



# Establishing A Reference Standard For Therapeutic Monoclonal Antibody Products

- **A Regulator's Perspective** -

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# Disclaimer

The opinions of this presentation represent the speaker's experience.

The contents *do not necessarily reflect* FDA official policy.



# Topics du jour

- **Scope**
- **Considerations**
- **Development and Qualification**
- **Case Study**
- **Final remarks**



# Scope

**Characterization, stability, and qualification of reference standards (RS) used for the release and stability testing of products regulated by DMA, such as:**

- **Monoclonal antibodies for in vivo use**
- **Therapeutic Fc-fusion proteins**

# General Considerations

- **The use of a well-characterized reference material, as a comparator, is critical for the verification of lot-to-lot quality of monoclonal antibody products.**
- **Neither USP nor other pharmacopeia (e.g., Eu.Ph., JP, etc) have established reference standards to serve as comparators for monoclonal antibody products.**
- **Reference standards, for monoclonal antibody products, generally evolve throughout the product development and manufacturing phases.**



# **Reference Standards (RS) For Monoclonal Antibody Products Considerations**

**- The Regulator's Perspective -**



## **Types of reference standard material.**

- ***Primary* RS: Established from representative clinical material. Can be used as the sole RS or used to qualify secondary RS lots.**
- ***Secondary (working)* RS: A lot, representative of the clinical material, that has been qualified against the primary RS.**



# How is the reference standard used?

## Examples of RS use are:

- **Lot release testing**
- **Lot stability testing**
- **Comparability studies**
- **Qualification of new reference standard**
- **Assay system suitability requirements (as needed)**
- **Assay site transfer**



## Characterization of the RS.

- **The primary RS should be subject to an extended physicochemical and biological characterization.**

**RS characterization may include, but not be limited to, glycosylation profile, charge variants, and determination of the kinetic binding constants.**

- **Reference standard characterization may be revised as product manufacturing evolves, but the RS should be well-characterized by licensure (i.e., BLA).**



## **Documentation supporting the RS.**

- **A description of the RS manufacture (i.e., source) and characterization.**
- **The qualification, re-qualification, and stability protocols should detail the assays and their acceptance criteria.**
- **A description of the conditions under which the RS is stored.**



**Reference Standards (RS)**  
**For Monoclonal Antibody Products**  
**- Regulatory Considerations -**



## Regulatory Considerations

### ICH Q6B

"[.....] at the time of submission, the manufacturer should have established **appropriately characterized in-house reference materials** which will serve for biological and physicochemical testing of production lots [.....] to ensure lot-to-lot consistency."

### ICH Q7

"Where a primary reference standard is not available from an officially recognized source, **an in-house primary standard should be established. Appropriate testing** should be performed to establish fully the identity and purity of the primary reference standard. **The source of each primary reference standard should be documented**<sup>12</sup>"



## Regulatory Considerations (con't)

### ICH Q7

**"The suitability of each batch of secondary reference standard should be determined prior to first use by comparing against a primary reference standard.**

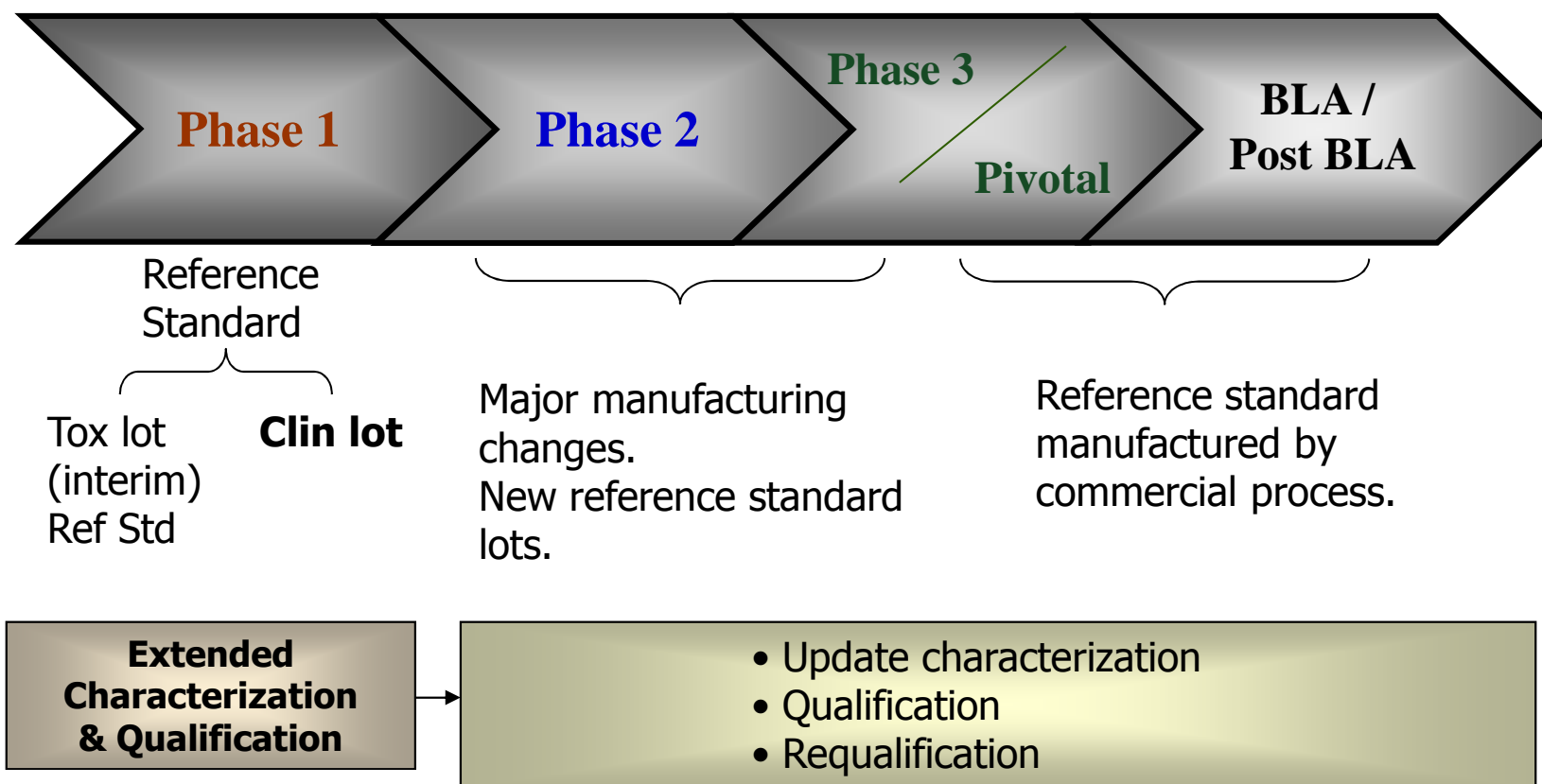
**Each batch of secondary reference standard should be periodically re-qualified in accordance with a written protocol."**



**Reference Standards (RS)**  
**For Monoclonal Antibody Products**  
**- Development & Qualification -**



# Reference Standard Evolution Through The Phases Of Product Development



# Examples of attributes to consider when characterizing the RS

## ➤ General characteristics

- Appearance ▪ visible particles ▪ pH ▪ strength ▪ polysorbate concentration (if applicable)

## ➤ Physical structure

- amino acid sequence ▪ protein structural levels ▪ molecular mass ▪ PTM

## ➤ Identity

- Peptide mapping ▪ binding-based assays ▪ immunogenic-based assays

## ➤ Biologic Activity

- Potency assay ▪ binding kinetics (i.e.,  $K_{off}$ ,  $K_{on}$ ,  $K_D$ )

## **Examples of attributes to consider when characterizing the RS (con't)**

- **Purity profile**
  - Intact protein (e.g., monomer %) ▪ aggregates ▪ fragments
- **Product variants**
  - Glycoforms (e.g., oligosaccharide, monosaccharide, & sialic acid content) ▪ charged variants
- **Process-derived impurities profile**
  - DNA ▪ protein A ▪ host cell protein
- **Stability**
  - Evaluated annually ▪ stored under long term and/or accelerated conditions ▪ tested against pre-set specifications



## RS Qualification Protocol

*As per ICH Q7 "Appropriate testing should be performed to establish fully the identity and purity of the primary reference standard."*

**The qualification protocol should include a description of:**

- **Test type (e.g., MALDI-ToF MS)**
- **Test purpose (e.g., oligosaccharide quantification)**
- **Test requirement (e.g., single vs. multiple RS aliquots to be tested)**
- **Test acceptance criteria (as per ICH Q6B)**

## **RS Requalification Protocol**

***As per ICH Q7 " [...] reference standard should be periodically re-qualified in accordance with a written protocol."***

**The requalification protocol should include information on:**

- **Time of re-qualification (e.g., annually)**
- **Test type**
- **Test purpose**
- **Test requirement**
- **Test acceptance criteria**
- **Data analysis strategy (e.g., real-time data trend analysis compared to historical data using linear regression)**



## RS Requalification

- e.g., Potency -

- **Document a plan for how the potency of the reference standard will be monitored over time. For example:**
  - **Comparison of RS re-test activity to historical data with acceptance criterion for acceptable range, or**
  - **Establishment of acceptance criteria for activity observed upon multiple replicate RS re-test results**
  
- **In order to manage product drift over time, the RS qualification potency assay's acceptance criteria should be narrower than that used for lot release.**



# **Reference Standards (RS)**

## **For Monoclonal Antibody Products**

### **- Case Studies -**



## **Case Study # 1: Characterization of Primary RS**

**A BLA submission, for a liquid product, described that the RS characterization was performed using the same methods described in the lot release program plus volume determination.**

### **Observations**

- **There were no other in-depth characterization of the product structure or PTM (e.g., glycoforms analysis).**
- **The Sponsor was asked to justify why no additional tests are required to characterize the primary (or new) reference standards.**

*The Sponsor indicated that a new reference standard was about to be introduced. A full characterization and comparability study was performed for the new RS.*

## Case Study # 2: Reference Standard Stability Program

**A BLA submission described the stability plan for the primary reference standard lot including the tests and acceptance criteria to be used.**

SDS-PAGE, Reduced (% IgG)  
SDS-PAGE, Non-reduced (% IgG)  
  
Gel permeation HPLC  
    Monomer (%)  
    Aggregates (%)  
  
Imaged capillary Isoelectric focusing  
    Main peak (%)

### Observation

- **The stability protocol is based on purity testing, but it did not evaluate other quality attributes, such as appearance, pH, and potency, that could drift with time even under long-term storage conditions.**
- **The stability plan does not indicate storage temperature or evaluation time points.**

***The Sponsor agreed to revise and update the stability protocol.***



## Case Study # 3: Reference Standard Requalification Protocol

A PAS described a modification in the biannual requalification protocol for a lyophilized product.

Requalification Plan		
	Current	Proposed
Attribute	Test	Test
Appearance	color/clarity/appearance	color/clarity/appearance
	particulate analysis	_____
Identity	peptide mapping	peptide mapping
	CZE	_____
Purity	CE-SDS	_____
	HIC	HIC
	IEC	IEC
	SEC	SEC
	Oligosaccharide content	_____
Potency	Bioassay	Bioassay
Quantity	UV detection	_____
Osmolality	Compendial	_____
pH	Compendial	Compendial
Safety	Container closure (dye leakage)	_____



## Case Study # 3: RS Requalification (con't)

### The amended requalification plan

- Kept the scheduled (every 2 years) performing time as approved at licensure.
- Eliminated testing for protein content and osmolality, but maintained pH testing.
- Eliminated visible particulates, but maintained test for color/clarity/appearance.
- Eliminated CZE as identity test, but maintained peptide mapping.
- Eliminated CE-SDS and oligosaccharide content as purity tests, but maintained HIC, IEC, and SEC.
- Eliminated container closure integrity (CCI) test.

### Observation

- Although most changes were justified based on the Sponsor's extended knowledge and were found to be acceptable, it was advised that the requalification plan maintain the particulate analysis and CCI test.

*The Sponsor agreed to maintain visible particulate and CCI tests.*



**Reference Standards (RS)**  
**For Monoclonal Antibody Products**  
**- Final Remarks -**



## Final Remarks

- **Therapeutic monoclonal antibody product development requires the use of a properly qualified *in-house* reference standard (RS).**
- **The RS should be representative of the current manufacturing process.**
- **The RS should be thoroughly characterized:**
  - **Physicochemical characteristics (e.g., protein structure, charge variants, glycoforms, etc)**
  - **Functional characteristics (e.g., binding kinetics, potency, immunochemical properties)**
    - ❖ **Quantitative assays' acceptance criteria in some cases should be narrower than release specifications, to manage drift over time.**
  - **Process-derived impurities**
  - **Stability**



## **Final Remarks (con't)**

- **The RS qualification and re-qualification should be performed per established protocols.**
- **The RS development, characterization, qualification, and requalification, should be appropriately documented.**
- **The RS should be used for product release and stability testing to ensure lot-to-lot consistency.**
- **It is expected that several RS lots will be created as product development evolves, but by the time of the BLA submission, the RS lot should be representative of the commercial process.**



# Acknowledgement

**Many thanks to**

The Division of Monoclonal Antibody  
(DMA) Regulatory Branch