Current Regulatory Expectations

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Use of Reference Standards in the Transition from
In Vivo to In Vitro Methods for Vaccine Release Testing
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The Opportunity and the Challenges

The good news:

- The transition from an in vivo to an in vitro potency method for **legacy marketed vaccines**, is in the context of a long-established, well controlled manufacturing process within a consistency model.
- A fit for purpose, stability indicating in vitro potency assay will have been demonstrated to have at least equivalent and typically superior discriminating ability relative to the in vivo method, hence will enhance the quality control of the vaccine.

The regulatory expectation:

- The overall control strategy must have sufficient characterization of the quantity and quality of the antigen via the potency assay, **and supportive orthogonal methods**, to ensure consistent and clinically effective commercial lots throughout the lifecycle of a product, and manufacturing changes. Key to this will be in-house reference standards.

Reference Standards are Critical for Vaccine QC

Reference standards (RS) are used to:

- Calculate relative test results by comparing the absolute readout of a test sample to that of a RS (e.g., Bioassays, ELISA, immunogenicity assays, etc.)

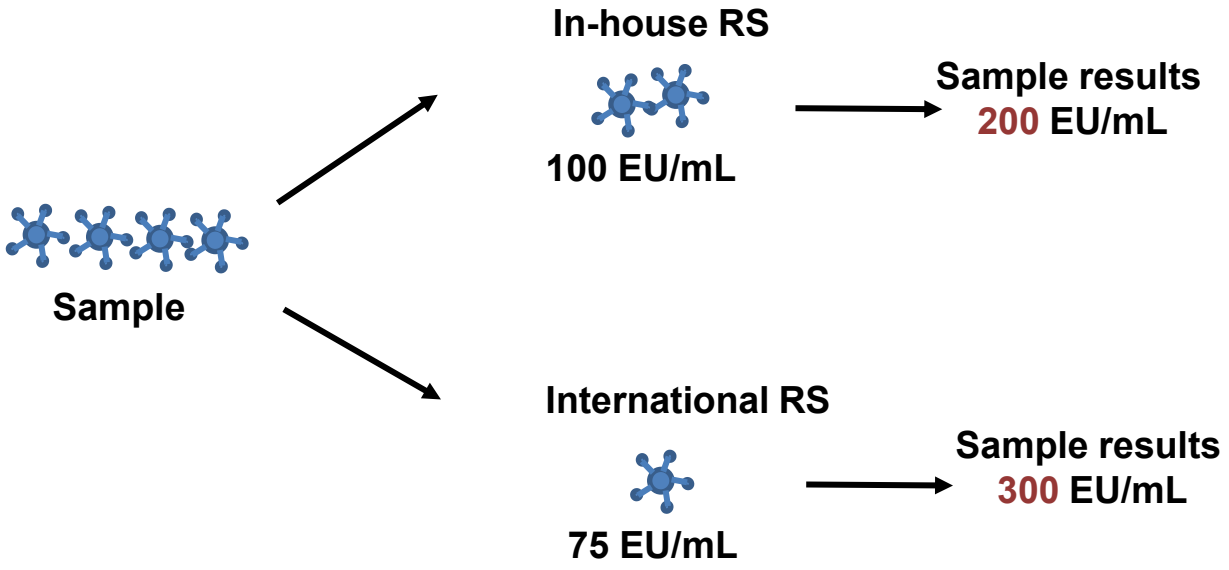
The regulatory expectation of an in-house RS (IHRS) management program is:

- To ensure that all IHRS replacements are comparable to the **1st IHRS** that is linked to clinical materials shown to be safe and effective (efficacious) in clinical studies.

- A key strategy to ensure consistent and clinically effective commercial lots throughout the lifecycle of a product.

Characteristics of Potency Reference Standards (RS)

Potency RS almost always uses arbitrary units (e.g., ELISA Units (EU)) rather than absolute units → The numerical potency value of the sample has no meaning other than in relation to the RS.



Numerical potency values cannot be compared independent of the reference standards used in assays.

Components of In-House Reference Standard (IHRS) Programs

Criteria for candidate material:

- Similar dose-response curves for IHRS and test samples with the in vitro method.
- Composition and storage conditions → maximize IHRS stability.

Qualification of an IHRS replacement:

- Assign a value to the candidate against the current IHRS based on a large dataset (test runs) → minimize measurement uncertainty.
- Confirm equivalence of candidate against the current IHRS in potency assays using common samples → based on statistical analysis (e.g., use of two one-sided t-test (TOST)).

Stability monitoring of IHRS:

- Establish a stability program for an IHRS that can detect changes* over time.

***While atypical, some biological materials can increase in potency over time, particularly early in their storage period.**

Challenge 1: Detect Potential Drift Due To Successive Bridging

Is the current equivalence assessment sufficient?

- The equivalence assessment routinely performed by vaccine manufacturers compares IHRS candidate against current IHRS.

- Measurement uncertainty associated with each qualification of an IHRS replacement can accumulate over several successive bridging.
  - Propagation of error

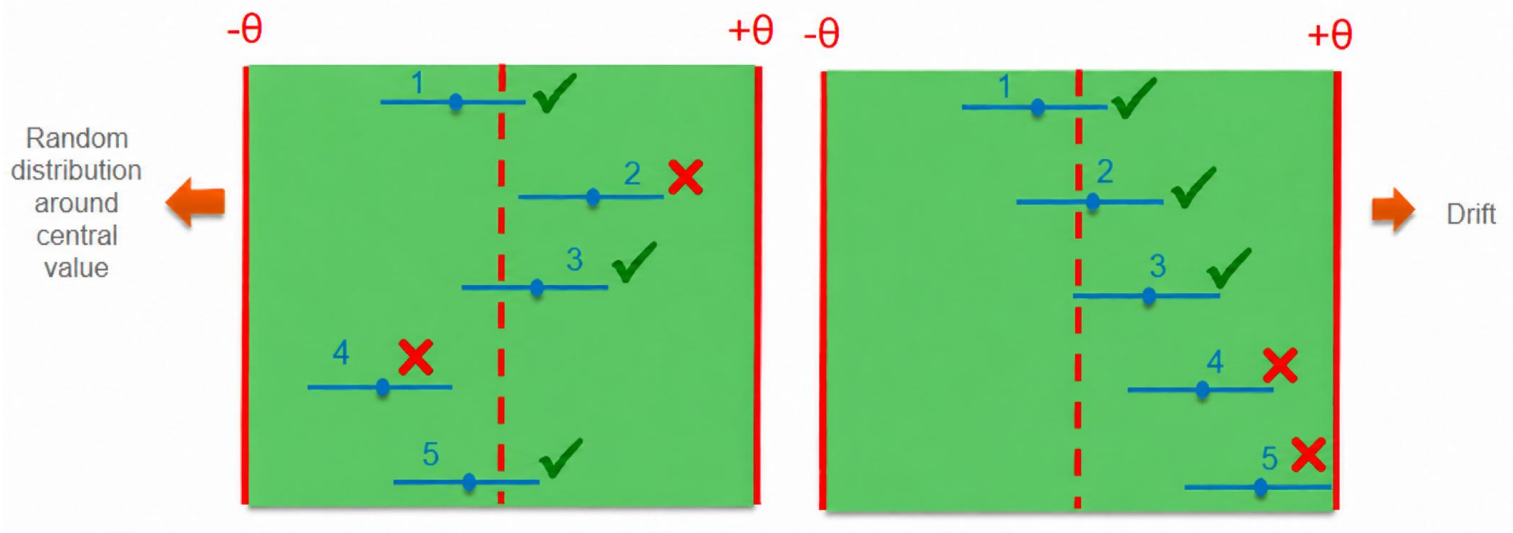
- This practice ensures the candidate IHRS (X^{th} IHRS) is equivalent to the previous IHRS but does not ensure that the X^{th} IHRS is comparable to the **1^{st} IHRS**.

Challenge 1: Detect Potential Drift Due To Successive Bridging (cont.)

How can this be improved?

- Examining the outcomes of all equivalence assessments for all IHRS replacements may allow the detection of a drift in relation to the 1st IHRS.

TOST results for all RS replacements (Simulated data)



However, it's challenging to estimate the drift between the IHRS candidates and the 1st IHRS, as it could be impractical or infeasible to test them side-by-side, due to stability of IHRS or availability if previous IHRS lots.

Challenge 2: Stability Monitoring of Reference Standards

Vaccine IHRS are typically complex biologics that may undergo conformation changes or degrade even under optimal storage conditions.

There are many challenges to implement an effective stability monitoring program for IHRS, due to:

- Use of arbitrary unit.
- Lack of suitable measurements for trending purposes.

It is often assumed that an IHRS retains its assigned potency throughout its use.

Greatest Challenge in IHRS Management is Tools to Detect Drift

Potential drift of IHRS and its replacements is due to:

- Measurement uncertainty during calibration of IHRS replacements (challenge 1).
- Conformation changes and/or degradation of IHRS during storage (challenge 2).

Limitations of current monitoring strategies:

Current strategy	Limitation
Trending of positive controls (control chart)	**Positive controls often have similar stability characteristics as IHRS and are measured against IHRS (and its replacements).**
Use of orthogonal methods	**Those methods require the same IHRS or may be highly variable.**
Stability prediction based on Arrhenius equation (WHO TRS 932, Annex 2)	**Arrhenius equation is only applicable to a first-order reaction rate. However, the decay rates of many vaccines at different temperatures don't follow a first-order kinetics.**
Trending of assay readouts, such as ED_{50}, GMT.	**The results are often too variable.**

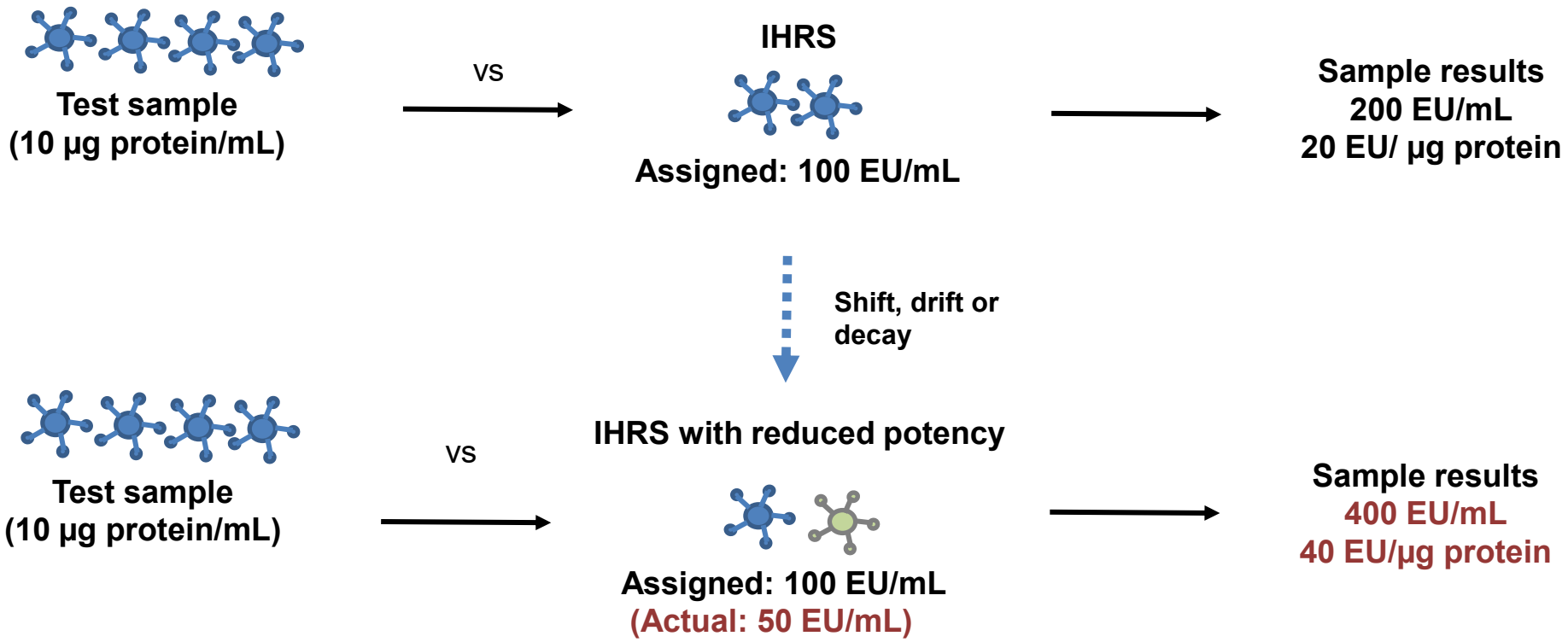
How to Improve Monitoring of Vaccine IHRS?

Link potency to other quality attributes for the same antigen:

- Multiple assays are usually performed to verify critical quality attributes of an antigen (e.g., ELISA, total protein, and purity are usually performed for protein-based antigens).
- Vaccine manufacturing process has inherent variability:
  - The antigen concentration (by ELISA or protein) at DS manufacturing stage is more variable → no impact on final product quality.
- "Specific activity" [e.g., antigen (e.g., by ELISA) to total protein is an intrinsic quality attribute of a protein-based antigen*.
  - "Specific activity" of an antigen is expected to remain relatively stable over time.
  - Antigen content by ELISA is susceptible to issues with relative measures, including potential drift of IHRS replacements.
  - Total protein content can be measured accurately and precisely (e.g., by Kjeldahl or other suitable methods) over time.
  - Trending of "specific activity" of vaccine lots → indirect monitoring of IHRS and their replacements.

*** Note that the protein determination method must be independent of reference standard, and that since many vaccines contain multiple antigens → it's challenging to monitor "specific activity" at DP manufacturing stage.**

Impact of a Decayed IHRS on Testing Results

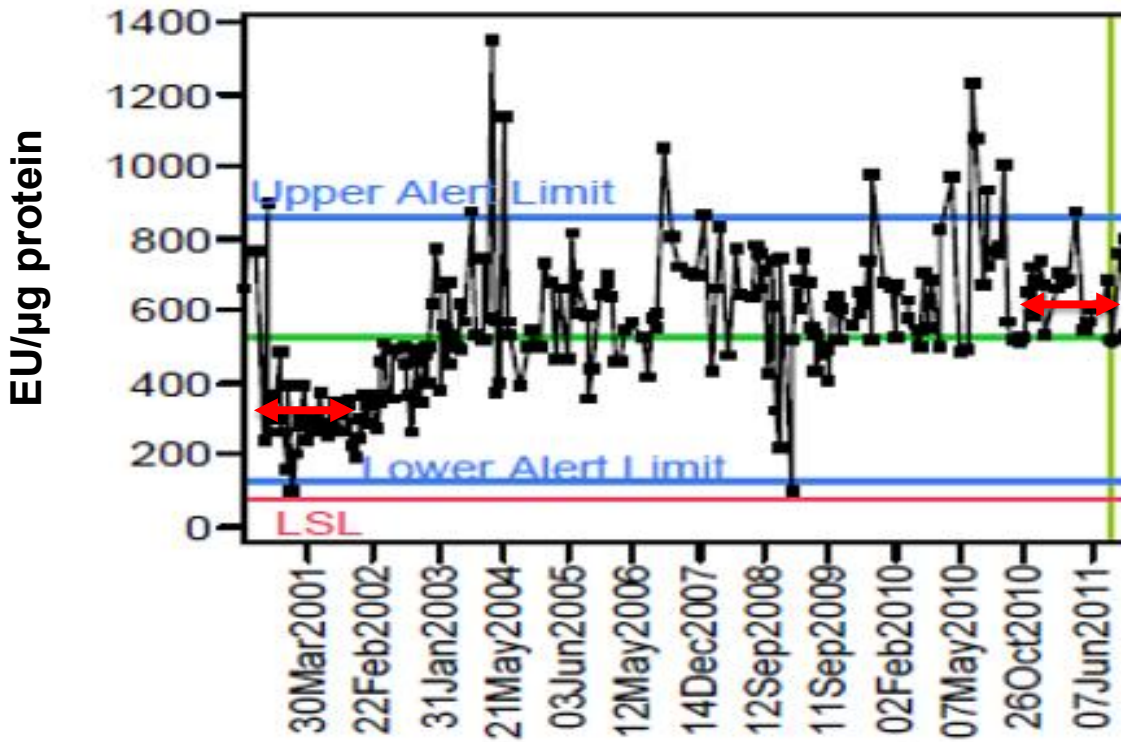


Vaccine lots tested against decayed IHRS:

- Higher "specific activity".
- Higher relative potency results for the same sample

In conclusion, trending of "specific activity" (mean and range over time) of a purified antigen (DS) can improve monitoring of IHRS.

Trending "Specific Activity" of a Protein DS Detects Potency Decay of IHRS



EU: ELISA unit

Protein content: based on total nitrogen

Conclusion: An approximately 50% reduction in IHRS potency over 10 years.

Proposed Approach to Improve Stability Monitoring of Potency RS

As part of demonstrating manufacturing consistency during product development and early commercial manufacturing stage:

- Establish the range and the mean value of “specific activity” (e.g., potency to protein ratio) based on a sufficient number of lots.

Trend “specific activity” data during routine commercial manufacturing, as part of IHRS monitoring.

Re-assess the impact on “specific activity” when introducing manufacturing changes.

Considerations for International Standards (IS) When Available

The availability of an IS relevant to your product does not relieve a manufacturer's responsibility to establish and maintain its own IHRS program.

Independent of whether an IS has an assigned unitage (arbitrary or IU) consider an IS as a key element of in an IHRS program, where the IS stability and quality attributes are being monitored independently and acquire that data as well as control chart your own.

Use an IS or commercially obtained RS as part of the Manufacture's IHRS monitoring program, but It is recommended that it should not be **the only anchor** for the program.

Following the 2023 Ottawa IABS Reference Standard meeting recommendations*, WHO has revised TRS 932, Annex 2- Recommendations for the preparation, characterization and establishment of international and other biological reference standards+.

*https://doi.org/10.1016/j.biologicals.2024.101756

+https://www.who.int/publications/m/item/recommendations-for-the-preparation--characterization--establishment-and-use-of-who-international-biological-reference-standards-(2026)

Conclusions

The composition and storage conditions of IHRS may be different from the vaccine product:

- IHRS and test samples should have similar dose response curves.

- It is important to preserve the integrity of IHRS and reduce the need for frequent replacements that may lead to drift.

Effective IHRS management → ensure IHRS replacements are comparable to 1st IHRS:

- The assigned value of an IHRS replacement is based on a large dataset.

- Equivalence assessment should also examine the outcomes of all previous equivalence assessments → improve the detection of a drift in relation to the 1st IHRS.

- Establish, monitor, and trend the range and the mean value of a “specific activity” for each antigen during routine commercial manufacturing and the qualification of an IHRS replacement → improve the detection of a drift.

Many thanks to our colleagues for excellent discussions on this topic!

Dr. Richard Siggers
Dr. Chad Irwin
Dr. Julie Joseph

Questions?