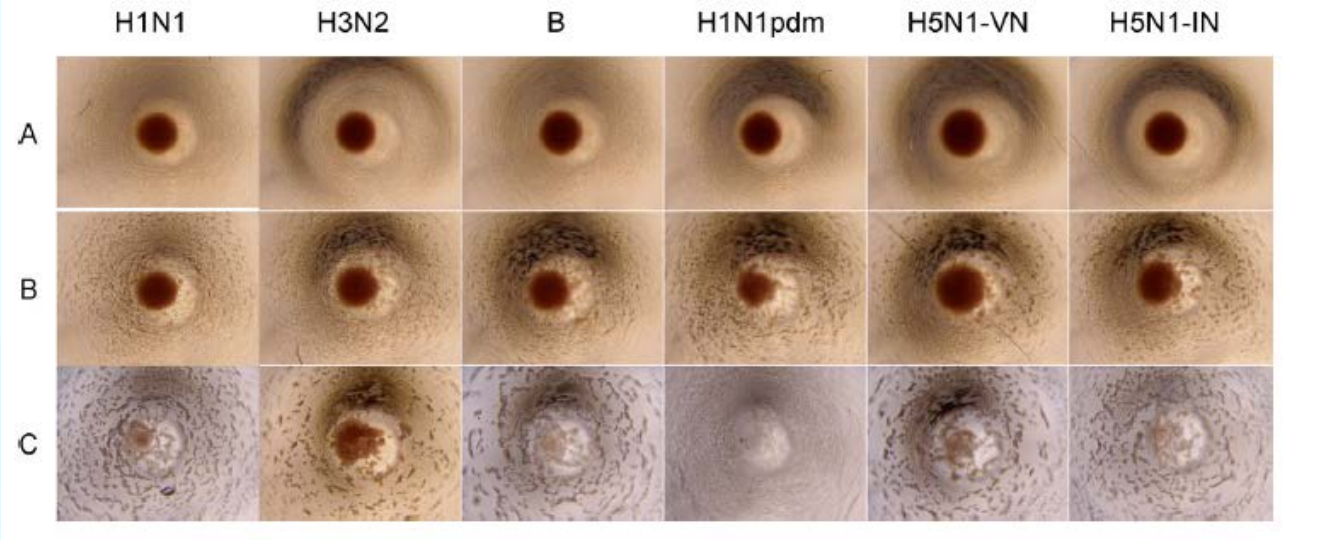
Sample ID	H1N1 (seasonal)		H3N2		B (non E.T.)	
	mHAI	HAI	mHAI	HAI	mHAI	HAI
Virus titre	128	64	128	64	512	128
PC (NIBSC)	4096	2048	32,768	16,384	8192	1024
NC (NIBSC)	<8	<8	<8	<8	<8	<8
Human 01	8	<8	<8	<8	8	<8
Human 02	64	32	<8	<8	32	<8
Human 18	32	8	32	32	1024	256
Human 34	128	32	128	64	512	128
Human 51	<8	<8	16	8	<8	<8
Human 54	<8	<8	16	8	32	<8
Human 89	32	8	16	16	128	16
Human 97	64	32	32	16	16	<8
Ferret M14	<8	<8	<8	<8	128	32
Ferret M16	<8	<8	64	32	128	64
Ferret F20	8	8	256	128	32	16
Ferret F22	16	8	256	256	8	<8
Ferret F24	32	16	128	64	128	32
Ferret M28	<8	<8	<8	<8	<8	<8

Microscopic Read-out Patterns for Seasonal and Avian Subtypes

No virus

With Ab – last well showing100% inhibition

With virus, no-specific Ab

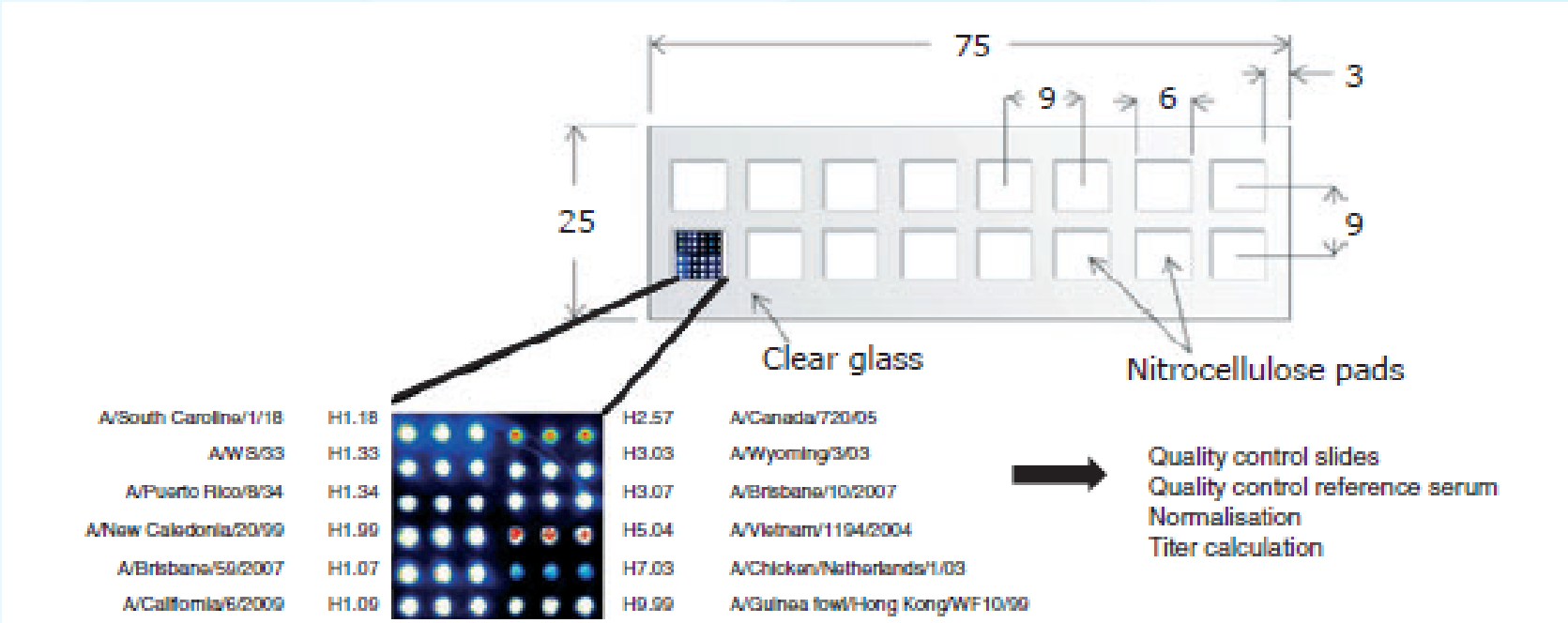


Profiling Human Antibody Response to Influenza using a Protein Microarray

(Koopmans et al., Clin Micro Infect 2011; Boni et al., JID 2013)

- Need for new antibody methods because existing assays difficult to standardize and HI and VN assays may not detect all antibodies relevant for protection
- HA1-based protein microarray developed:
  - HA1 used for improved specificity
  - Small volume (10 µl) sera
- High-throughput testing
  - Can test up to 100 antigens in one day
  - Provides a profile of prior exposure of individual
- Comparison with standard HAI assay
- Used to estimated population-based antibody levels to H7N9 virus in sera collected in Vietnam from 2010-12 (Boni et al., 2013)

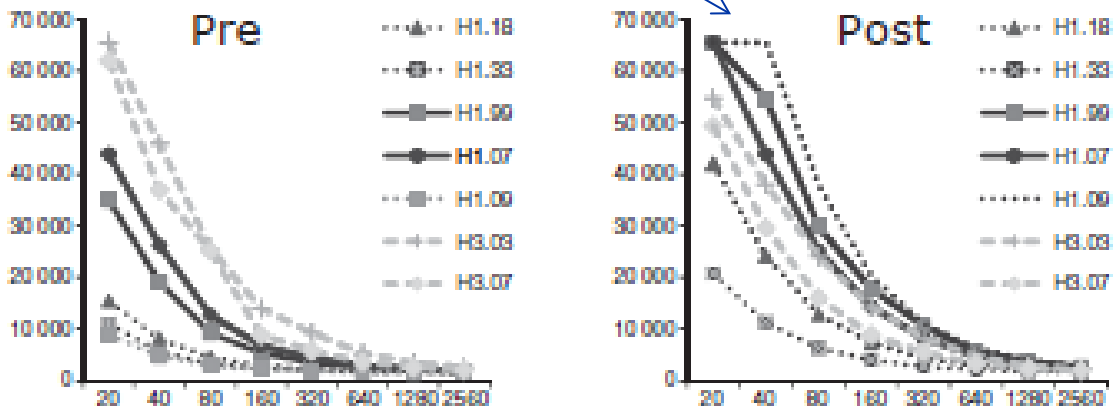
HA1 Microarray Set-up on Nitrocellulose Coated Slides



- Commercially available HA1 proteins produced in HEK293 cells
- Triplicate wells contain optimized amounts of HA1 spotted onto nitrocellulose pad
- 4 X 16 pad slides yields 64 wells
- Stable at RT for up to 3 weeks
- 2-fold serial serum dilutions from 1:20 to 1:5120 incubated for 1 hr at 37°C
- Bound antibody detected by goat anti-huIgG conjugated to fluorescent dye
- Signals quantified by a ScanArray microarray scanner

Protein Array (PA)Titer Estimation in Acute and Convalescent Sera from H1N1pdm09-infected Child

Max readout set at ~65,000

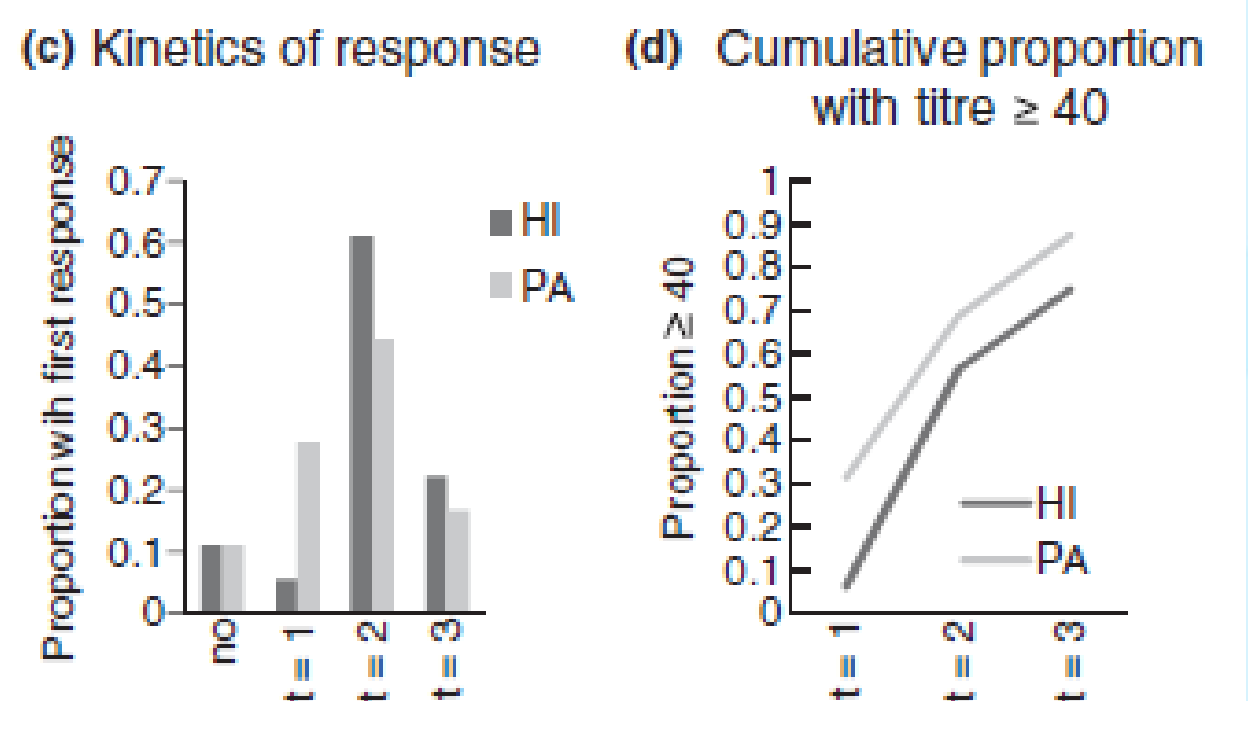


Antigen	Titer	
	Pre (2008)	Post
H1.18	10	24
H1.33	10	10
H1.99	10	70
H1.07	26	54
H1.09	10	90
H2.57	10	10
H3.03	56	44
H3.07	45	31

Min readout set at 3,000

- Titers read as dilution achieving 50% maximum binding signal for sample
- Titers with lowest dilution giving readout below minimum threshold were scored as 10

Kinetics of Antibody Response in 18 H1N1pdm09 Cases/contacts Measured by PA and HI Assay

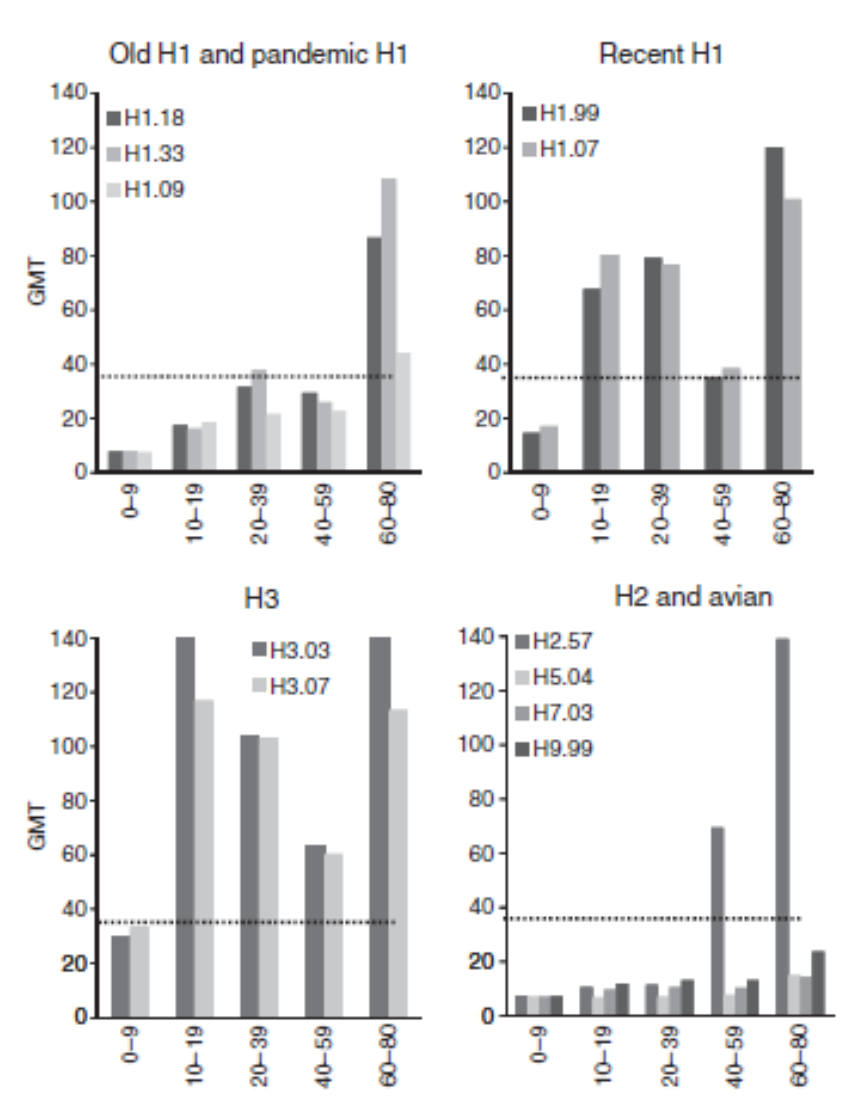


- Kinetics of response similar with PA antibody developing slightly faster
- Five persons had detectable antibody at baseline versus one by HI, suggesting assay detects cross-reactive antibody
- PA detected antibody in one person who was HI antibody negative

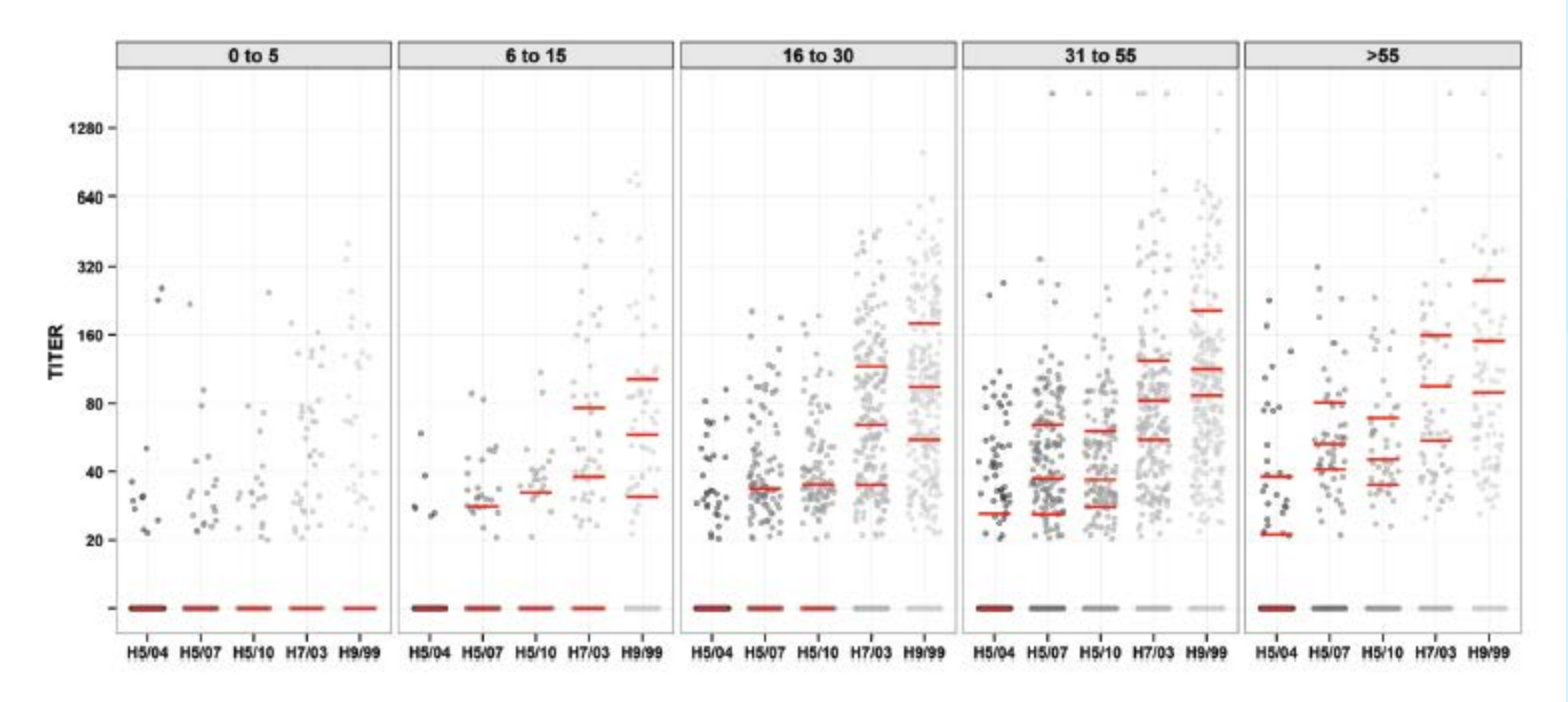
Age-related Geometric Mean PA Titers in 122 Persons Sampled in 2008

Titers of ≥40 to H5/H7/H9
Detected at low frequency

≤10 yr: 0/43
11-20 yr: 2/21
21-40yr: 2/19
41-60yr: 3/27
>60yr: 3/12



Use of Protein Microarray to Detect Antibodies to Avian H5, H7 and H9 Viruses in Vietnamese Population Sampled in 2010-12



- Scatter plots showing individual antibody titers to 5 different avian viruses by age group
- Red lines show 70th, 80th and 90th quantiles; a single red line indicates that these quantiles are all set at 10
- Antibody titers to avian viruses increase with age

Assays in Development at CDC: Rapid Influenza Immunity Tests

Rapid Influenza Immunity Tests

Goal

- Development of point-of-care assay and high-throughput assay to evaluate influenza immune status

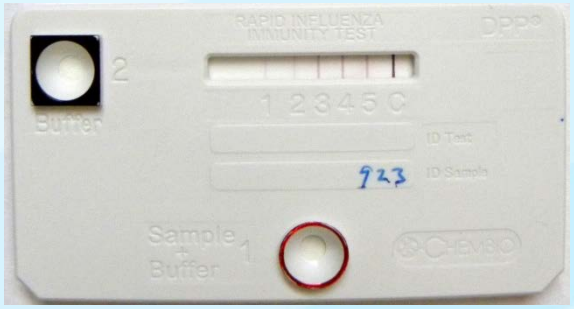
Applications

- Screening of at risk populations for antibodies to novel influenza viruses
  - Field testing in Bangladesh Live Bird Market worker surveillance program

- Evaluation of immune status prior to intervention in the event of limited vaccine and antivirals supplies

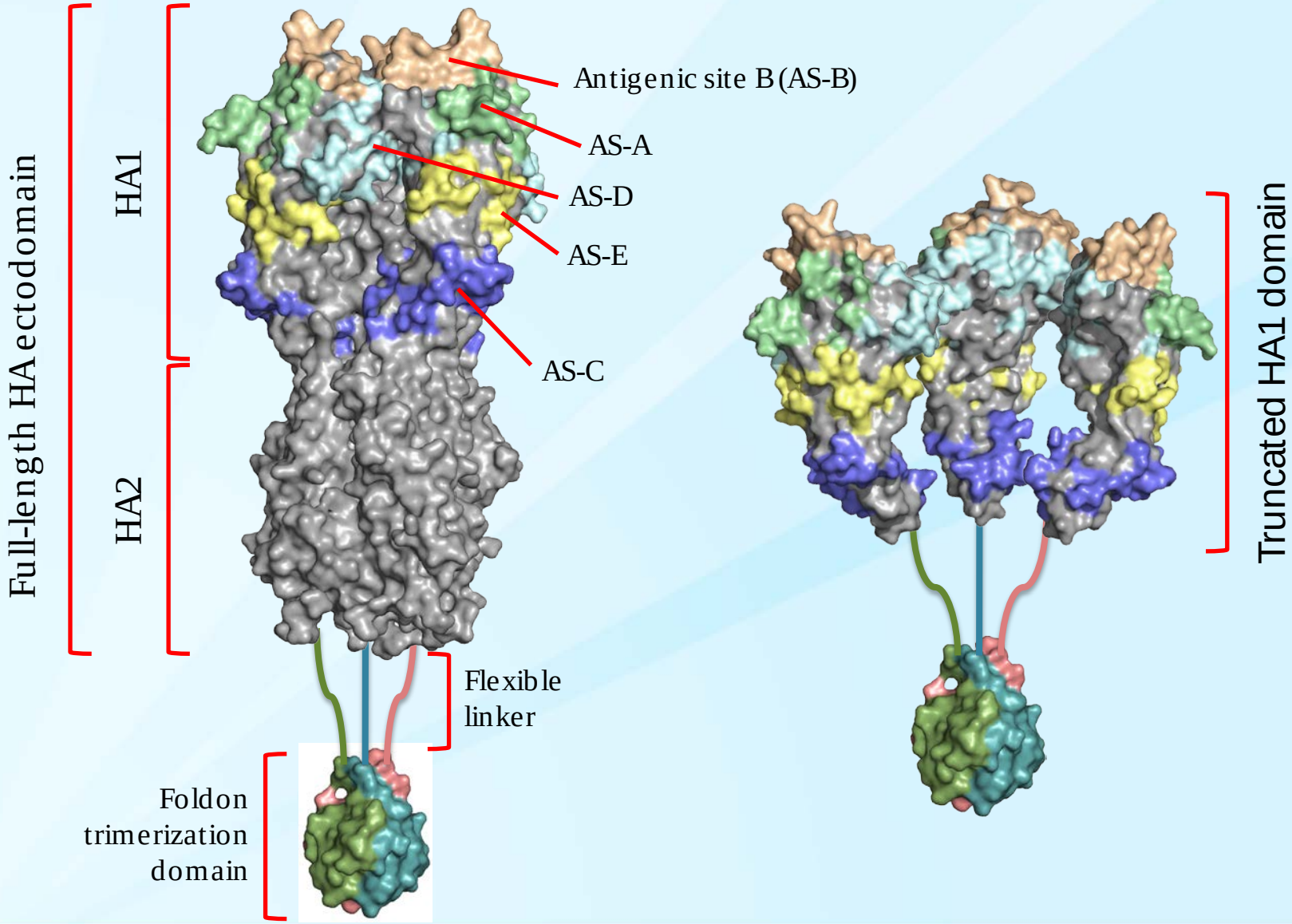
Rapid Tests Under Development

- **Point of care: Dual Path Platform (DPP) Lateral Flow**
  - Easy to use
  - Stable at high temperature and humidity
  - Rapid
  - Subtype specific
  - Low sample volume (finger stick)
  - Whole blood specimens

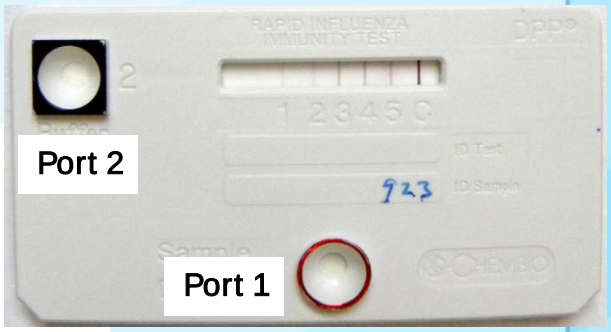


- **Laboratory: Luminex xMAP technology**
  - High through-put
  - Rapid
  - Subtype specific
  - Multiplexed
  - Automated
  - Low sample volume
  - Standardized reagents

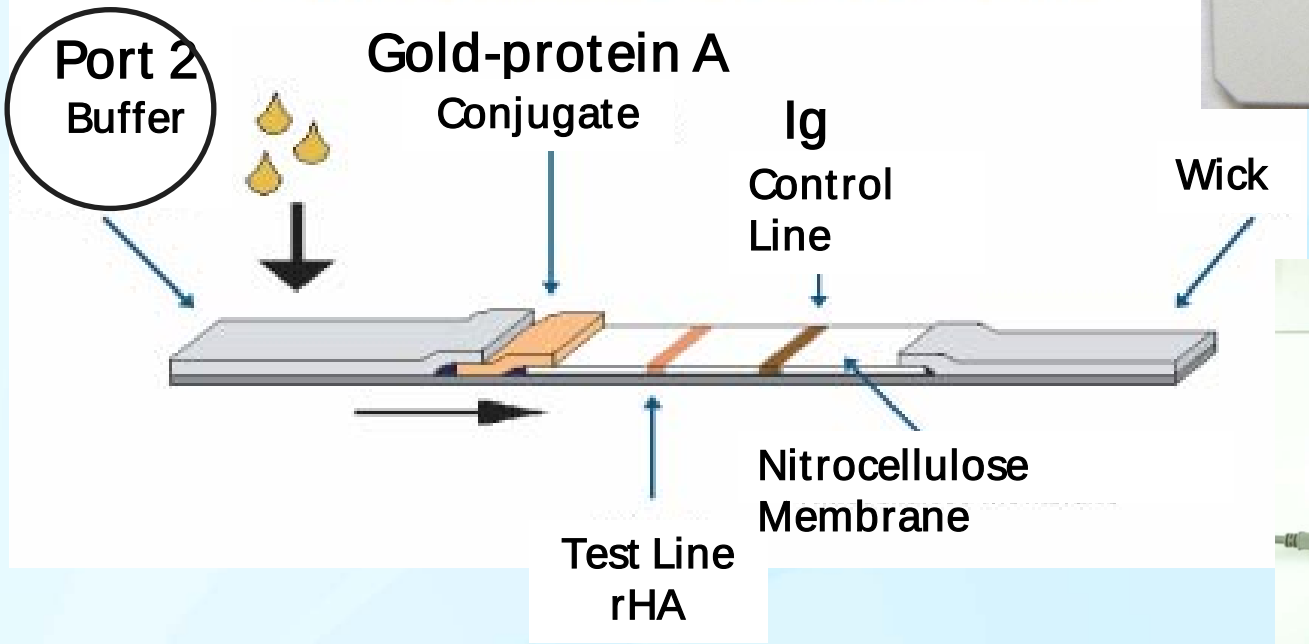
HA versus HA1



Basics of a dual path lateral flow assay for antibody detection



Step 2: Add 5 drops buffer to port 2



Port 1
(Sample)

Step 1: Add10 µl of serum or 20 µl of whole blood to sample diluent vial ; add 55ul of mixture to port 1. Wait 5 min

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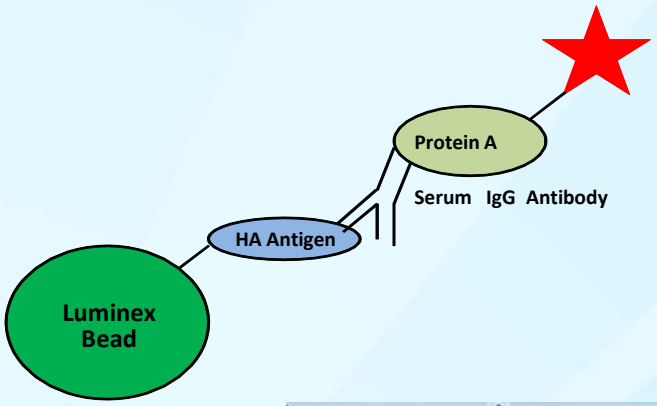
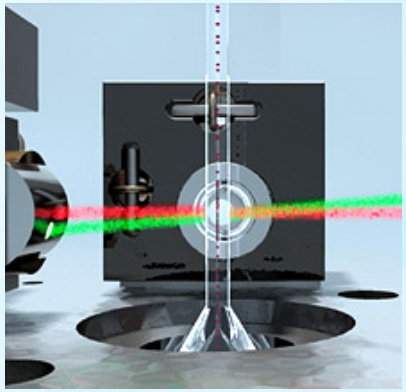


Ab binding to rHA detected via protein A labeled with R-Phycoerythrin

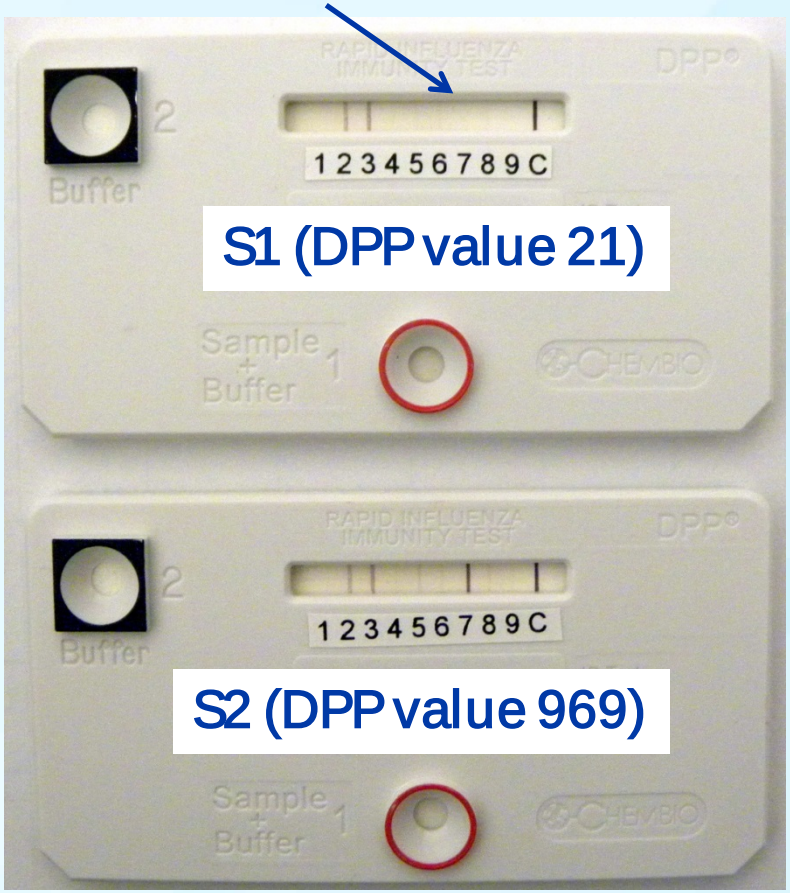


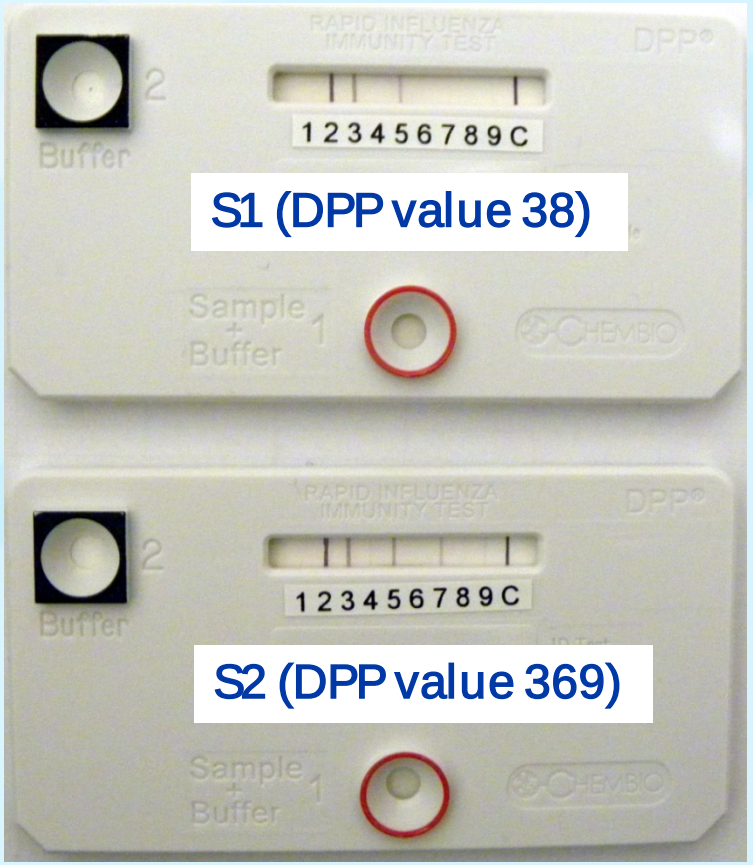
- One laser identifies the bead type.
- Second laser measures signal from R-Phycoerythrin.

Reader

Detection of H5 Antibody in H5N1 Vaccinated Persons Using the DPP Assay

H5 HA in line 7





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