Standards and beyond: challenges of application of old methods to next generation products

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Recombinant Flu vaccines

- Propagation of the influenza virus in chicken embryos is the predominant way to manufacture influenza vaccines.

- Highly purified recombinant subunit vaccines could resolve problems associated with:
  - the use of embryonated chicken eggs for inactivated vaccines, such as getting high yield egg-adapted reassortant and possible antigenic change during egg adaptation (one of the reason of low vaccine effectiveness in 2012-2013 season, VRBPAC 2013 transcript)
  - necessity to attenuate pathogenic strains of the influenza virus (for live vaccines)

- A recombinant influenza vaccine has a greater potential to provide necessary number of doses faster during pandemics and also makes influenza immunization a reality for people with people with real or suspected egg allergies, regardless of severity (ACIP).
Clinical trials of Flu vaccine were performed in US Army.

The first licenses for civilian Flu vaccines were issued to several companies in the US.

SRID method for measuring potency of Flu vaccine was implemented.

First recombinant product (insulin) is approved (Eli Lilly).

Difficulties in adaptation of classical and widely accepted release bioassay (SRID), optimized for traditional products to the new recombinant vaccine were among many reasons for a more than 30 years lag in market appearance of recombinant Flu vaccine.

First recombinant Flu vaccine is approved (Protein Sciences Corporation).

What does SRID measure was identified.

The single-radial-immunodiffusion (SRID) assay has been adopted world-wide in 1978.

SRID measures amount of hemagglutinin (HA): viral surface protein. Antibodies against HA protects from the Influenza.

SRID was optimized to provide consistent results when tested against all of the licensed influenza vaccine preparations at time of implementation: whole virus (discontinued) and split vaccines. Both vaccines were propagated in eggs and inactivated by chemical agents (MS Williams 1993).
Advantages of SRID as bioassay

- Simple in performance
- Robust
- Reproducible
  - An international collaborative study (Wood et al. 1981) demonstrated test reproducibility (geometric coefficient of variation between laboratories less than 10%).
  - Two EU collaborative studies in 1989 and 1990 have reaffirmed test reproducibility (J. Wood Textbook of Influenza 1998).
- Correlation with clinical efficacy
  - Numerous clinical trials have validated the test (Ennis et al. 1977; Pandemic Working Group of the MRC 1977; Nicholson et al. 1979)
  - Numerous follow up clinical studies have confirmed that the test measures immunologically active HA (J. Wood Textbook of Influenza 1998).
- Measure biologically relevant potency
- Ability to detect individual strains in multivalent vaccine
- Proven stability-indicating capacity
Disadvantages of SRID

- SRID requires the production of antibodies to a strain-specific HA (up to 4 months).

- SRID is relative bioassay and requires reference standard with assigned potency, which production and standardization must be performed after production and characterization of strain-specific antibodies.

- For seasonal influenza virus vaccines, which typically contain three constantly changing sub-types, **new antisera and reference standard must be made and standardized each time a new strain is incorporated into the vaccine formula during annual reformulation.**

- Each of these steps are time consuming and costly. More importantly reagent preparation may be a major factor in delaying of supplies of a vaccine during pandemic.

- **Suboptimal for vaccine which are produced in other than egg-based systems:** in cell culture (J. Wood, Textbook of Influenza, 1998 Chapter 25) and using recombinant DNA technology.
This presentation summarizes points to consider for adaptation of established standards, which are produced for traditional Flu vaccine, for measuring of potency of novel innovative and often fundamentally different products.
Description of SRID assay

1. **Standard (Reference Antigen)**
   - Detergent treatment (Zwittergent 3-14)
2. **Sample (Vaccine)**
   - Passive diffusion at room temperature
3. **Agarose with embedded specific antibodies**
4. **Antibody-Antigen lattice**
5. **Wash out unbound antigen and antibodies**
6. **Coomassie staining**
Calculation of Flu vaccine potency

Potency of sample is assigned by comparing ring diameter with the standard with US FDA assigned potency.
Standard and Vaccine in SRID assay

**Standard**
(Reference Antigen)

whole virus

**Vaccine**

HA rosettes

SRID predominately measures HA trimer

(DBSQC/OCBQ/CBER/FDA at PhRMA 2012;
Semenova et al, BioAssays 2013)
Different vaccines – the same standard

Flu vaccines, which are required to use SRID as a potency bioassay

- **Inactivated vaccine**
  - Inactivated either by alkylating or crosslinking agents
  - Predominately egg-produced

- **Split vaccine**
  - Virion split by detergent
  - Virion contains 38-44 % of HA
  - (Ruigrok, Textbook of Influenza 1998)

- **Whole vaccine**
  - Discontinued at the end of 1970’s due to side effects

- **Recombinant vaccine**
  - Produced in insect cells
  - Contains >90 % of rHA

- **Subunit vaccine**
  - After splitting vaccine is enriched for surface antigens (HA and NA)

Standard for SRID is a freeze-dried inactivated whole virus, initially optimized for split and whole vaccines.
Reagents are prepared by the four WHO Essential Regulatory Laboratories (ERLs):

- Australia – Therapeutic Goods Administration (TGA)
- Japan – National Institute for Infectious Disease (NIID)
- United Kingdom – National Institute for Biological Standards and Control (NIBSC)
- USA – Center for Biologics Evaluation and Research (CBER)

One of ERLs (lead ERL) prepares Primary Liquid Standard (PLS) and a large batch of freeze-dried antigen (inactivated whole virus), which are distributed to all other ERLs for independent calibration.

Data generated by the ERLs are collected by the lead ERL and compiled for the final potency value agreement and confirmation. Manufacturers’ data may be considered, if available. The lead ERL has final authority to assign a potency value.

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION (Geneva, 17-21 October 2011), Generic protocol WHO 2012
Primary Liquid Standard (PLS)

PLS is inactivated whole virus

PLS is calibrated by physicochemical means

Scheme is based on “Generic protocol for the calibration of seasonal/pandemic influenza antigen working reagents” WHO 2012

HA is 38 - 44 % of total virion mass

(Textbook of Influenza, R.W.H. Ruigrok 1998)
Points to consider for recombinant Flu vaccine at PLS calibration step

- Co-migration effect for other proteins (different proteins at the same band on SDS-PAGE) can be as high as 25% (Getie-Kebtie et al, An. Biochemistry 2011).

- General guidance for confirmation of accuracy of PAGE band analysis for ERLs: HA content should be between 20% and 50% of total protein. Recombinant protein is > 90% of HA in the sample and free from influenza viral proteins.

- Ratio between HA and total protein for PLS is assigned based on band densitometry analysis of sample which have less than 50% of HA, thus co-migration effect from other proteins is possible. Even moderate co-migration effect could put potency number “off” for highly pure HA recombinant vaccine.
Freeze-dried working reference standard

- Freeze-dried antigen (working reference standard)
  - PLS with assigned HA content
    - SRID assay
      - Assigning potency to working reference standard
        - Distribution to manufacturers of Flu vaccine
Points to consider for recombinant Flu vaccine

- As reported previously, the SRID gave unreliable potency results when different chemical agents used for inactivation of the virus were used for preparation of reference standard and vaccine samples (R. Gupta, and W. McCormick, US FDA, WHO/FDA/HC Workshop, June 2010, Ottawa, Canada). These agents modify proteins in different way, and therefore inactivated viral preparations may have different gel mobility. The recombinant HA vaccine does not require an inactivation step.

- Egg-based standards contain significant amount of other viral and host proteins, which may be involved in formation of stable complexes. This further increases complexity of the sample post treatment with inactivating agents.

All these facts may contribute to a higher or lower gel mobility of the highly purified recombinant HA proteins compared with egg-based reference antigens.
Points to consider for recombinant Flu vaccine (cont)

- The protein ring intensity and appearance, which is also likely dependent on the way of HA presentation in the sample, may be different between standard and measured samples, produced in heterologous systems.

Such differences could create problems in automated reading of the gels, which negatively affects accuracy and precision of the bioassay.
CONCLUSIONS

- Regulations and guidance, developed and optimized for traditional products, may need adjustment when applied to novel innovative and often fundamentally different products.

- Points to consider outlined above may require either an adaptation of traditional method for the new products, for example through the preparation of compatible reference reagents, or the development of standard-independent physicochemical assays measuring the physiologically active form of the proteins present in the vaccine.