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Global Availability of Standards: examples of collaborations on reagents production and availability

Case study: Influenza Vaccine Potency Testing Reagents

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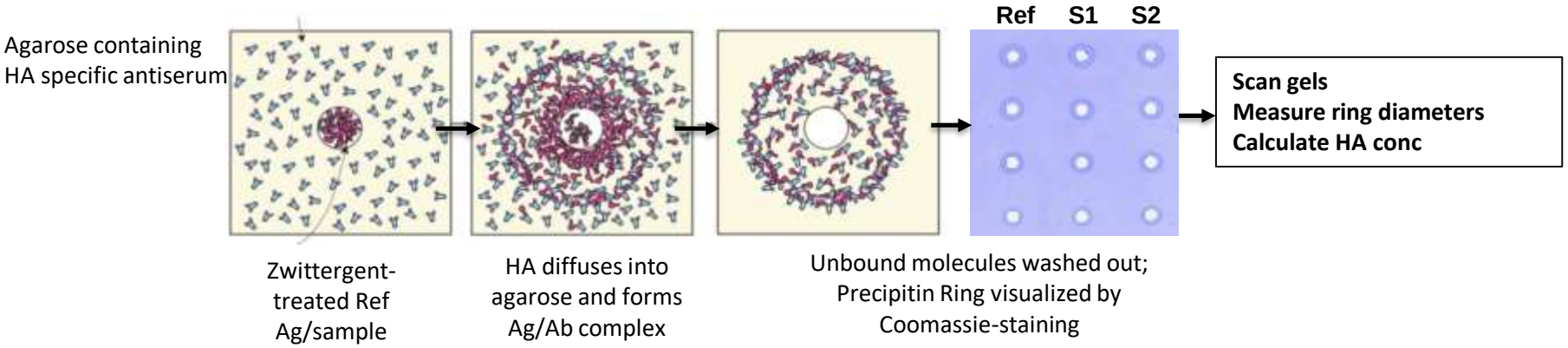
on behalf of the WHO Influenza ERLs

Inactivated influenza vaccine potency test: Single Radial Immunodiffusion Assay (SRID)

Principle of assay:

- zwittergent-treated vaccine diffuses into agarose containing HA-specific antibodies
- at an optimal concentration, HA and antibodies form a lattice/precipitin ring
- the diameter of the ring is proportional to HA concentration
- HA (µg/mL) of the unknown sample is calculated from a reference antigen standard curve



Reagents required for SRID

- Calibrated reference antigen
  - Specific for each strain/Candidate Vaccine Virus (CVV)
  - Suitable for each vaccine-production platform

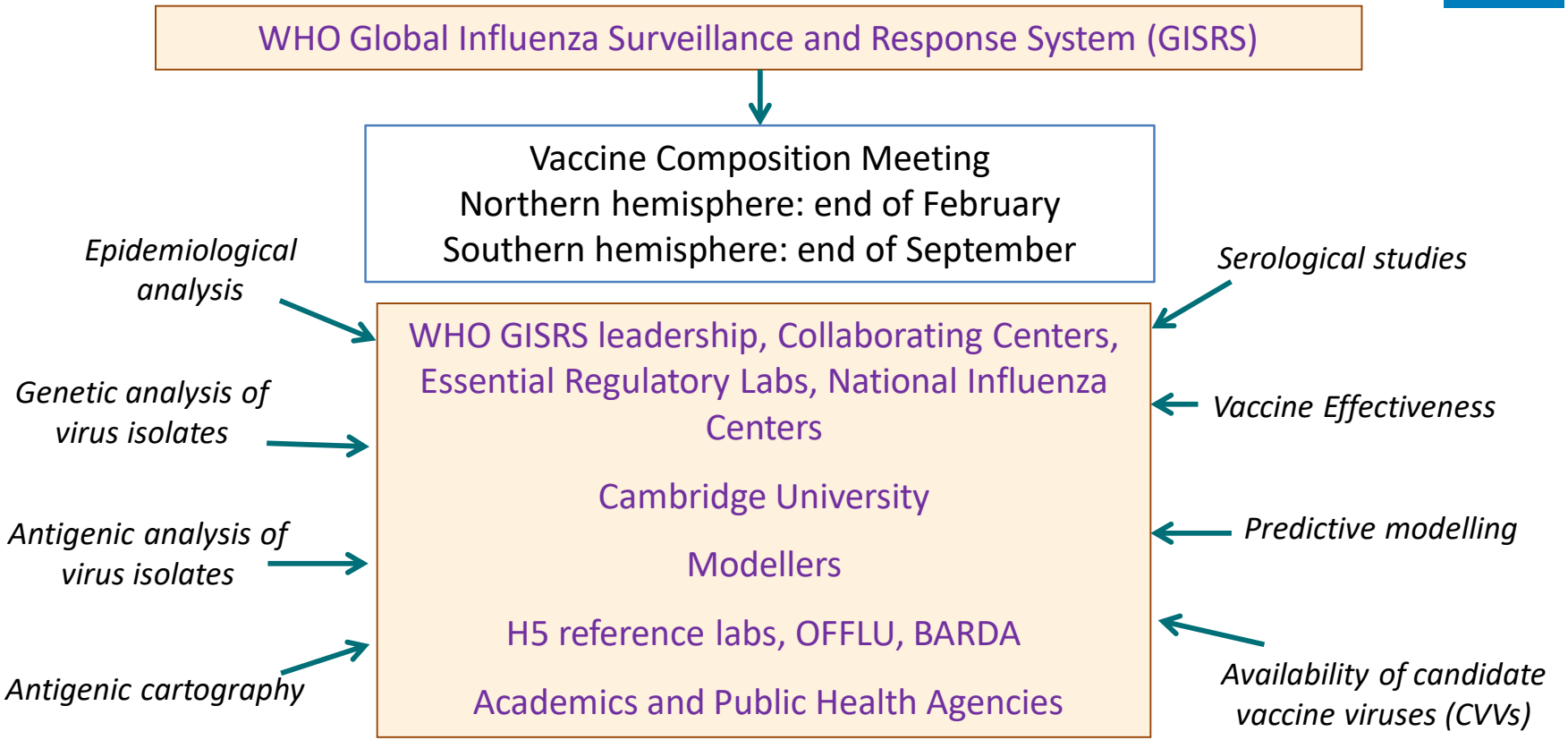
- HA-specific antiserum
  - Cross-reactive within a group of antigenically ‘like’ viruses
  - Suitable across vaccine-production platforms

Reference reagents are prepared, calibrated, and distributed by

the four WHO Essential Regulatory Laboratories (ERLs):

Center for Biologics Evaluation and Research (CBER)/Food and Drug Administration (FDA), USA;
Medicines and Healthcare products Regulatory Agency (MHRA), UK;
National Institute for Infectious Diseases (NIID), Japan;
Therapeutic Goods Administration (TGA), Australia

Influenza strain recommendations for vaccine production



Recommended composition of influenza virus vaccines for use in the 2024-2025 northern hemisphere influenza season

Egg-based influenza vaccines:

- an A/Victoria/4897/2022 (H1N1)pdm09-like virus
- an A/Thailand/8/2022 (H3N2)-like virus; and
- a B/Austria/1359417/2021 (B/Victoria lineage)-like virus.

Cell- or recombinant-based influenza vaccines:

- an A/Wisconsin/67/2022 (H1N1)pdm09-like virus;
- an A/Massachusetts/18/2022 (H3N2)-like virus; and
- a B/Austria/1359417/2021 (B/Victoria lineage)-like virus

For quadrivalent vaccines:

- a B/Phuket/3073/2013 (B/Yamagata lineage)-like virus in addition to the viruses recommended for trivalent formulation

Candidate Vaccine Virus used for vaccine production

The CVV used for vaccine production must be the recommended wild-type virus or a virus that is “antigenically-like” the recommended virus

- Another wild-type virus that reacts similarly with ferret antisera against the recommended wild-type virus
- A reassortant, high yield (HY) virus that contains genes for HA and NA of the recommended virus or another “antigenically-like” virus and other gene segments from a high growth/yield donor virus, e.g., A/PR/8/1934 (H1N1)
  - Reassortants are produced in some ERL and manufacturer’s laboratories and sent to WHO Collaborating Centers (CCs) for antigenic analysis
  - Reverse genetics is used to produce CVVs for pandemic and live, attenuated seasonal vaccines

Wild-type and reassortant viruses approved for vaccine production are listed on the WHO website

CVVs and Reference Antigens for A(H3N2) component of influenza virus vaccines for use in the 2024-2025 northern hemisphere

Global Influenza Programme (who.int)

Candidate vaccine viruses antigenically-like A/Thailand/8/2022 (egg-derived)

Parent virus	Type of virus (CVV name)	Reference antigens available
A/Thailand/8/2022	Wild type virus	
	reassortant IVR-237	2023/145B (TGA)* 23/220 (MHRA)
A/California/122/2022	Wild type virus	
	reassortant SAN-022	H3-Ag-2314 (CBER)* 23/226 (MHRA)
	reassortant CBER-54A	
A/Brisbane/837/2022	Wild type virus	
	reassortant IVR-246	
A/Brandenburg/15/2022	Wild type virus	
A/Sichuan-Gaoxin/1144/2023	Wild type virus	
	reassortant CNIC-2302D	
A/Sichuan-Jianyang/35/2023	Wild type virus	
	reassortant CNIC-2303A	
	reassortant CNIC-2303C	
A/California/45/2023	Wild type virus	

*Primary calibrated antigen

Candidate cell culture-based vaccine viruses antigenically-like cell cultured A/Massachusetts/18/2022

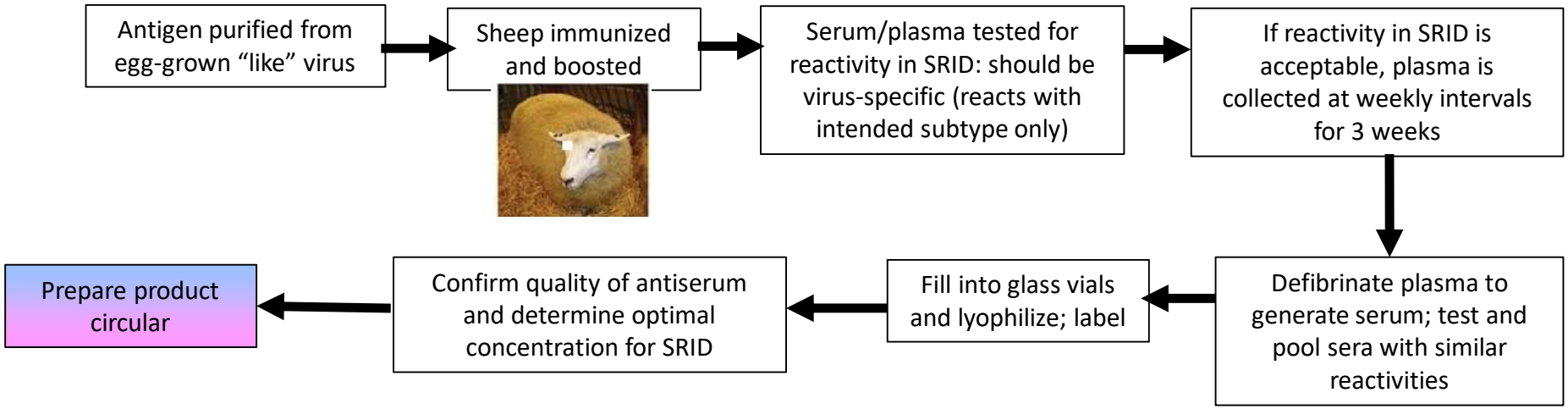
ccCVV	Type of virus (CVV name)	Reference antigens available
A/Sydney/1304/2022	Wild type virus	H3-Ag-2312 (CBER)
A/California/123/2022	Wild type virus	
	Cell reassortant (CVR-178)	
A/California/45/2023	Wild type virus	H3-Ag-2404 (CBER)
A/Massachusetts/18/2022 HA sequence: EPI_ISL_16968012	recombinant HA	H3-Ag-2405 (CBER)

- Reagents are prepared only for CVVs used to manufacture vaccines
- Reagents for the same CVV may be available from more than one ERL

Preparation of reference antigens for potency testing

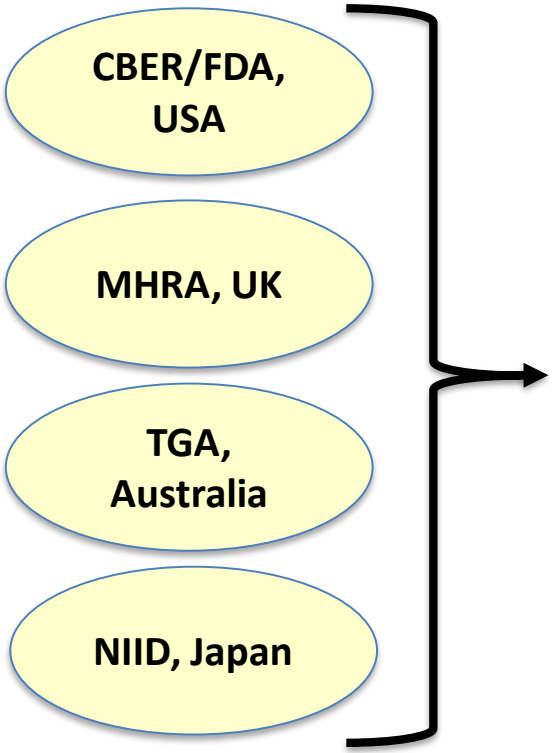
- The reference antigen used as standard in potency assay is produced using the same CVV as used for vaccine production
- The reference antigen is produced on the same platform as the vaccine
  - Reference antigens are lyophilized preparations of inactivated, whole egg- or cell-grown virus or rHA
- Reference antigens and PLS are usually donated by manufacturers; ERLs are responsible for calibrating the reference antigens
- The first (primary) lyophilized reference antigen is calibrated using a “primary liquid standard” (PLS); subsequent lots are calibrated using the primary lyophilized reference antigen (secondary calibrations)

Preparation of HA-specific sheep antisera at CBER



- Production of HA-specific antiserum is a lengthy process (~8-12 weeks)
- When an ERL is not able to produce their own antiserum in a timely manner, the reference antigen may need to be calibrated using homologous antiserum from another ERL. Qualified heterologous antisera have been used when it is not possible to share homologous antiserum.

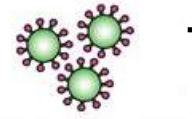
Calibration of Influenza Vaccine Reference antigens is performed collaboratively by ERLs



- ERLs follow WHO guidelines (ECBS protocol) to calibrate reference antigens
- "Lead" ERL produces antigen-specific antiserum, performs investigational testing, ships PLS, lyophilized antigen, and antiserum to other ERLs
- Each ERL determines HA concentration in PLS by physicochemical method; the average HA concentration is assigned as the value for PLS
- Each ERL measures HA concentration of Reference antigen by SRID, using PLS as the standard
- The Lead ERL assembles the results and assigns average as the reference antigen potency value
- Reference antigen values are reported to IFPMA and WHO, and documented in Tables on the MHRA Influenza Hub (Sharepoint site) and WHO webpage

Primary Calibration of Reference Antigen

1. Determine HA concentration of the Primary Liquid Standard (PLS)



PLS: *Whole inactivated egg- or cell-grown virus or rHA*

- Total protein concentration measured (e.g. Lowry/BCA assays)
- %HA determined from SDS-PAGE analysis

→ Calculate HA concentration (µg/mL)

or

- HA concentration (µMoles) determined by Isotope Dilution Mass Spectrometry (IDMS)

→ Calculate HA concentration (µg/mL)

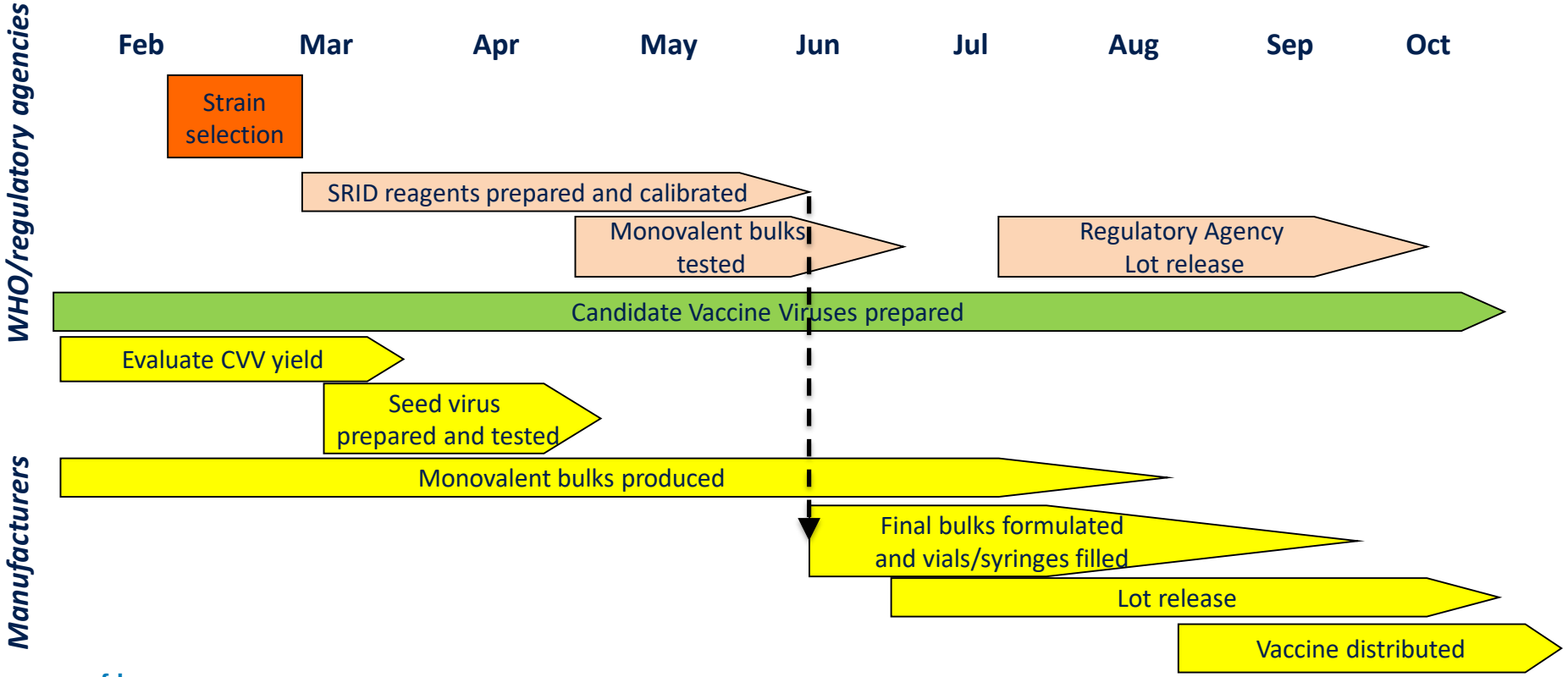
2. Determine potency of lyophilized reference antigen

Ref Ag: Lyophilized Whole Inactivated Virus

- Reconstitute lyophilized antigen
- Perform SRID using HA-specific antiserum and PLS as standard

→ Compile results from all ERLs → Assign potency value (µg/mL) and report to WHO and IFPMA

Timeliness of reference reagent calibration is critical



Notes on reagent preparation (1)

- ERLs try to produce reagents needed by manufacturers as quickly as possible
- With an increasing choice of CVVs, the number of reagent needed has increased in recent years
- Capacity of ERLs is limited and some prioritisation may be needed
- Early communication between manufacturers needing reagents and ERLs is crucial
- Antigen reagent can only be made if a manufacturer is willing to donate bulk material for the chosen CVV
- Frequent communication between ERLs is critical for efficient collaborative activities

Notes on reagent preparation (2)

- ‘Rules’ for material donated for reagent production:
  - Donated reference antigen may be supplied to ERL as bulk liquid to be lyophilized by the ERL, or as filled, lyophilized vials
  - Donor remains anonymous
  - There is no guarantee that any donated material will be made into a reagent (e.g. more than one manufacturer may donate material for the same CVV)
- ERLs provide status updates to influenza vaccine manufacturers through:
  - Biweekly telecons with vaccine manufacturers (WHO/IFPMA call)
  - Reagent spreadsheet that is available at the MHRA “Influenza Hub” Sharepoint site
  - ERLs submit reagent lot numbers and potency values to both WHO and IFPMA as soon as calibrations are finalized
  - Reagents are available to all manufacturers at the same time under the same conditions

Looking ahead:
Future influenza vaccine potency measurement

- SRID potency assay is not perfect:
  - Takes a long time to produce HA-specific antiserum
  - Requires production of large volumes of antisera
  - Requires calibration of standards (reference antigen) for each CVV used by manufacturers and vaccine production platform
- Improvements to SRID are being evaluated:
  - Use of alternative antigens to immunize sheep, e.g., recombinant HA
  - Use of heterologous antisera
  - Use of heterologous antigens
- WHO working groups, manufacturers, ERLs have independently as well as jointly been exploring the use of alternative potency assays

Requirements of an alternative potency assay

- Quantitative
- Stability-indicating
- For seasonal influenza vaccines, should discriminate between all vaccine viruses in the trivalent or quadrivalent formulation
- Practical and cost-effective; advantageous if small volumes (or no) antiserum is used in the assay
- The potency values measured should correlate with levels of HA-inhibiting antibodies produced post-vaccination (or vaccine efficacy)

Influenza vaccine manufacturers need to discuss potency assay(s) they plan to use and whether equivalence to SRID measurements or clinical dosing study is needed to support the use of an alternate assay with regulatory agencies

Examples of alternative potency assays

- Enzyme Linked ImmunoSorbent Assay (ELISA):
  - many formats, including some commercially available assays (VaxArray)
  - use HA-specific capture and detection monoclonal or polyclonal antibodies
- Antibody capture followed by IDMS:
  - HA conformation-dependent antibodies specific for each subtype/lineage in vaccine to separate HA that is denatured from native form
  - Measure HA concentration of the fraction that was bound by antibodies by IDMS
- Surface Plasmon Resonance:
  - either antibodies or glycan on chips to which native HA binds
  - measure the signal based on mass of the complex that forms
  - Signal is proportional to HA concentration
- Limited tryptic digestion (or combination of enzymes that digest protein):
  - conditions are used that result in digestion of denatured forms of HA
  - subsequent IDMS measurement of the intact (native) form of HA

Examples of collaborative and individual studies that evaluate suitability of alternative potency assays

- Novel antibody independent receptor-binding SPR-based assay for rapid measurement of influenza vaccine potency, Khurana et al., Vaccine 2014;32(19):2188–97
- Rapid determination of influenza vaccine potency by an SPR-based method using subtype or lineage specific monoclonal antibodies, Narayan et al., Front. Immunol. 2023 (14).
- Development of an enzyme-linked immunoassay for the quantitation of influenza haemagglutinin: an alternative method to single radial immunodiffusion, Bodle et al., Influenza Other Respir Viruses 2013; 7(2):191–200
- Titer on chip: new analytical tool for influenza vaccine potency determination, Kuck et al., PLoS One 2014; 9(10):e109616
- Limited tryptic digestion-isotope dilution mass spectrometry (LTD-IDMS): a reagent free analytical assay to quantify hemagglutinin of A(H5N1) vaccine material, Cooper, et al. Anal Chem 2020
- Conformationally selective biophysical assay for influenza vaccine potency determination. Wen, et al. Vaccine 2015;33(41):5342–9.
- Immunocapture isotope dilution mass spectrometry in response to a pandemic influenza threat. Pierce et al., Vaccine 2017;35(37):5011–8.
- Assessing the stability-indicating properties of alternative potency assays for inactivated influenza vaccine, Ekimov et al., Vaccine 41 (2023) 4639-4647

Summary

- Preparation of reference reagents for influenza vaccine potency testing is a collaborative effort, requiring
  - Information and expertise of all GISRS partners
  - Materials from manufacturers
  - Intensive laboratory effort and regular communication between ERLs
- Preparation of reference reagents takes time
  - Ways to improve SRID are being evaluated
  - Alternative potency assays are being evaluated
  - The use of a potency assay that does not rely on production of homologous antisera may be critical in the event of a pandemic

Acknowledgments

ERL Influenza Reagents Teams:

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Questions?


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