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**Proposed Generic Protocol for the Calibration of Seasonal/Pandemic  
Influenza Antigen Working Reagents by WHO Essential Regulatory  
Laboratories**

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## 1. Preamble

This document provides a generic protocol for the calibration of influenza antigen working reagents by the four WHO Essential Regulatory Laboratories (ERLs). It represents the consensus of the ERLs on the process of assigning a potency value to a newly established influenza antigen reagent for use in potency testing of inactivated influenza vaccines. An influenza antigen working (or reference) reagent is a preparation of inactivated whole virus that has been freeze-dried and calibrated as outlined in this document.

The calibration process involves the preparation of a primary liquid standard (PLS) and a large batch of freeze-dried antigen by one of the ERLs. The PLS is distributed to all other ERLs for independent calibration. Samples of the freeze-dried antigen are also distributed to the other ERLs and are calibrated against the PLS using SRD.

## 2. Essential Regulatory Laboratories (ERL)

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

The participation of all ERLs is assumed, as is current practice, with a minimum of three ERLs contributing data for each calibration. The laboratories will agree a timeline for completion of all calibration tests, with an expectation that most calibrations will be completed within 15 working days.

The lead ERL is the ERL that has produced the freeze-dried antigen reference reagent and has sent out materials as specified in point 3 (below) to the other ERLs for use in calibration. The lead ERL will inform WHO timely about the availability of a new reagent and about progress of calibration.

## 3. Reagents Supplied to Collaborating ERLs

For each antigen reagent to be calibrated, the following is to be supplied:

- at least 30 ampoules of freeze dried antigen
- 10 vials of antiserum; if more than one lot is shipped, 10 vials of each lot
- A batch, preferably with two aliquots, of a whole virus primary liquid standard (PLS), e.g. an in-house live or inactivated preparation or liquid pre-freeze-dried antigen. The PLS is to be supplied with an estimated protein concentration.

## 4. Internal Compliance Testing and documentation

The lead ERL performs the following tests on the supplied materials prior to distribution, provides test data upon request, and supplies interim documentation (e.g.: Instructions for Use [IFUs]):

- a protein estimation on the PLS, preferably confirmed using more than one method with a confirmatory value  $\pm 20\%$  of the estimated value
- an estimated range of working dilutions of the specific antiserum
- when possible, SRD assays of the PLS and the freeze-dried antigen using the specific antiserum are performed to confirm their antigenic identity, the estimated potency

value of the freeze-dried antigen and a qualitative assessment of SRD zones. If specific antiserum becomes available only after distribution of PLS and freeze-dried antigen, SRD with a cross-reactive antiserum is performed, if possible.

## 5. Antigen Reagent Supply to Vaccine Manufacturers

Upon request, vaccine manufacturers are to be provided with freeze-dried antigen reference reagents as soon as they are available and prior to final calibration. These may be supplied with interim estimated values for use in SRD potency assays. Manufacturers' data (e.g. comparison of SRD values with manufacturers' in-house methods for preliminary yield analysis of monovalent bulks) can be supplied to ERLs for review.

## 6. ERL Calibration Methodology

- *PLS protein estimation*: this should be performed by a recognised assay, e.g. Lowry or Total Nitrogen Determination, according to the local ERL SOP/methodology. Assays should include an appropriate protein control, preferably a common control shared between the ERLs.
- *PLS PAGE assay*: the PLS should be treated as appropriate prior to the PAGE analysis, e.g. reduction or deglycosylation. Assays should be performed according to the local ERL SOP/methodology, with a minimum of two independent assays, preferably performed by different analysts. Protein bands should be visualised using Coomassie blue based staining.
- *PAGE band analysis*: Analysis of PAGE gels should be performed according to the local ERL SOP/methodology, recording any varying parameters from their usual procedures.

### General guidance for confirmation of accuracy of PAGE band analysis:

- the ratio HA1: HA2 is approximately 3:2
- the HA content should be between 20-50% of total protein
- the analysis is to be repeated if there is > 20 % variation between replicates

### SRD:

- Preferably, 3-6 assays should be performed involving more than one operator.
- Assays should be performed using the local ERL SOP/methodology.
- Freeze-dried antigen should be analysed using the PLS as standard antigen on plates containing the appropriate antiserum.
- The final potency value of the freeze-dried antigen is derived using the mean potency values of all assays.

### Complete ERL data sheet:

- A sample sheet is attached to this protocol.
- The data sheet is to be sent to the lead ERL.
- Supplementary data may also be included.

## 7. Assignment of calibrated potency value by the lead ERL

Data generated by the ERLs are collected by the lead ERL and compiled for the final potency value agreement and confirmation (a sample table is attached). Manufacturers' data may be

considered, if available. Before the assigned value is made public (e.g. through ERL website, WHO website, IFU), the final data sheet and proposed calibration value are sent to all participating ERLs for comment and/or approval. The lead ERL has final authority to assign a potency value.

## **8. Calibration of secondary and replacement reagents**

The first antigen reagent to be developed (primary reference antigen) for a given candidate vaccine virus (CVV) is calibrated according to the process outlined in points 2 to 7 above. When another antigen reagent for the same CVV is produced, either as a replacement to replenish stocks or as an alternate reagent provided by another ERL, it is calibrated against the primary reference antigen reagent to ensure equivalence of antigen reagents and to ease the switch from one lot of reagent to a new one. The process for calibration (cross-calibration) of these types of secondary reagents is abbreviated: calibration uses the primary reference antigen reagent as the relevant calibrant; calibration against PLS is not performed under these circumstances. In all cases, calibration is performed using the SRD assay. The exact process for cross-calibration varies between ERLs; use of more than one laboratory, either within the same institution or by using external laboratories (e.g. other ERLs, national control laboratories with proven experience in influenza vaccine potency testing), is encouraged. If this is not feasible, more than one operator within the calibrating ERL laboratory will be engaged in the calibration process.

## **9. Review of this document**

This document will be reviewed periodically, at least once per year, by the ERLs to ensure it reflects best practice within ERLs and updated methodology that may be implemented in the future. Review may occur through electronic means or during meetings between ERL representatives.

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