A Comparative Examination of Influenza 2 day ELISA and 3 day HA Consensus Microneutralization Assays : *A(H3N2) and A(H5N1) update*

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CONSISE
CONSORTIUM FOR THE STANDARDIZATION OF INFLUENZA SEROEPIDEMIOLOGY

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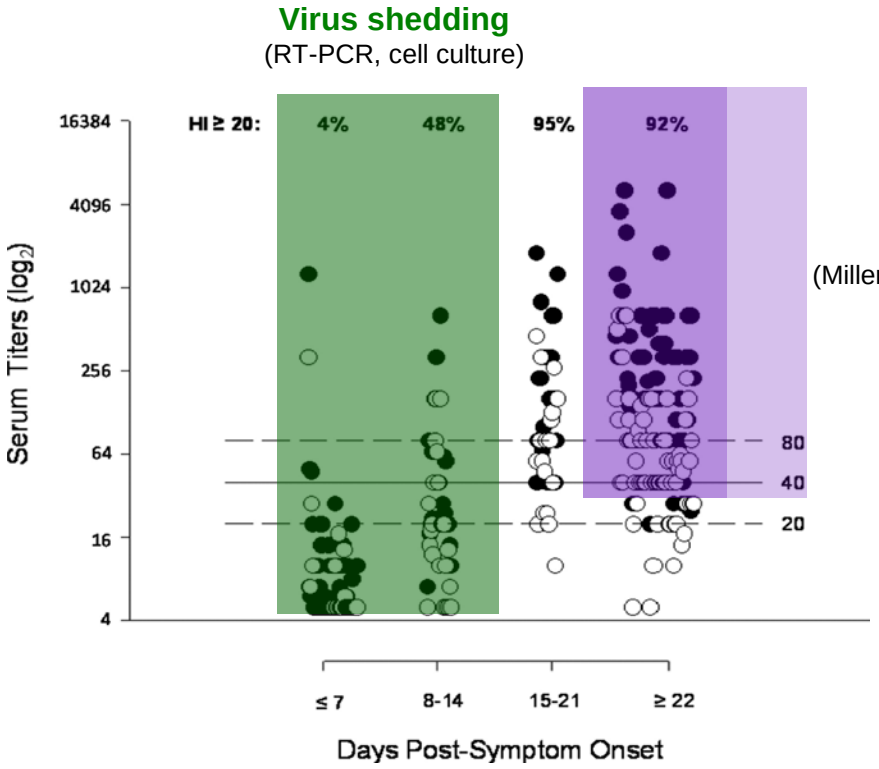
Serological Studies



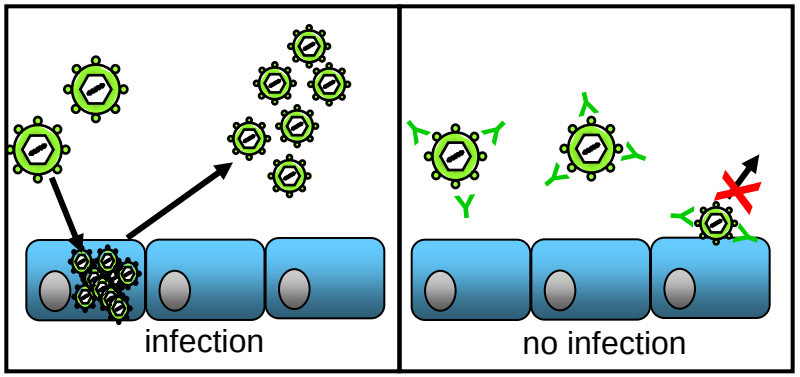
Veguilla et al 2011. J. Clin. Microbiol.
(Miller et al 2009 Lancet; Chen et al 2010 PLoS One)

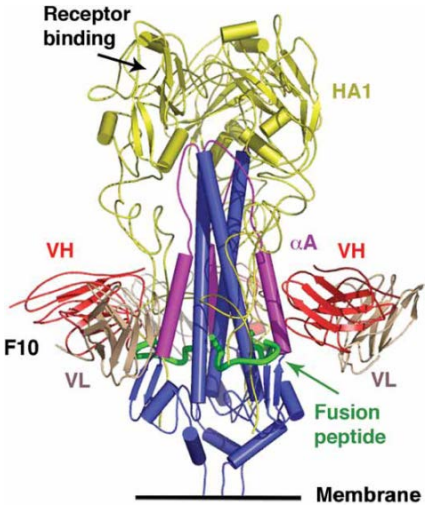
- *Serological studies can confirm past infection in the absence of positive virological testing and regardless of clinical presentation, thus detecting both symptomatic and asymptomatic infection.*
- *CONSISE Laboratory Working group formed to 'co-ordinate and standardize the international laboratory serological response'.*

Laurie et al 2012

Microneutralization (MN) Assay

- Detects functional antibodies that block hemagglutinin binding to sialic acid residues on cells (antibodies specific or antigenic regions or stem region of hemagglutinin protein).
- Only live influenza viruses may be used → implications for viruses recommended for use only at high containment.
- Criteria for seropositivity has been established for infection with A(H1N1)pdm09 virus.
- Correlates of protection not established.
- Useful to detect antibodies specific for avian influenza viruses.
- More sensitive than the Hemagglutination Inhibition assay at lower end of titres.
- 2 day, 3 day and 7 day MN assay protocols used in different laboratories.



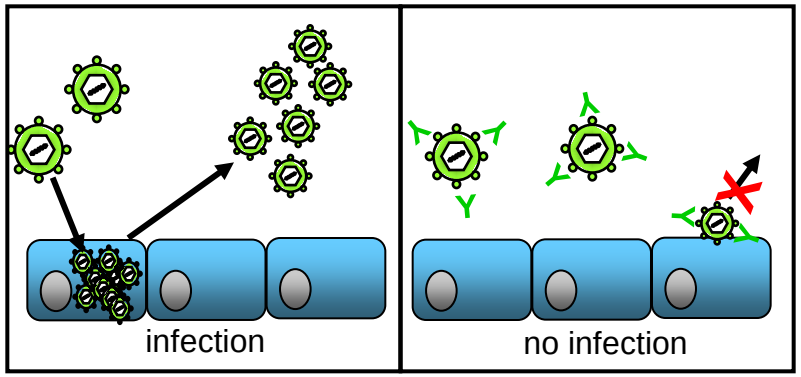


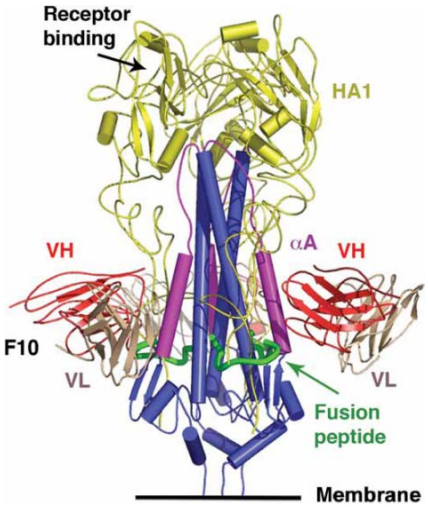
Rowe et al 1999; Stephenson et al 2007; Veguilla et al 2011; Laurie et al 2012

Adapted from slide by J. Katz,International Seroprevalence Meeting, February 2011, Ottawa, Canada

Microneutralization Assay Comparison

- Serological data from different locations is often compared during an outbreak to estimate the impact of a novel infection on a population.
- Standardization of assays is important for combining and comparing data.
- MN assays vary in use of protocols and determination of endpoint titres amongst laboratories worldwide.
- Comparison of MN assays using shared sera and A(H3N2) viruses found more consistency in laboratories using shorter assays, with viral antigen detection (Stephenson et al 2007).
- Knowledge on reproducibility, intra- and inter-laboratory variability of different MN assays is limited.





Adapted from slide by J. Katz,International Seroprevalence Meeting, February 2011, Ottawa, Canada

Summary of CONSISE MN Assay Comparison for A(H1N1)pdm09

- Ten laboratories shared their protocols of the 2 day ELISA and 3 day hemagglutination (HA) MN assays and a consensus protocol was developed for the 3 day HA MN assay.

- Twelve laboratories from eight countries participated in the laboratory evaluation of the 2 day ELISA (WHO) and 3 day HA consensus MN assays.

- There were differences in the sensitivity of the assays between laboratories and between the MN assay methods.

- The ratio of titres between the 2 day ELISA and the 3 day HA MN assays was similar for the International Standard that was included in the study and the in-house serum samples.

- Overall, in most laboratories, there was good correlation between the results obtained using the two assay protocols.

Our results indicate that the 2 day ELISA (WHO) and 3 day HA consensus protocols for MN assays may be considered interchangeable for assays of antibodies to the influenza A(H1N1)pdm09 virus.

What about other subtypes of influenza A viruses?

Microneutralization Assay Laboratory Comparison : A(H3N2) and A(H5N1)

Study Plan

- Each laboratory should attempt to assay antibody levels in a small panel of sera using both consensus MN assay protocols: 2 day ELISA (WHO) and 3 day HA

Virus strain

- A(H3N2) - a representative wild type or reassortant virus of the vaccine strain A/Perth/16/2009 or A/Victoria/361/2011 from laboratory's own stocks.
- A(H5N1) – a representative wildtype or reassortant virus from the clade the laboratory's sera panel 'matches'.

Sera

- Approximately 10 sera comprising low, medium and high titer antibody levels.
- Sera could be from seroepidemiology studies or from vaccine studies.
- If available, the inclusion of a ferret antiserum is recommended.

Laboratory materials

- Local resources and laboratory materials shall be used
- The same cell line should be used for both 2 day and 3 day MN assays

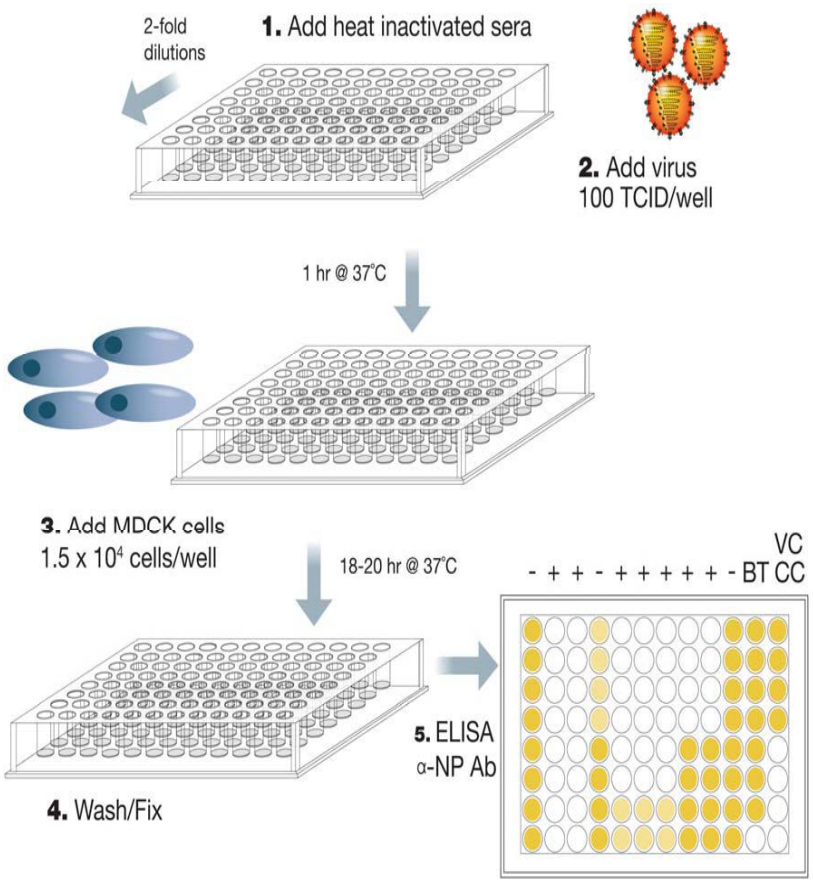
Number of assays

- At least three comparative assays using each protocol on different days is requested

Microneutralization Assay Comparison

2 day ELISA (WHO) protocol

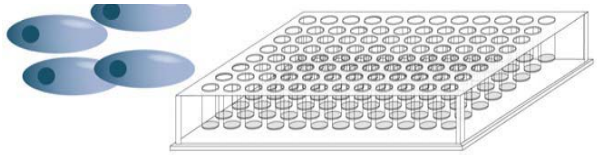
(Rowe et al 1999 J Clin Microbiol 37(4):937)



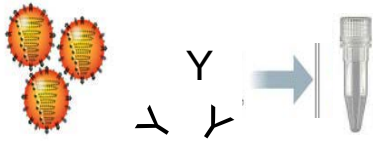
http://www.who.int/influenza/gisrs_laboratory/2010_12_06_serological_diagnosis_of_influenza_by_microneutralization_assay.pdf

Consensus 3 day HA protocol

1. Prepare cells to form monolayer at least 24h before required.

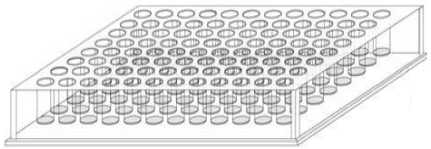


2. Heat inactivate sera. Dilute, add virus (100 $TCID_{50}$)



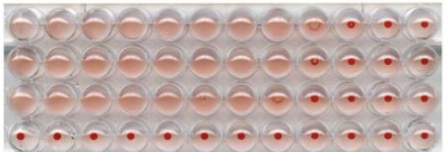
1h @ 37 °C

3. Add virus:serum to confluent MDCK monolayer. Incubate 1-2h, 37 ° C



4. Remove virus:serum, replace with media containing trypsin. Incubate 3 days @ 37 ° C, 5% CO_2

5. HA agglutination



Specific parameters were required for dilutions, calculations and incubation times. Other parameters were recommended.

A(H3N2) and A(H5N1) Microneutralization Assay Comparison – laboratories that have submitted data to date