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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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’.

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; Vegoilla 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

1. Add heat inactivated sera
   2. Add virus 100 TCID/well
   3. Add MDCK cells: 1.5 x 10⁴ cells/well
   4. Wash/fix
   5. ELISA α-NP Ab

Consensus 3 day HA protocol

1. Prepare cells to form monolayer at least 24h before required.
2. Heat inactivate sera. Dilute, add virus (100 TCID₅₀)
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% CO₂
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

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- Istituto Superiore di Sanita (ISS), Italy
- National Institute of Infectious Diseases (NIID), Japan
- Naval Health Research Center (NHRC), United States of America
- Siriraj Hospital, Mahidol University, Bangkok, Thailand