The problem

- Influenza vaccines aimed at inducing broad immunity are based on various principles and induce different types of immune responses
- A single assay will not suffice for all approaches
- Determination of breadth of response
  - Retrospective (historical) and prospective (antigenic drift and shift)
- Correlates of protection for new vaccines may not be known yet
- Biological assays are inherently variable
  - Serologic assays for influenza have proven quite variable in past studies and full standardisation has not been achieved

Approaches towards standardisation

- Use of standardised assay protocols
- Use of standards
- Regular proficiency assessment of laboratories

Use of standardised assay protocols

- Harmonisation of assay procedures is feasible
- Harmonisation/standardisation of critical reagents may be harder (or impossible)
- Aspects to be considered in harmonisation:
  - Type of sample
  - Sample storage and preparation
  - Supply of critical reagents, preparation of critical reagents
  - Experimental procedure
  - Method of acquiring read-out (manual, instrumentation)
- Harmonisation of methods does not always lead to improved agreement between laboratories:
  - E.g. EDQM study for HI and SRH, 2007 – 2009: common SOPs for both methods did not lead to good agreement between labs (Wood et al, Pharmeur Bio Sci Notes, 2011, 1:36–54)
Example: CONSISE

- "Consortium for the Standardisation of Influenza Seroepidemiology"
- Mission statement: CONSISE is a global partnership aiming to standardise the seroepidemiology of influenza and other respiratory pathogens, and to develop comprehensive investigation protocols for use in optimising the control of influenza and other respiratory pathogens.
- Comprised of two interactive working groups: Epidemiology and Laboratory; and a Steering Committee
- More than 100 members from over 40 countries
- CONSISE shares study protocols and laboratory assay protocols and other information on the internet with free access. Membership is free and open to all individuals whose activities may contribute to those of CONSISE
- http://consise.tghn.org

Example: CONSISE – development of consensus protocols

- HI assay and MN assay protocols are published in the Global Influenza Surveillance Network Manual for the laboratory diagnosis and virological surveillance of influenza.
- However, laboratories use variations in the specific details of the assay protocols, and variations in the determination and expression of endpoint titres.
- To improve assay standardisation, CONSISE developed new consensus protocols.
  - Parameters were identified within each assay with potential variables listed
  - Laboratories entered the variable they preferred, adding a new variable if not specified.
  - All details were collated, de-identified and the data summarised.
  - Consensus protocols were developed; parameters classified as ‘required’ or ‘recommended’.
- Consensus protocols for HAI and MN assays are published on the CONSISE website.
- Member laboratories have aligned their assay parameters to the consensus protocols
- A planned comparative study will evaluate the use of consensus protocols vs in-house protocols

Standardisation of assays for new vaccines

- Some work has been done on selected assays
  - E.g.: validation of granzyme B assay and multiplex cytokine assay in 4 laboratories (Gijzen et al, Vaccine 2010, 28:3416-3422)
- European FP7 funded project UNISEC: attempts to standardise assays for CMI on-going

Use of standards – biological standardisation

- SI units based on artefacts (kg) or definitions (metre)
  - Do not apply to biological activities
- Biological activity can only be measured in a biological assay
- Mass does not capture the activity of a biological analyte
- International Unit:
  - A fraction of a vial of a biologically active substance, arbitrarily defined as having the biological activity of 1 IU
- Method not defined
  - Use of standard permits measurement in IU, irrespective of method (within limits)
Use of standards

- Different levels/types of standard
  - WHO International Standards – highest level
  - National/regional/pharmacopoeial standards
  - Working reagents
  - In-house standards
  - Run controls

Example: International Standards for antibody to influenza virus

- International Standard for antibody to influenza H5N1 virus
- International Standard for antibody to influenza H1N1pdm09 virus
- 2nd International Standard for antibody to influenza H1N1pdm09 virus

Variation between laboratories

- HI assay
- VN assay

Application to vaccines inducing broad immunity

- Where antibody response is targeted, antibody/serum standards may be feasible
  - May have to be product specific if particular epitopes are targeted
- Where specific biological analytes (e.g. cytokines) are measured, standards may be available or feasible
- Cell standards for CMI assays have been made and more could be prepared
  - Freeze-dried cells for intracellular cytokine staining
  - Freeze-dried cells for ELISpot
Proficiency testing /external quality assessment

- Testing a laboratory’s quality system with samples of known but undisclosed content
- Provides a snapshot of a lab’s performance
- Provides a comparison of a lab’s performance with that of other labs
- Can highlight problems
  - But does not solve them
- Can drive move to standardisation

Examples for EQA

- WHO EQAP for PCR detection of influenza A and B viruses
- UK NEQAS EQA schemes for detection of various pathogens
  - Includes a few serological EQA schemes (e.g. measles and mumps, HIV)

Looking ahead

- Different approaches to standardisation of immunological assays for vaccines inducing broad immunity can be taken:
  - Standardisation of protocols and use of standards
  - EQA may only be appropriate later (once a number of laboratories are using an assay routinely)
- Networking and international communication about new assays will be beneficial
  - CONSISE could be the venue for these activities
- Down-selection and prioritisation of potential assays will be required
- Start standardisation early before methods drift too much

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