Alternative potency assays for inactivated influenza vaccines

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Potency testing of inactivated influenza vaccines: the status quo

• Single radial immunodiffusion (SRD or SRID) in use for several decades
• Collaborative studies and proficiency studies: variability of SRD often acceptable (RSD ~ 10 %), but can be much higher in some studies
• By and large, vaccines standardised using SRD seem to have been OK
Principle of SRD

Before diffusion

After diffusion (stained blue)
The SRD assay

SRD zone size is proportional to antigen concentration
SRD zone size is inversely proportional to antiserum concentration
The SRD requires reagents

- Calibrated reference antigen
  - Specific for each strain/HGR
- Antiserum reagent
  - Cross-reactive within a group of antigenically ‘like’ viruses
- Made, calibrated and distributed by the four ERLs (CBER, USA; NIBSC, UK; NIID, Japan; TGA, Australia)
The pandemic of 2009

• Massive pressure on vaccine production and release time lines
  – Clinical trials required or desirable
  – Some clinical trial lots were released based on alternative assays (SDS-PAGE, HPLC)

• New interest in alternative potency assays
Alternative potency assays

- Physico-chemical assays
  - HPLC
  - SDS-PAGE
  - Mass-spectrometry

- Biological assays
  - ELISA/EIA, Titre on a chip
  - Surface plasma resonance (SPR)
  - Immunocapture isotope dilution mass spectrometry (IC-IDMS)
Physico-chemical assays

- Total amount of HA quantitated
- Detect and measure HA regardless of conformation
- Usually, do not distinguish between native and denatured antigen
- Unlikely to be stability indicating
- Combinations of methods may permit distinction between native and denatured HA
Physico-chemical assays – pros and cons

- Advantages
  - Rapid
  - May be automatable and high through-put
  - Probably quite reproducible – collaborative studies needed

- Disadvantages
  - HA measured regardless of conformation/antigenicity/immunogenicity
  - May still require reference reagents
  - Some methods not useful for trivalent vaccines
  - Some methods are expensive, technically difficult and difficult to implement
Biological assays

- Measure a biological activity or reactivity (usually antibody-based assay)
- Antigen must be in a conformation that is recognised by the antibody/antibodies
- Potentially able to distinguish native from denatured antigen
- Therefore, stability indicating
- Combination with physico-chemical methods possible (e.g. IC-IDMS)
Biological assays – pros and cons

• Advantages
  – Potential to measure biologically active HA
  – Likely stability indicating
  – Some assay formats well known (e.g. ELISA) – easily implementable

• Disadvantages
  – Need reagents
    • Is it possible to develop assays that use more broadly cross-reactive reagents?
  – Some assays expensive and technically complex
1. Origin of document
At the Eleventh meeting between WHO Collaborating Centres, Essential Regulatory Laboratories and IFPMA (International Federation of Pharmaceutical Manufactures and Associations)/EVM (European Vaccine Manufacturers) influenza vaccine manufacturers at NIBSC, 1 February 2011, participants discussed characteristics any new potency test for inactivated influenza vaccines should display. A list of essential and desirable features was developed and refined at the Twelfth meeting between WHO CCs, ERLs and IFPMA/EVM influenza vaccine manufacturers at NIBSC, 19 July 2011. The present document presents the outcome of these discussions, and is intended as an aid for the evaluation of future potency tests.

2. List of assay features
Characteristics for an improved potency assay

• **Essential features:**
  – Biological relevance of analyte
  – Accuracy and robustness
  – (sub)type specificity
  – Flexibility and maximum practicability

• **Additional important features:**
  – Applicability to existing and novel vaccines
  – Robustness of reference reagents
  – Flexibility and practicability
Essential features

• Biological relevance of analyte:
  – Biologically relevant potency measure
  – Correlation with clinical efficacy
  – Stability indicating
  – Bridging to single radial immuno diffusion (SRD) test

• Accuracy and robustness:
  – Precision and accuracy
  – reproducibility

• (sub)type specificity

• Flexibility and maximum practicability:
  – Applicable world-wide
  – Quick availability/usability following a strain change
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Additional important features

• Applicability to existing and novel vaccines:
  – Applicable to existing and novel vaccines
  – Unaffected by adjuvants
  – Measuring low doses

• Robustness of reference reagents:
  – Independent of strain specific reagents
  – Reduced amount of reagents (required for assay)
  – Robustness of reagent supply, speed of supply, volume/quantity of supply

• Flexibility and practicability:
  – Usable in in-process control (i.e. in presence of other proteins, contaminants)
  – Efficient regulatory review
  – Accelerating lot release
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What is needed?

• International coordination and information sharing
  – Risk: loss of global standardisation
• Improvements possible for current potency assay
  – Activities on-going
• Thorough assessment of potential alternative methods guided by document ‘Characteristics for an improved potency assay for inactivated influenza vaccines’ (ECBS, 2011)
The way forward

• Down-selection of assays:
  – Field of potential alternative potency assays is wide
  – Not all assays can be evaluated in depth and by many laboratories
  – Shortlist required

• Evaluation of promising assays by various laboratories
  – ‘inner core group’ formed to steer coordination of activities (HHS, CBER, NIBSC, IFPMA) and to organise sharing of assays
  – Questionnaire/survey sent out to manufacturers to gauge interest in assessing alternative methods
  – Study to evaluate assays to start soon
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