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Scientific Considerations for the Lifecycle Management of Vaccine Reference Standards and the Impact of Animal Assay Use

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Global Availability of Critical Reagents for Biologicals Testing:
Current Status, Challenges, and Possible Solutions
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Outline

- Background
- Challenges in management of reference standards
- Proposed approach
- Considerations for and challenges with international reference standards
- Conclusions

Reference standards play a critical role in vaccine quality control

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., ELISA, immunogenicity assays).

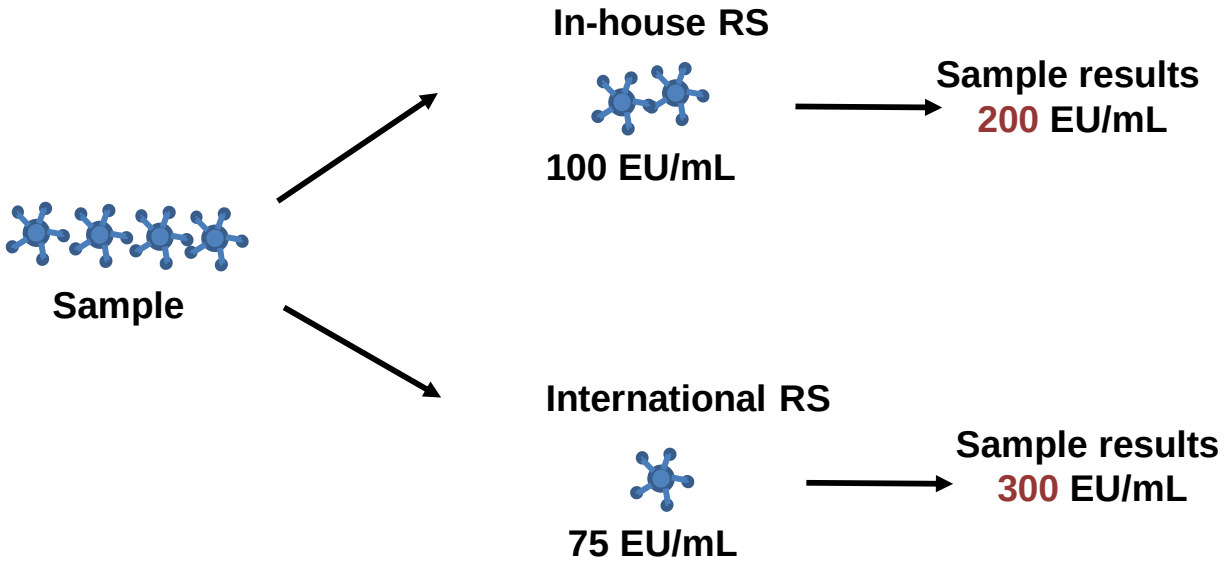
Aim of an in-house RS (IHRS) management program:

- 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 (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 an in-house reference standard (IHRS) program

Criteria for candidate material:

- Similar dose-response curves for IHRS and test samples.
- 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**.

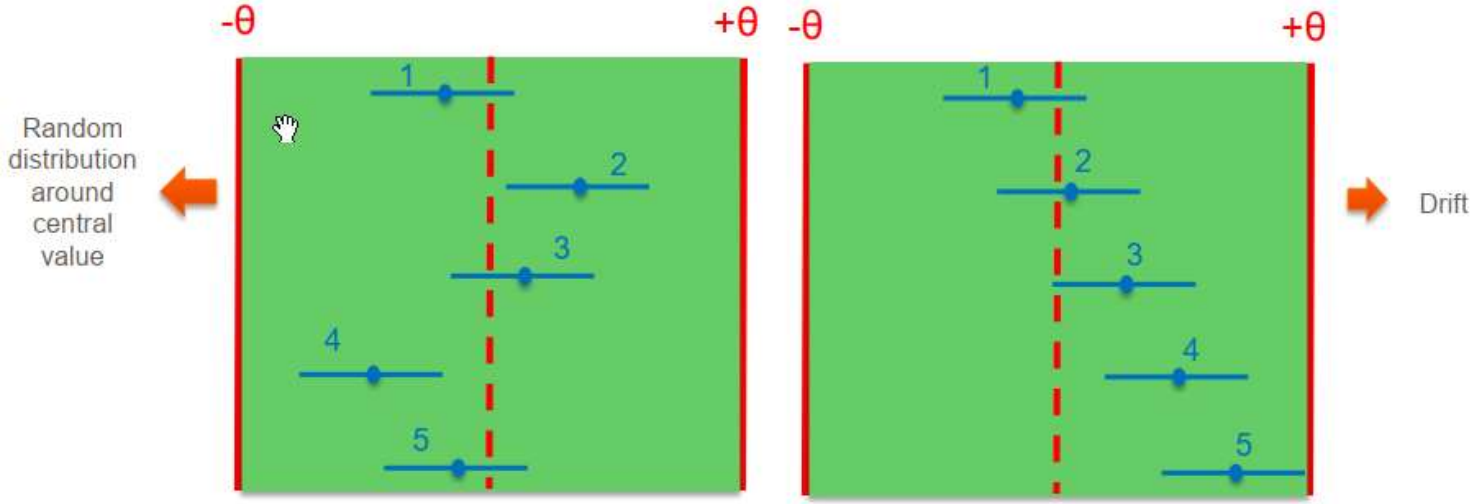
Challenge 1: detect potential drift due to successive bridging (cont.)

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)

(Courtesy of GSK, November 2015 at BRDD)



However, it is challenging to estimate the drift between the IHRS candidate and the 1st IHRS, as it could be impractical or infeasible to test them side-by-side.

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 lack of 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 the monitoring of IHRS?

Link potency to other quality attributes for the same antigen:

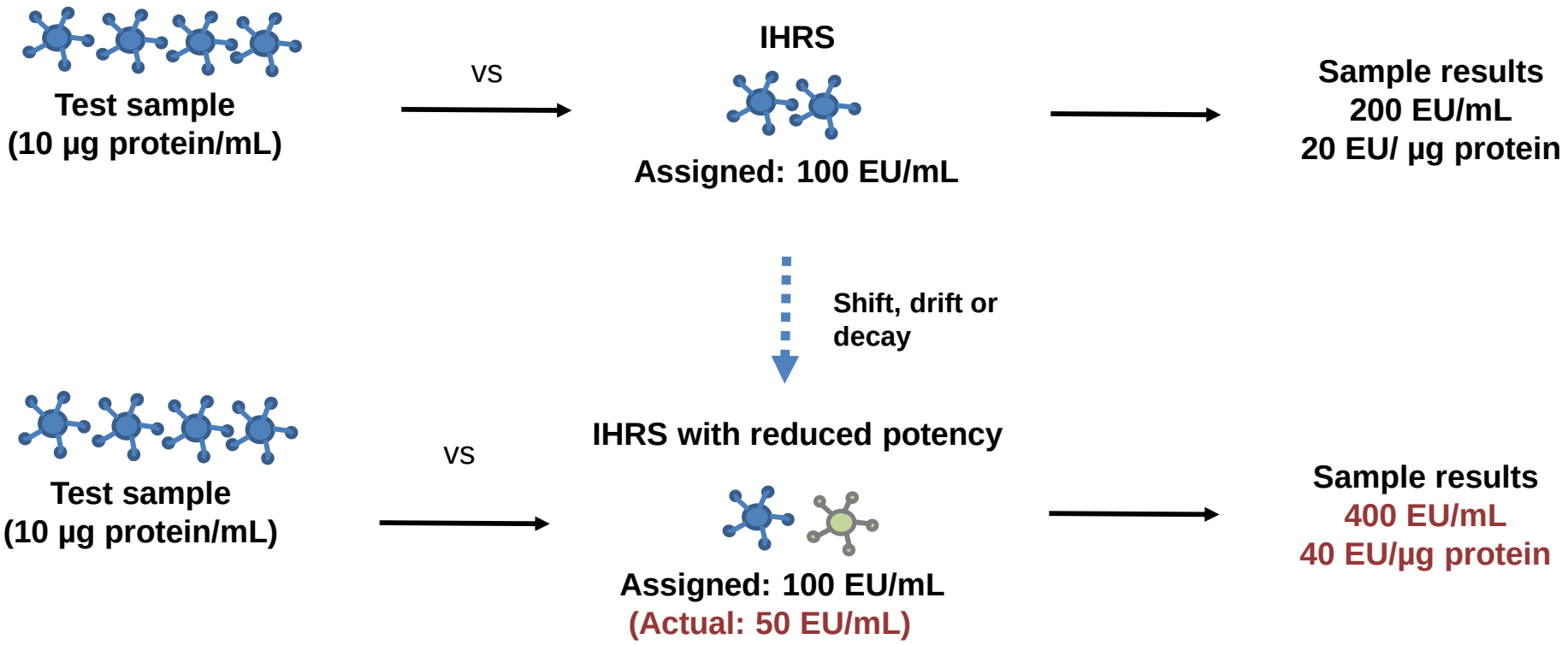
- Multiple assays are usually performed to verify critical quality attributes of an antigen (e.g., ELISA, total protein and purity are usually performed for protein-based antigens).

- 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 (by ELISA) to total protein (by Kjeldahl) ratio] 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 method) over time.
  - Trending of "specific activity" of vaccine lots → indirect monitoring of IHRS and their replacements.

***Many vaccines contain multiple antigens → impractical 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.

Impact of a decayed IHRS on product quality

When a drug product is formulated based on protein content (e.g., by Kjeldahl):

- Assigned potency results of DP lots by ELISA → trend upward.

In reality: No impact on DP potency!

When a drug product is formulated based on antigen content (by ELISA):

- Assigned potency results of DP by ELISA → no trend.

In reality: Reduced antigen in DP lots!

Example: IHRS for a new protein-based vaccine

1st IHRS for potency using ELISA:

- Assigned arbitrary value: 100 ELISA Unit (EU) per mL.
- Protein content: 20 µg/mL as measured using Kjeldahl method.
- IHRS: stored at -80˚C.

DS Manufacture consistency (based on 35 clinical/commercial lots):

- Protein purity range: 95±2%.
- Mean/range of “specific activity”: 5.0±1.0 EU / µg protein.

Example – dataset 1 (stability monitoring of 1st IHRS)

10-year trending of "specific activity" of commercial DS lots against the 1st IHRS (simulated)

IHRS					Commercial DS lots	
Years in use	Assigned Potency (EU/mL)	True Potency (EU/mL)	Measured Protein content (µg/mL)	Calculated EU per µg protein	Relative potency (EU/mL)	Mean EU per µg protein (50 lots)
2	100.0	100.0	20.0	5.0	100.0	5.0 ± 1.0
4	100.0	90.0			111.1	5.6 ± 1.0
6	100.0	80.0			125.0	6.3 ± 1.0
8	100.0	70.0			142.6	7.1 ± 1.0
10	100.0	60.0			166.7	8.3 ± 1.0

Scenario

Assume: 20 µg protein/mL

- ✓ The data set incorporated a 10 EU/mL potency decay every 2 years.
- ✓ "Specific activity" of commercial DS lots → an upward trend.

Example – dataset 2 (monitoring of IHRS and its replacements)

Trending of "specific activity" (Simulated data)

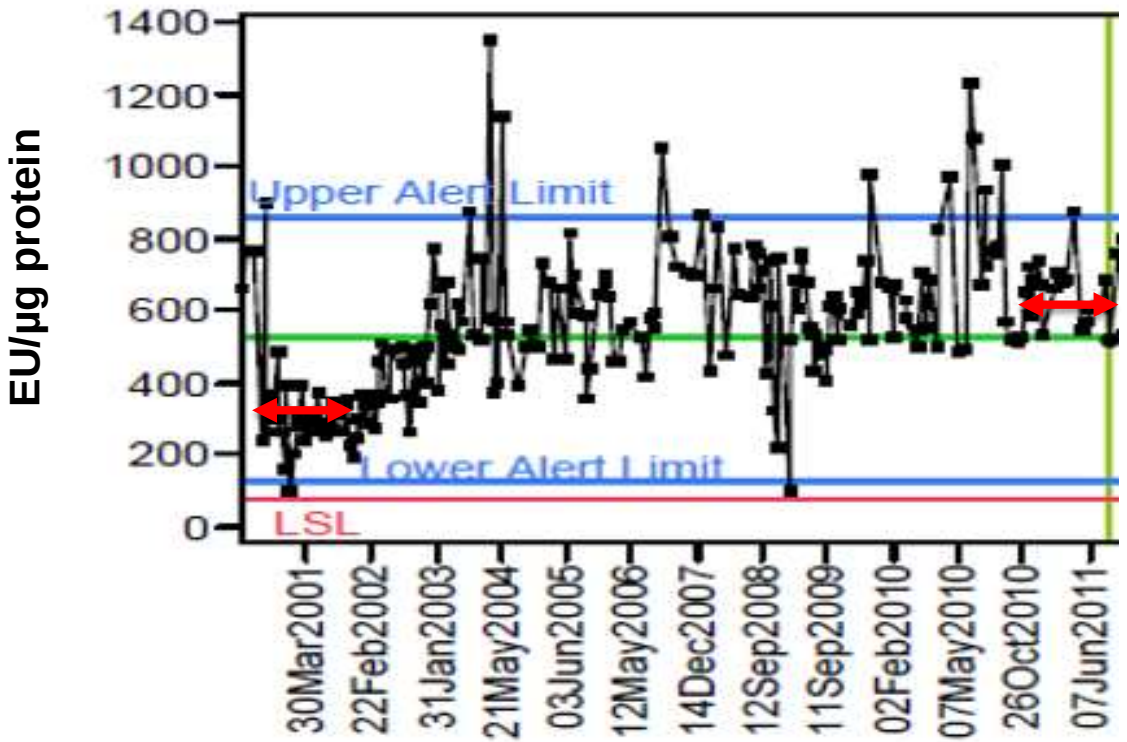
	IHRS and its replacements				Commercial DS lots	
	Assigned Potency (EU/mL)	True Potency (EU/mL)	Measured Protein content (µg/mL)	Calculated EU per µg protein	Established range for EU per µg protein)	Mean EU per µg protein (50 lots)
IHRS1	100.0	100.0	20.0	5.0	5.0 ± 1.0	5.0 ± 1.0
IHRS2	100.0	90.0	18.0	5.6		5.6 ± 1.0
IHRS3	100.0	80.0	16.0	6.3		6.3 ± 1.0
IHRS4	100.0	70.0	14.0	7.1		7.1 ± 1.0
IHRS5	100.0	60.0	12.0	8.3		8.3 ± 1.0

Simplified data

Related to true potency

- ✓ The data set incorporated a 10EU/mL potency decay for each new IHRS.
- ✓ "Specific activity" of IHRS replacements and commercial DS lots) → an upward trend.

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 a potency assay IHRS

Link the arbitrary unit of a potency assay IHRS with another relevant vaccine quality attribute that can be measured accurately and precisely (e.g., protein content etc.).

Monitor "specific activity" of a purified antigen → indirectly monitor the potency assay IHRS and its replacements.

Select an appropriate assay to measure the relevant quality attribute (e.g., protein content by Kjeldahl):

- The assay does not use the same IHRS for calculation (independent of IHRS).
- The assay performance is consistent over a long period time (conformational or structural changes to antigen protein do not impact the test results).
- For example, Kjeldahl method (based on total organic nitrogen) is more suitable for protein content determination than HPLC method (relative value using the same IHRS) for monitoring of "specific activity".

Proposed approach to improve stability monitoring of potency RS (cont.)

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.

Trending of "specific activity" of an antigen: Applications and limitations

Trending of "specific activity" of an antigen during commercial manufacturing, where the potency is measured against an IHRS has the potential to detect drift of the IHRS.

- Comparison of "specific activity" between the 1st IHRS and its xth replacement has the potential to detect drift.

"Specific activity" (mean and range) is antigen specific and product specific.

- It is not practical to trend "specific activity" in DP that contains multiple antigens.
- It is not possible to trend "specific activity" of International Standard and its replacements, as the candidate materials are supplied by different manufacturers.
- "Specific activity" of an IHRS lot remains unchanged through its use → assigned potency value and protein content remain the same.

Another important tool - International Standard (IS)

An IS can be a useful tool, but it has similar challenges as IHRS management:

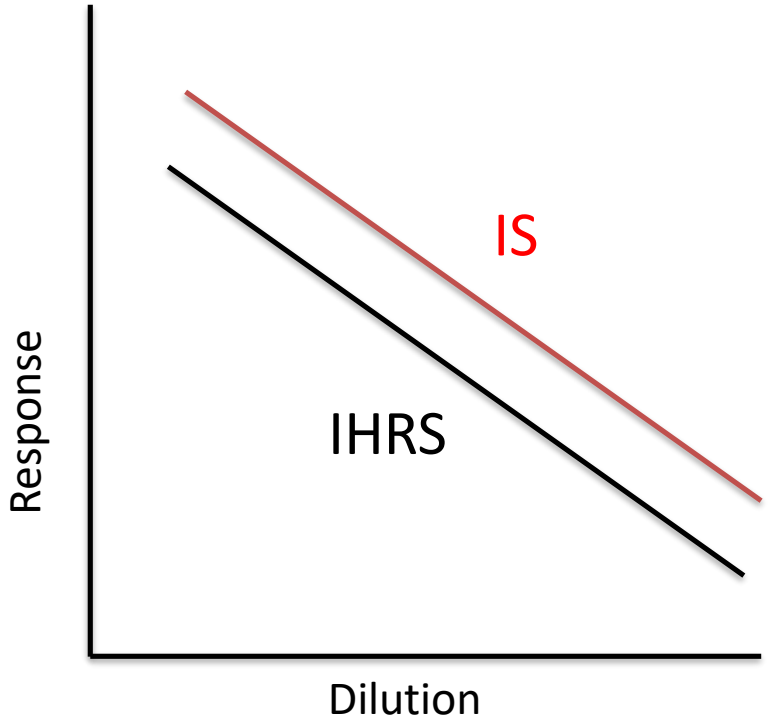
- IS for potency assay often uses arbitrary rather than absolute units → Measurement uncertainty is not applicable to 1st IS.
- IS replacements are calibrated against existing IS → Measurement uncertainty is applicable to IS replacements (2nd, 3rd…).
- IS and its replacements may be supplied by different manufacturers → no "specific activity" or manufacturing data to detect drift.

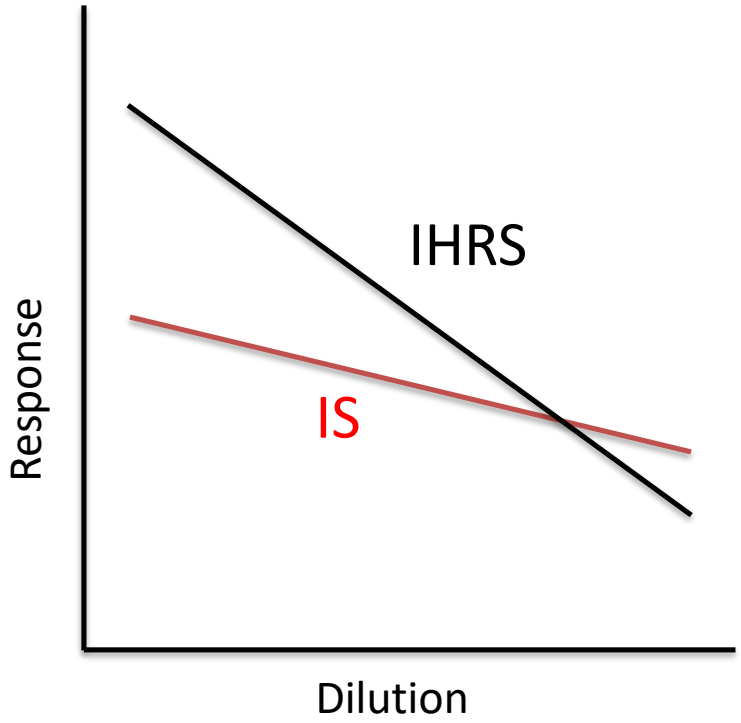
When investigating inconsistent results between an IHRS and an IS replacement:

- Consider that IS may decay and IS replacements have measurement uncertainty.
- Identify the root cause of the inconsistency.
- The resolution must ensure the comparability between the IHRS replacements and the 1st IHRS which is linked to clinical performance of the vaccine.

The dose-response curves for IS and IHRS may differ

- IS and IHRS may be supplied by different manufacturers and behave differently in assays
- Initial and replacement IS may also come from different sources

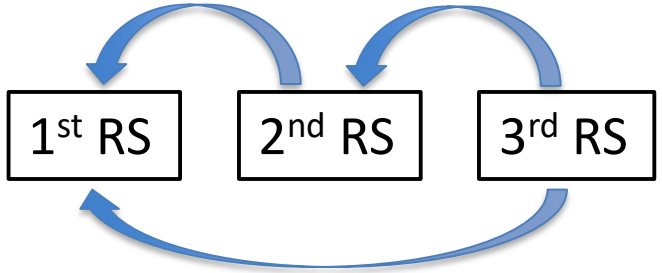




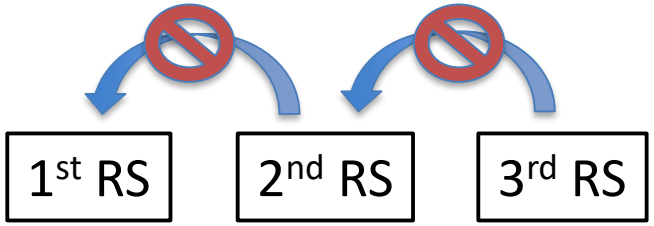
RS Replacements Should be Commutable to 1st RS

- IS and IHRS face similar challenges
  - Biological material that can decay
  - Introduction of replacement RS should consider method uncertainty and potential for drift
- Aim of IHRS management program should be to ensure that all IHRS replacements are commutable to the 1st IHRS that is linked to clinical studies
  - Consider how using IS may affect this linkage

Scenario 1: replacements are linked (successive or linked to initial RS)



Scenario 2: RS replacements independently established



Challenges with IS cont'd

HC and other NRAs became aware of issues related to IS calibration or the implementation of an IS that unintentionally risked changes in vaccine antigen content.

Workshop convened through the International Alliance for Biological Standardization (IABS) in collaboration with WHO, EDQM, FDA and EU NRAs to consider the issues.

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

Some key messages from NRA and manufacture case studies:

- NIBSC (now UK Medicines and Healthcare products Regulatory Agency (MHRA)): Questioned "the appropriateness of harmonized specifications for some vaccines" based on IU, particularly when animal potency assays were involved, due to the inherent variability of animal assays.
- EU National Control Laboratories (NCLs): "A consequence of strict interpretation and compliance to current WHO TRS [No. 932. Annes 2] is that the IU for a standard ceases to exist from a traceability standpoint when a replacement is made."
- Manufacture's case studies: "In both cases, the … [implementation of a] new IS had a significant impact on product consistency… [and] … caused an average reduction in product potency of 35%." "In the second case, the replacement IS … led to [an]… under formulation of the drug product."

Recommendations from the IABS Reference Standard Workshop

Four key recommendations from the workshop:

- Standards should be clearly defined and classified by their intended use(s) and corresponding practices should cover chemistry, manufacture, and control (CMC) and bioanalytic applications.

- When a standard is used as a calibrant, its selection, qualification, and stability should consider the relationship to the original assigned value and to the product specification so as to ensure quality.

- The approach taken for assigning units to IS for vaccines, **including whether a unitage is necessary**, should be carefully considered to ensure that it meets the needs of different stakeholders.

- Collaborative studies should require prior qualification of participating laboratories. To be included in the assignment of IU, testing labs should have sufficient experience and demonstrated proficiency with the types of tests performed, conduct method validation, and should be able to demonstrate that they regularly practice the test over time.

Additional Considerations for International Standards (IS)

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 an additional 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 program but do not be dependent on that.

As per the Ottawa IABS 2023 Reference Standard meeting*, WHO is revising 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**

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 great discussions on this topic!

Dr. Richard Siggers
Dr. Chad Irwin