Overview and Future Plans: Laboratory Working Group

John Wood and Othmar Engelhardt

CONSISE Open Meeting, Cape Town South Africa

4 September 2013

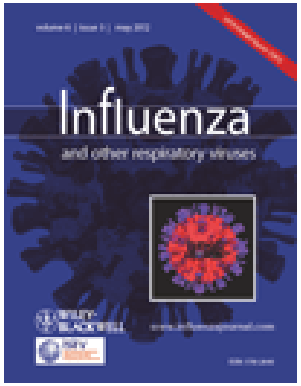
Background:

1st International Influenza Seroprevalence Meeting, Ottawa, Canada, February 9-10 2011

- *Influenza serological studies to inform public health action: best practices to optimise timing, quality and reporting*

- Several conclusions and actions agreed
  - Formed the basis for subsequent discussions
- Meeting report

  Laurie et al. (2012) Influenza and Other Respiratory Viruses
- CONSISE Steering Committee
- Two Working Groups
  - Epidemiology Working Group
  - Laboratory Working Group



CONSISE Steering Committee – Laboratory Working Group

Eeva Broberg,

Katja Hoschler,

Jackie Katz,

Malik Peiris,

Wenqing Zhang

Othmar Engelhardt

Olav Hungnes,

Karen Laurie,

John Wood

Background:

1st International Influenza Seroprevalence Meeting, Ottawa, Canada, February 9-10 2011

– *Influenza serological studies to inform public health action: best practices to optimise timing, quality and reporting*

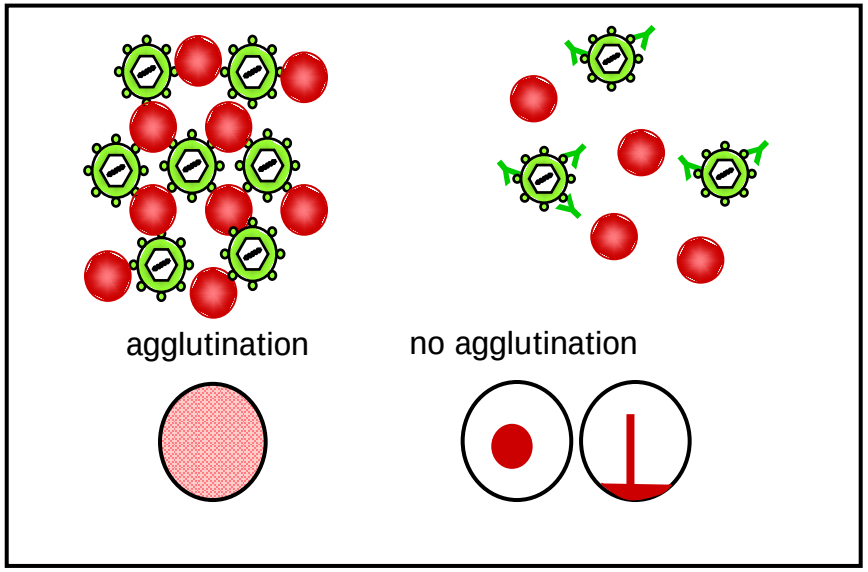
One of conclusions

Co-ordinate and standardise the international laboratory response

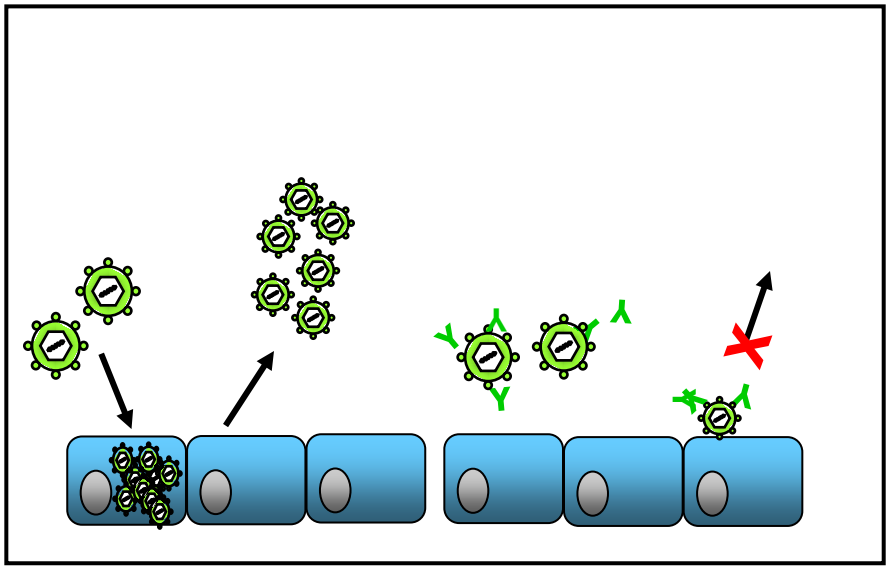
- develop an international network of laboratories for conducting serological studies and ensuring a common approach to generating comparable sero-epidemiological data
- establish commitment for production of international antibody standard and control panels
- establish collaboration/coordination between laboratory, clinical and epidemiological partners to access serum and virological samples rapidly in outbreak

Common assays for influenza serological studies

Haemagglutination Inhibition Assay



Microneutralization Assay



MN assay read-out:
- 7 day assay CPE on monolayer
- 3 day HA detection
- 2 day ELISA detection (WHO protocol)

Common assays for influenza serological studies

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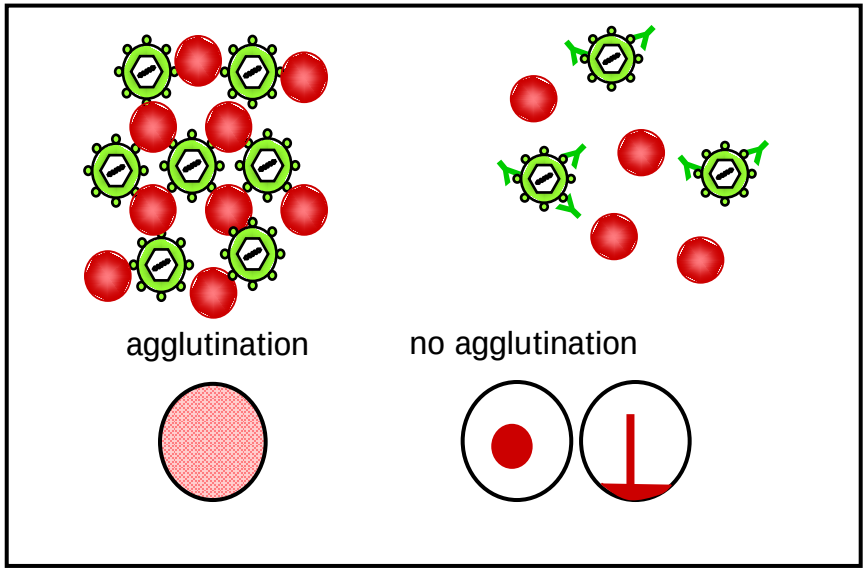

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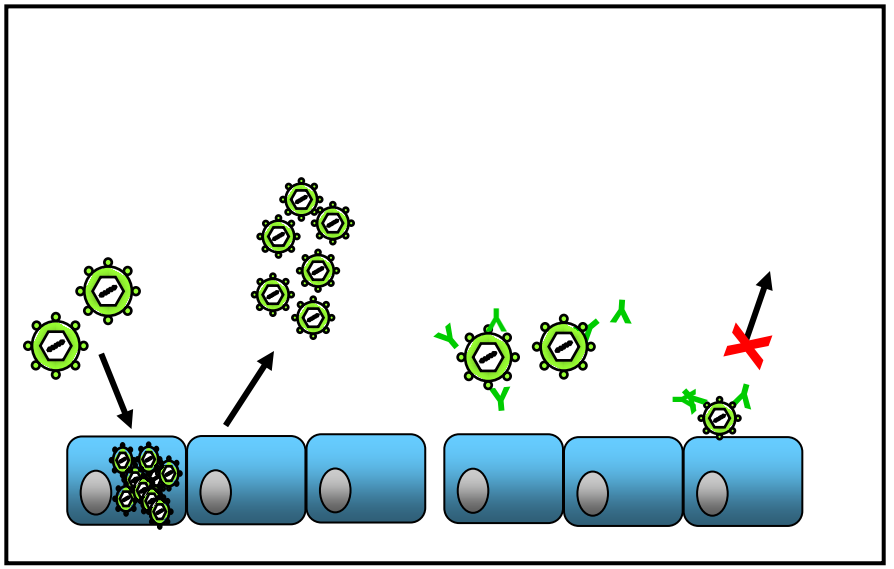


MN assay read-out:
- 7 day assay CPE on monolayer
- 3 day or HA detection
- 2 day ELISA detection (WHO protocol)

In collaborative studies HI/MN assay variability between laboratories can be substantial

How can they be standardized?

CONSISE Laboratory Working Group Strategy

"develop an international network of laboratories for conducting serological studies and ensuring a common approach to generating comparable sero-epidemiological data"

- Review laboratory protocols for MN and HI assays
- Develop consensus protocols using WHO protocols where possible
- Collaborative studies to compare different protocols
- If data supportive – use consensus protocols for subsequent seroepidemiology studies

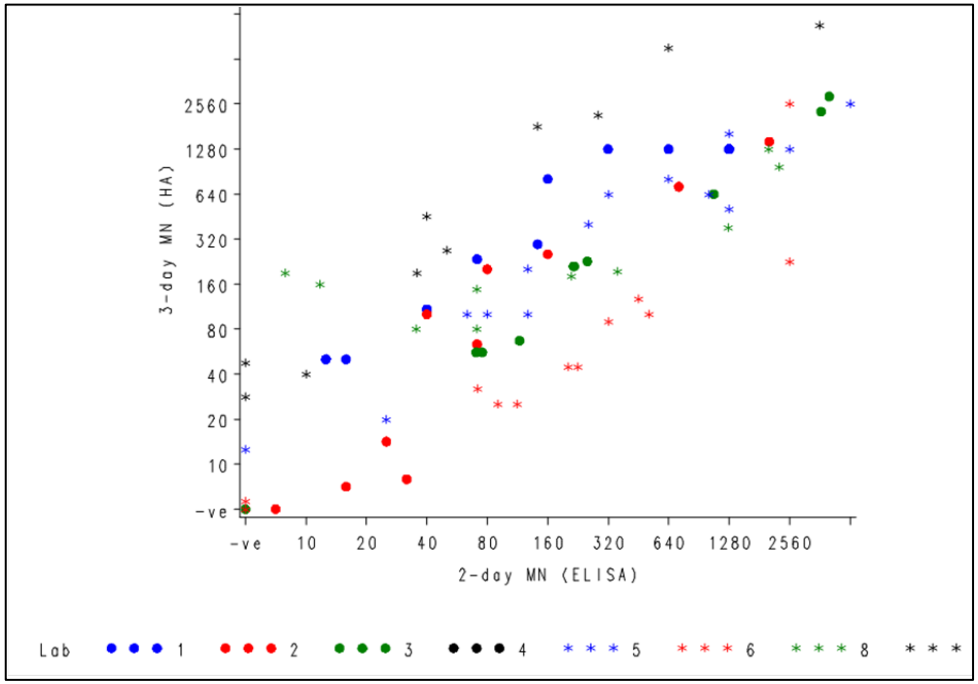
MN assay standardization

- CONSISE Working Group agree that 7 day virus neutralization assay is not appropriate for seroepidemiology studies
    - Takes too long and some evidence of poor reproducibility

- Karen Laurie (WHO CC, AUS) coordinated comparison of 2d ELISA WHO and 3d HA protocols – consensus protocols developed
- Laboratory comparison exercise for H1N1 pdm09 assays began 4 October 2012
    - Comparison of two methods where labs used their own serum samples
    - Results from 11 labs submitted to NIBSC (UK) for analysis

MN assay evaluation

Intra-laboratory Comparison : Correlation between 2-day ELISA and 3-day HA MN assays using in-house serum samples



- Ratio of titres between 3-day and 2-day assay similar in most labs
- Therefore, there were no underlying reasons that the two assays could not be comparable
- As conclusions were based on only one subtype, plans were made to extend study with data for seasonal H3N2 and H5N1

HI assay standardization

- CONSISE Laboratory Group is strongly in favour of keeping HI as the primary serology assay, but will assess how it can be better standardized.
- Karen Laurie and John Wood coordinated comparison of HI protocols and tried to develop consensus assay
  - Starting point: WHO protocol

CONSISE Laboratory Working Group Strategy continued

"establish commitment for production of international antibody standard and control panels"

- Examine antibody standards from different sources (human/animal/Mab) in planned collaborative studies

- Map the development of antibody standards in response to emerging novel influenza viruses
  - Develop and maintain an international laboratory network to rapidly produce and evaluate antibody standards

International antibody standards

Background

- Previous collaborative studies have shown that use of antibody standards can significantly reduce HI and MN assay variability between laboratories
- International Antibody Standards (WHO) have been prepared for influenza A (H5N1) clade 1 and A(H1N1)pdm09 by NIBSC, UK
- But it takes about 7 months to produce such standards
  - Can we do better?

Quality assessment

Background

- External Quality Assessment is used for Bacteriology, Mycology, Parasitology, Virology assays - a range of techniques examined
  - Serology schemes for Hepatitis B and C, HIV, Measles IgG, Rubella IgG
- A small group from CONSISE met with Dr Vivienne James from UK NEQAS
- Value of EQA is understood and appreciated by CONSISE Lab WG
- Consensus that formal EQA would be premature at the moment
  - CONSISE still exploring assay variables
- More emphasis currently on developing consensus protocols and standardisation
- Use of shared serum panels as a more realistic option at this point

Neuraminidase assays

Background

- Serum NA Inhibition (NI) titres correlate with reduced virus replication and disease symptoms and there is evidence that NA antibodies can protect against homo- and heterologous virus
- At the December 2011 Stockholm CONSISE meeting, Maryna Eichelberger (FDA, USA) described various NI assays including ELLA assay (referenced below)
- Some CONSISE laboratories have begun evaluation of sera from influenza vaccine trials using ELLA assays with encouraging results. Some of the difficulties related to the source of NA
- All CONSISE labs were encouraged to evaluate the ELLA assay.

Lambre CR, Terzidis H, Greffard A, Webster RG. Measurement of anti-influenza neuraminidase antibody using a peroxidase-linked lectin and microtitre plates coated with natural substrates. J Immunol Methods 1990;135:49-57.

Cate TR, Rayford Y, Niño D, Winokur P, Brady R, Belshe R, Chen W, Atmar RL, Couch RB. A high dosage influenza vaccine induced significantly more neuraminidase antibody than standard vaccine among elderly subjects. Vaccine. 2010 Feb 25;28(9):2076-9. doi:10.1016/j.vaccine.2009.12.041. Epub 2009 Dec 29.

New influenza serology assays

Background

- At January 2013 Hong Kong meeting, CONSISE members indicated that new MN serology assays using virus pseudotypes were being evaluated
- It was agreed that the CONSISE group should review the new serology assay being used

CONSISE involvement with influenza A (H7N9) and MERS-CoV serology assays

H7N9

- CONSISE TC in May 2013 led to posting of H7N9 HI/MN assay protocols from China CDC on CONSISE website
- A further CONSISE TC in July 2013 led to posting of CDC H7N9 modified HI assay protocol using horse erythrocytes on CONSISE website
- Link to WHO website for information on number of human cases http://www.who.int/influenza/human_animal_interface/influenza_h7n9/en/index.html

MERS-CoV

- WHO TC in June to assess MERS-CoV laboratory diagnoses included serology assays – CONSISE represented
- A variety of serology assays - need for serum panels