Lifecycle Management of Reference Standards Post-Commercialization: Case Studies and Best Practices

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Session III: Reference Materials: Navigating Regulatory and Scientific Best Practices

CMC Strategy Forum Europe

20-22 October

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Agenda

01	Introduction
02	Case Study 1: “Separating fact from fiction in protein content—because reference standards shouldn’t be tall tales!” Measured Value vs “True Value”
03	Case Study 2: “Leveraging Reference Standard Trending: “Glycan shifts revealed, control systems refined—science that adapts beyond launch” Adopting clinically relevant specifications
04	Concluding Remarks

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Disclaimer

The content presented in these slides reflects my professional perspective, informed by real-world scenarios encountered in previous roles where I served as a subject matter expert. Please note that all data included is not real and intended solely for illustrative purposes.

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Health Canada / Santé Canada

Canada

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

Dean Smith, Ph.D. & Tong Wu, Ph.D.
Vaccine Quality Divisions 1 & 3,
Centre for Vaccines, Clinical Trials & Biostatistics, Health Canada

IABS Webinar in collaboration with HSI
Global Availability of Critical Reagents for Biologicals Testing:
Current Status, Challenges, and Possible Solutions
July 2, 2024

YOUR HEALTH AND SAFETY... OUR PRIORITY.

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World Health Organization

Report of a WHO workshop on implementation of the WHO manual for the preparation of reference materials for use as secondary standards in antibody testing

14-16 November 2023, Denpasar, Indonesia.

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

SPECIFICATIONS: TEST PROCEDURES AND ACCEPTANCE CRITERIA FOR BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS
Q6B

Current *Step 4* version
dated 10 March 1999

CASSS

BioProcess International

FOCUS ON... QUALITY

Reference Standards for Therapeutic Proteins

Current Regulatory and Scientific Best Practices and Remaining Needs, Part 1

by Anthony Mire-Sluis, Nadine Ritter, Barry Cherney, Dieter Schmalzing, and Markus Blümel

USP–NF/PF

⟨11⟩ USP Reference Standards

Analytical Procedures and Methods Validation for Drugs and Biologics

FDA U.S. FOOD & DRUG ADMINISTRATION

Guidance for Industry

BEBPA — BIOPHARMACEUTICAL EMERGING BEST PRACTICES ASSOCIATION

SCIENTIFIC WHITE PAPER

Reference Standards for Potency Assays

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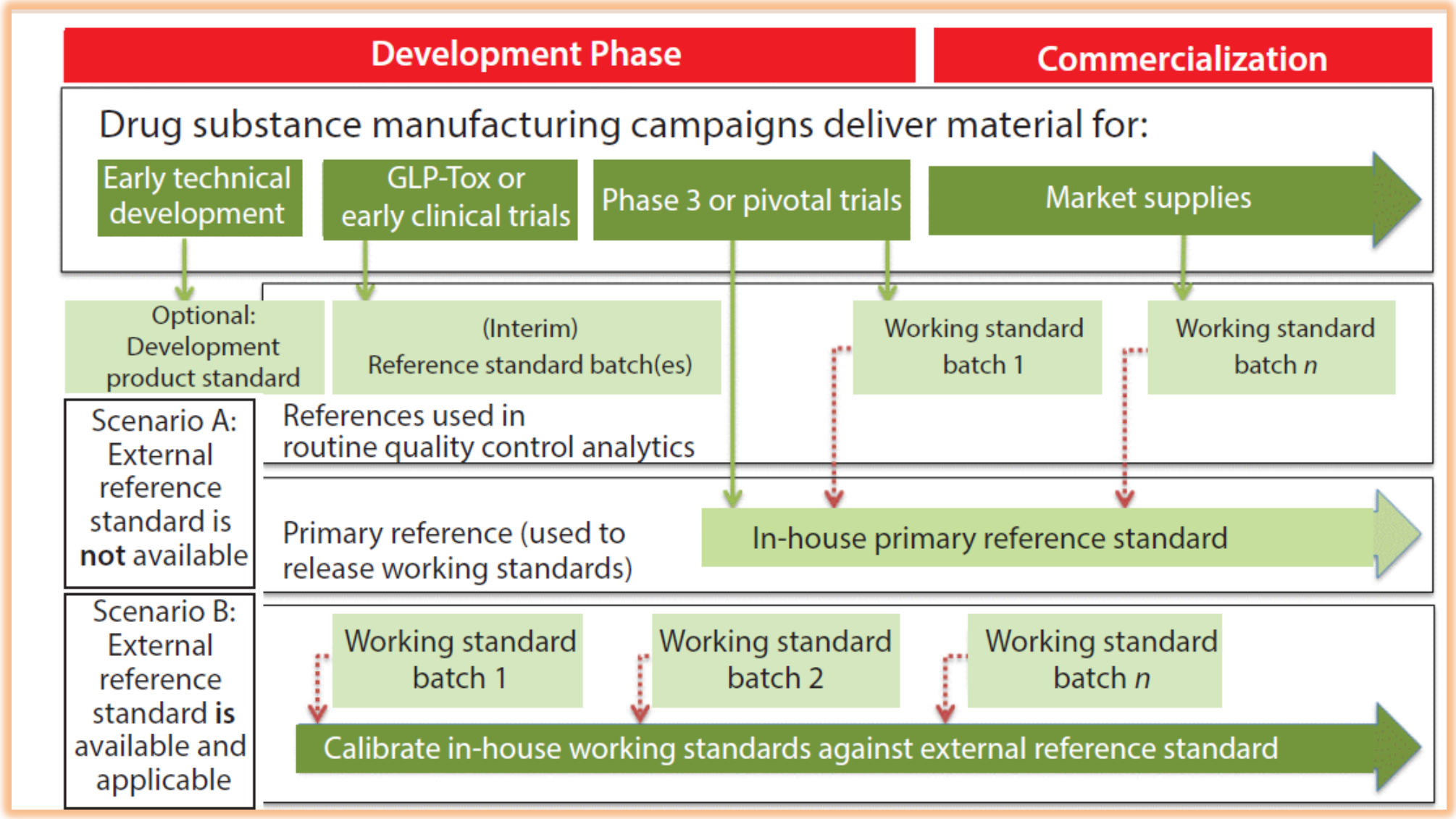
Reference Standard Lifecycle



Reference Standards for Therapeutic Proteins: Current Regulatory and Scientific Best Practices – Part I
Mire-Sluis A., Ritter N., Cherney B., Schmalzing D., Blumel M. (Bioprocess International, 12 (3), March 2014)

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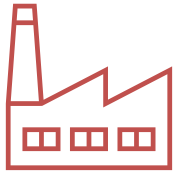
Examples of Reference Standard Life Cycle Management Post-Commercialization



Manufacturing Process Changes

Formulation

Yield improvements

Purification

Method Changes

Obsolete

Not suitable for intended use

New technology introduction (e.g. PAT, automation, MAM)

Primary and/or Working Reference Standard re-supply

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Case Study 1:
“Separating fact from fiction in protein content—because reference standards shouldn’t be tall tales!”

Measured Value vs “True Value”

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True Value

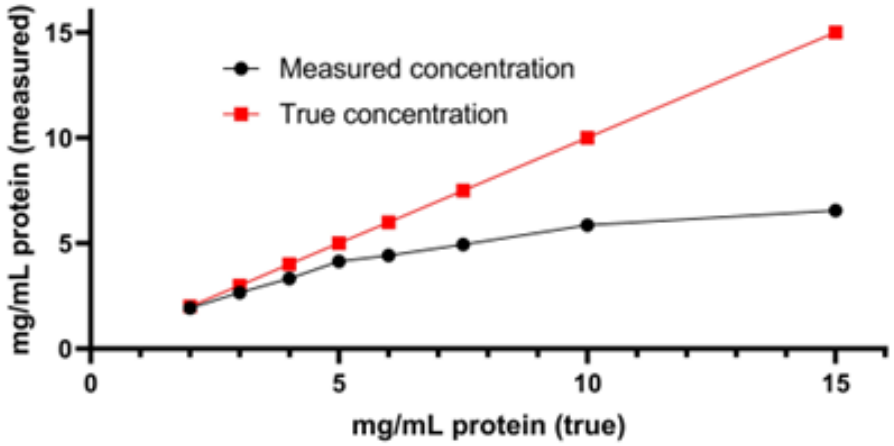
- Indeterminate
- Obtained by a perfect measurement
- Correct value of the measurand
- Value with no systematic errors

Measurement

- Value attributed to a measurand
- Includes uncertainty of measurement

Accuracy

- Closeness of agreement between a measured value and a true value
- Freedom from mistake or error
- Degree of conformity of a measure to a standard or a true value



Source: https://users.physics.unc.edu/~deardorf/uncertainty/definitions.html

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Problem Statement: Establish the “true value” for Protein Concentration of a Reference Standard

Manufacturing changes: **matrix components** and **target concentration.**

Formulation **changes interfered with the signal outputs** of the methods used to determine protein content for in-process release and stability testing. **Methods were updated** to suit intended purpose. **No bridging studies were conducted** between these different test methods.

Single-tier reference standards qualified using three different test methods qualified to suit formulation changes throughout clinical development

A **post-marketing commitment** for one major market required to develop and validate a method that could determine the **“true value” of protein content**. The same technical package justifying the method change intended for RoW.

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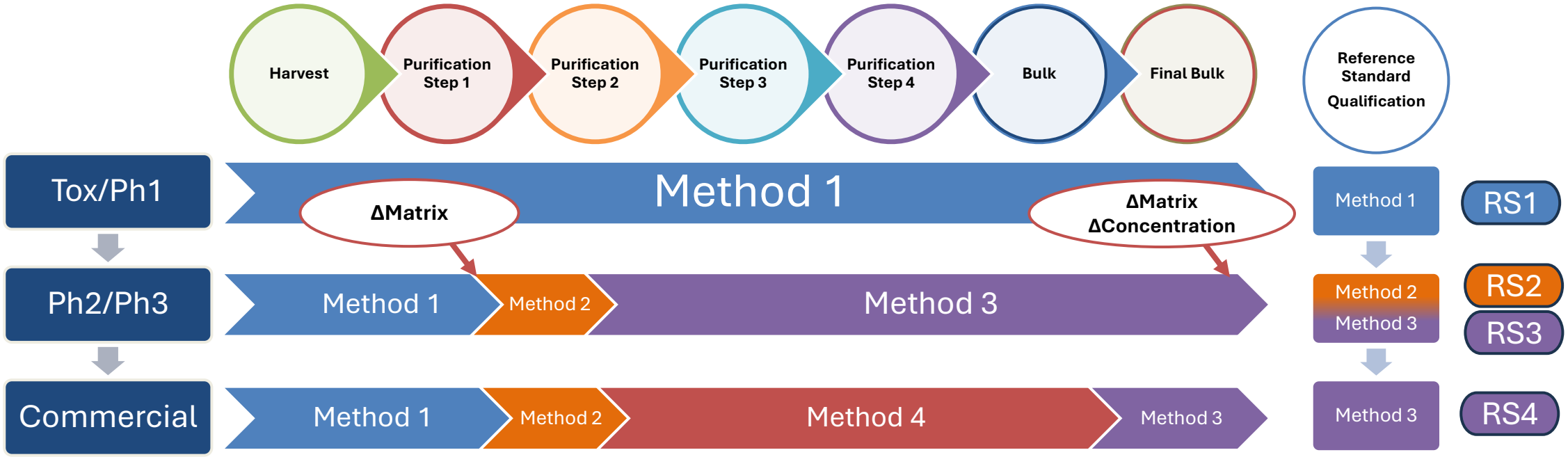
Analytical Method Changes: In Process, Release and Reference Standard Qualification



	Matrix Interference	Accuracy & Precision	Method Principle	Calibration Standard	Assay Control	Testing Location
Method 1	(+)	++	Fluorescence	(-)	Instrument	Mfg
Method 2	(+)	++	Refraction	(-)	Instrument	Mfg
Method 3	(+)	++	Colorimetric	RS	RS	QC
Method 4	(-)	+++	LC	RS	RS	QC

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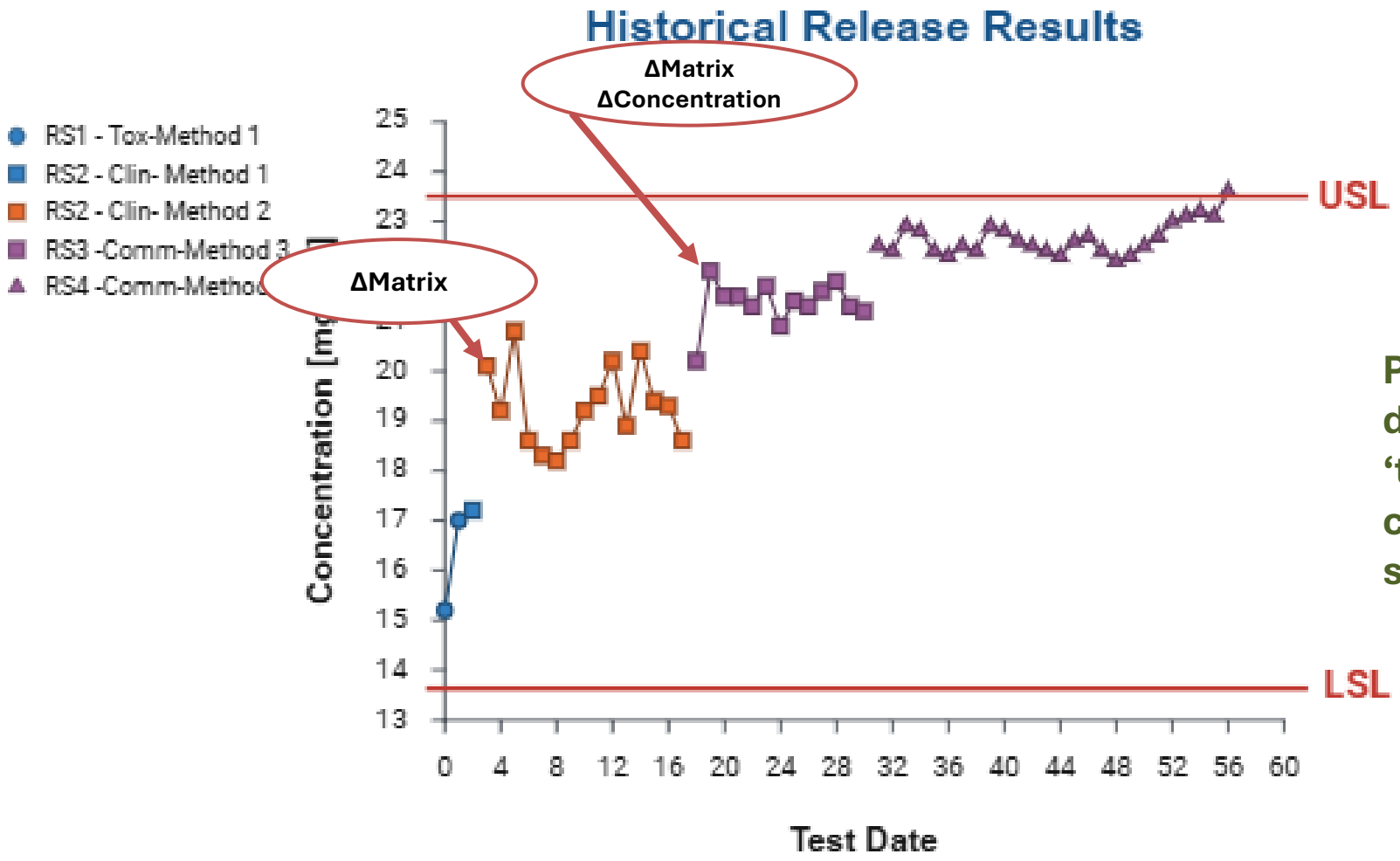
Historical Release Testing Results



Process and method changes disrupted the ability to trace the 'true value' of protein concentration across reference standard lots.

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Strategy for PMC Fulfillment

Establish a 2-tier reference standard program

- **Upgrade RS → PRS (extensively characterized, tested with historical and proposed method)**
- **Select WRS (representative of current process)**

Confirm "True Value" of PRS with orthogonal methods

- **Amino Acid Analysis**
- **Method 4 - LC (separates formulation components/higher precision and accuracy) using a well-characterized independent calibration standard (NIST)**

Method bridging: Demonstrate equivalency or superiority of Method 4 to Method 3 (used for release testing and qualification of RS for commercial process)

PRS/WRS Qualification criterion: Establish tighter acceptance criteria than release specification for qualification, including confidence intervals for statistically significant number of replicates to certify protein content.

Qualify WRS - use Method 4 & PRS as calibration standard

Process changes: Introduce process control to target concentration range within 10% of the specification target of the final product.

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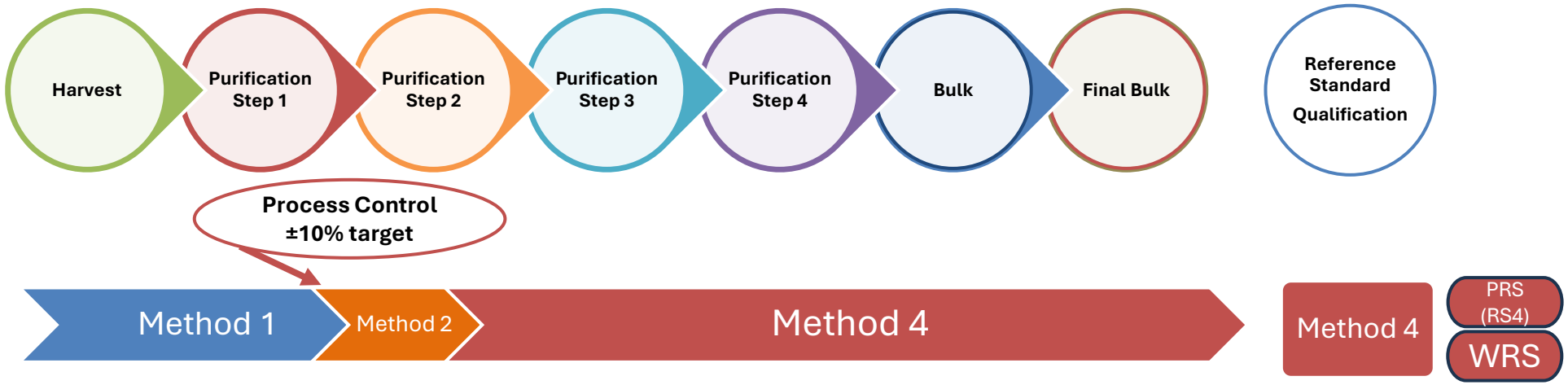
Analytical Methods and Process Changes Post- PMC



	Testing	Accuracy & Precision	Method Principle	Calibration Standard	Assay Control	Testing Location
Method 4	PRS Qualification	+++	LC	NIST	NIST	QC
	WRS Qualification	+++	LC	PRS	PRS	QC
Method 1	Routine Testing	++	Fluorescence	(-)	Instrument	Mfg.
Method 2		++	Refraction	(-)	Instrument	Mfg.
Method 4		+++	LC	WRS	WRS	QC

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Case Study 2:
Leveraging Reference Standard Trending: “Glycan shifts revealed, control systems refined—science that adapts beyond launch”

Adopting clinically relevant specifications

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Problem Statement: Repeated system suitability failures for sialylated N-glycan species preventing release of DS and DP lots

- Reference standard used as a comparator for glycan profile and charge heterogeneity
- System suitability failures registered at upper limit, ultimately leading to repeated assay failures.
- Glycan profile for sialylated species increased over time with concomitant decrease in neutral species.
  - No changes in relative percentage of mannose-6-phosphate or basic species
  - No new species detected

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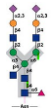
Background

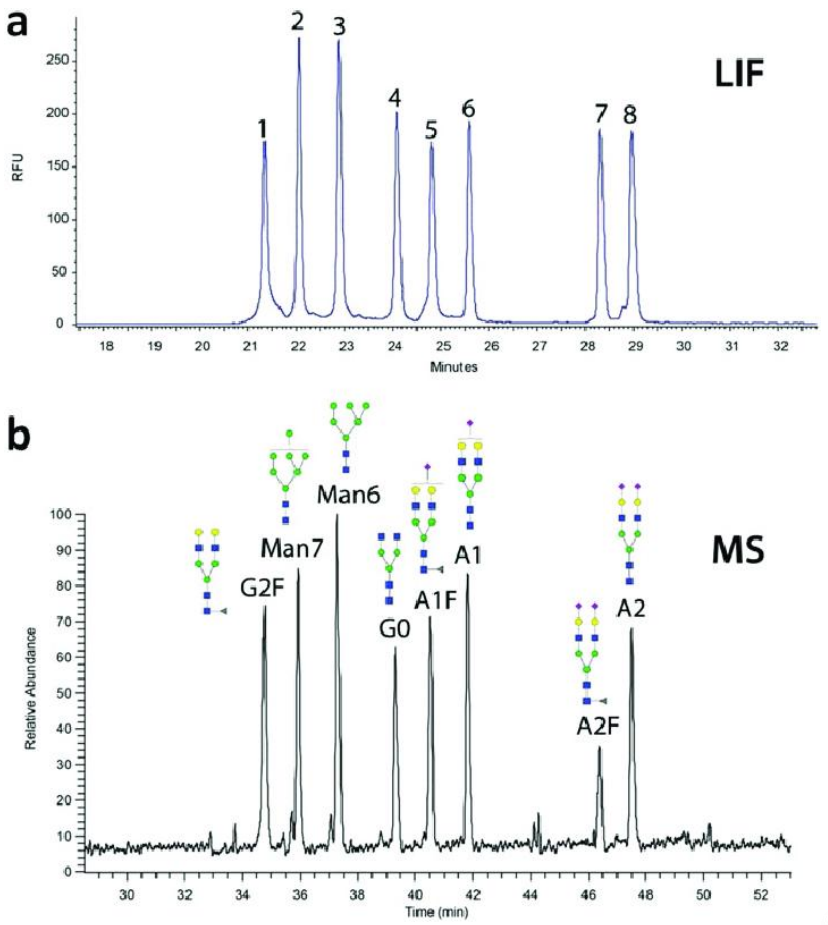
🧬 N-glycosylation is a post-translational modification which occurs at the bioreactor step. The modifications are not expected to change during further steps of manufacturing or under controlled long-term storage conditions.

🧪 N-glycan assay by CE-LIF was validated to monitor glycosylation and charge heterogeneity as indicators of process consistency.



Extended characterization

- **Orthogonal for potency** – an indirect measurement of potency. A minimum relative percentage of mannose-6-phosphate is required to maintain potency for receptor binding and entry to target cells
- **Orthogonal for charge heterogeneity** –thoroughly characterized peak groups with their abundance directly proportional to charge variants and pI.



Not real data. For illustration purposes only. Figure sourced from: Rapid Communications in Mass Spectrometry, June 2018; 32(11); DOI:10.1002/rcm.8150

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Justification of system suitability criteria and specification

System suitability criteria for reference standard were based on historical data sets for N-glycan peak groups (basic, neutral, acidic) and based on historical trends from representative material.

Release and stability specifications were based on historical data sets for N-glycan peak groups (basic, neutral, acidic) including clinical data and inclusive of certain glycan species related to known quality attributes (eg. sialylated, mannosylated).

Limited data set from manufacturing scale and process changes for intended commercial process – typical +/- 3SD approach.

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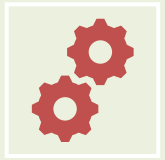
Strategy for Justifying Changes in Control System



Process data: Review historical data for PRS/WRS, DS and DP (release and stability)

Determine whether trends in N-glycan are similar

Determine if other trends in CQAs are observed

Assess analytical method performance

Optimize parameters known to control variability

Assess clinical relevance of N-glycosylation state

Determine whether trends in N-glycosylation impact bioavailability (C_{max})

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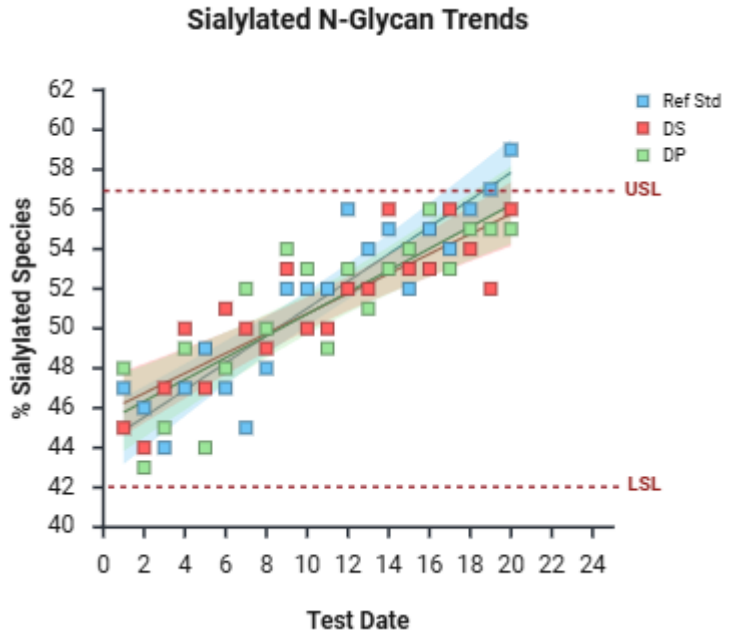
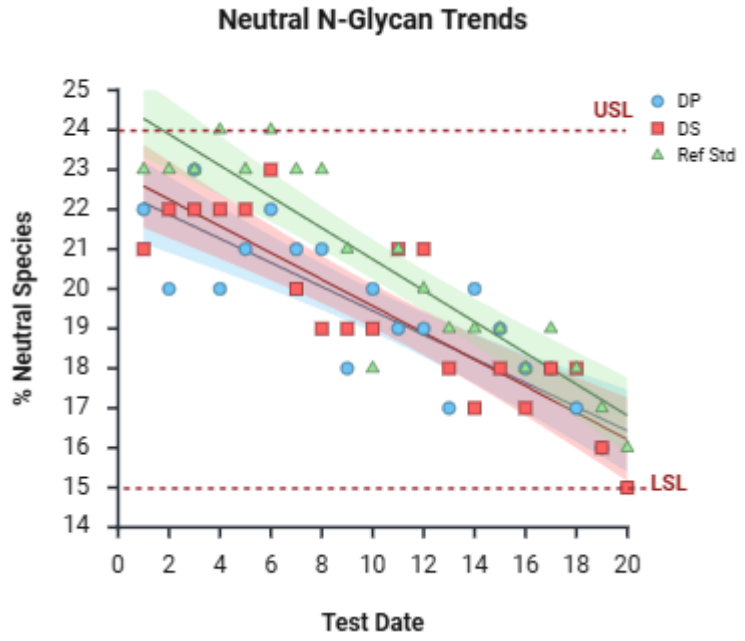
Historical Process Data Review

- Review of historical data for DS and DP and for stability indicate a similar shift in N-glycan species over time.
  - **Limited commercial scale data set (~ 5 lots)**
- Same PRS and WRS lots since licensure.
- No changes in process since licensure.
- No changes in potency or impurity profiles DS/DP release and stability or for PRS/WRS periodic re-qualification.

Conclusion: Shift in N-glycosylation is independent of inherent process variability.





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Analytical Method Performance Assessments

Method Principle

Preparative digestion with PNGase

Fluorescent labeling of released glycans

Separation of labeled glycans by charge and size

Experimental assessment of parameters known to impact analytical method performance was executed

Labeling reaction

- **Sample preparation**
- **Temperature**
- **Reagent ratios**
- **Capillary lots**

Conclusion: Subtle changes in incubation temperature, mixing and sample handling at labeling step were able to control variability associated with labeling of sialylated species, returning values for the qualified reference standard within expected sialylation limits.

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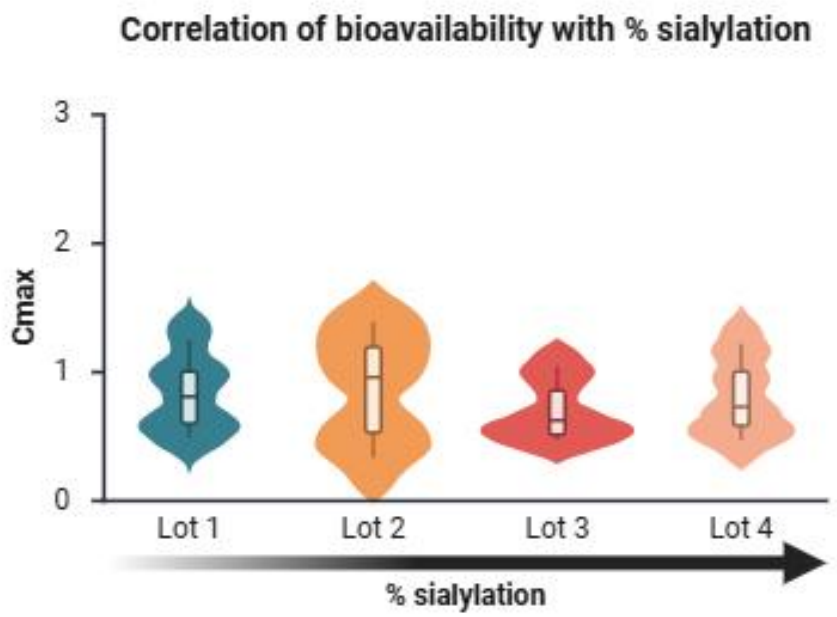
Assessment of clinical relevance



- Lots administered to subjects in Ph1-Ph2B trials.
- Available results reported for % sialylated species and potency were assessed against C_{max} reported for subjects administered these lots.
- Regardless of sialylation levels
  - No statistically significant differences in C_{max}
  - No changes in potency
  - No reported adverse events

Conclusion: No projected impact to efficacy or potency when % sialylation levels approach upper-level specification for % sialylation.

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Implemented Post-Approval Changes

Broadening of upper specification limit for % sialylation to represent clinical relevance and process consistency

Process data: Review historical data for PRS/WRS, DS and DP (release and stability)

Shift in N-glycosylation is independent of inherent process variability

No impact to other quality attributes (charge heterogeneity, potency, impurities)

Assess analytical method performance

Optimized parameters known to control variability

Assess clinical relevance of N-glycosylation state

No projected impact to efficacy or potency when % sialylation levels approach upper-level specification for % sialylation.

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Reference Standard Lifecycle Management: Why it matters?

Lifecycle management elements

⭐ **Two-Tier Reference Standard Program**
Supports consistency and traceability across development and manufacturing.

🔬 **Clinical Relevance Connection**
Ensures products maintain consistent therapeutic outcomes.

📊 **Trending Programs**
Monitors performance over time to detect shifts and maintain quality.

🧪 **Fit-for-Purpose Methods**
Tailors analytical methods to specific product and process needs.

⚙️ **Optimize and bridge methods**

🧬 **Robust Characterization**
Provides deep understanding of reference materials' properties.

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Thank You!

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