Overview and Future Plans: Laboratory Working Group

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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
Background:

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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**
- Agglutination
- No agglutination

**Microneutralization Assay**
- Infection
- No infection

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


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