



A Regulatory Perspective on the Use Reference Standards for Therapeutic Proteins

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IABS/FDA Reference Standards for Therapeutic Proteins: Their
Relevance, Development, Qualification and Replacement,
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Outline

- Background on Ref Stds
- Method Validation & System Suitability
- In Acceptance Criteria
 - Identity Assays
 - Purity and Impurities
 - Potency
- DS/DP Source Materials
- Qualification of a Biotech Ref Std
- Stability of Ref Stds



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Background



Relevance of a Reference Standard

- Why are they used?
 - Need to ensure that results of assays at different times and in different labs can be compared in a meaningful manner
 - Most results of quantitative analytical methods do not provide absolute values
 - Analytical methods provide relative values due to variability of the assay procedure, raw materials, equipment, personnel, etcetera
 - Accuracy/precision of methods will influence the need for a standard (e.g., protein content determination may not use a standard if based on extinction coefficient)



Roles of a Standard

- To calibrate an instrument (pH, SVP content, MS for MW determinations)
- For method validation
- To establish suitability of an assay result
 - Appropriate assay resolution
 - Appropriate sensitivity
 - Appropriate assay quantitation/ recovery
- To establish identity of the test sample
- To assess purity/ impurities in a direct comparison
- To calibrate relative potency

One standard will not be suitable for all intended uses and in fact many standards are used in testing samples



Basic Requirements for a Ref Std

- A reference standard “should have a quality appropriate to its use” and “is often evaluated for its intended purpose” (ICH Q6A)
- A Biotech Ref Std should be “suitable for its intended purpose although applicants do not always clearly describe how a reference standard is used in submissions
- This information can be inferred from the specifications but the details are typically available only in the SOP that describes the analytical method. Applicants are only required to **reference** SOPs in an application not to submit them unless requested.



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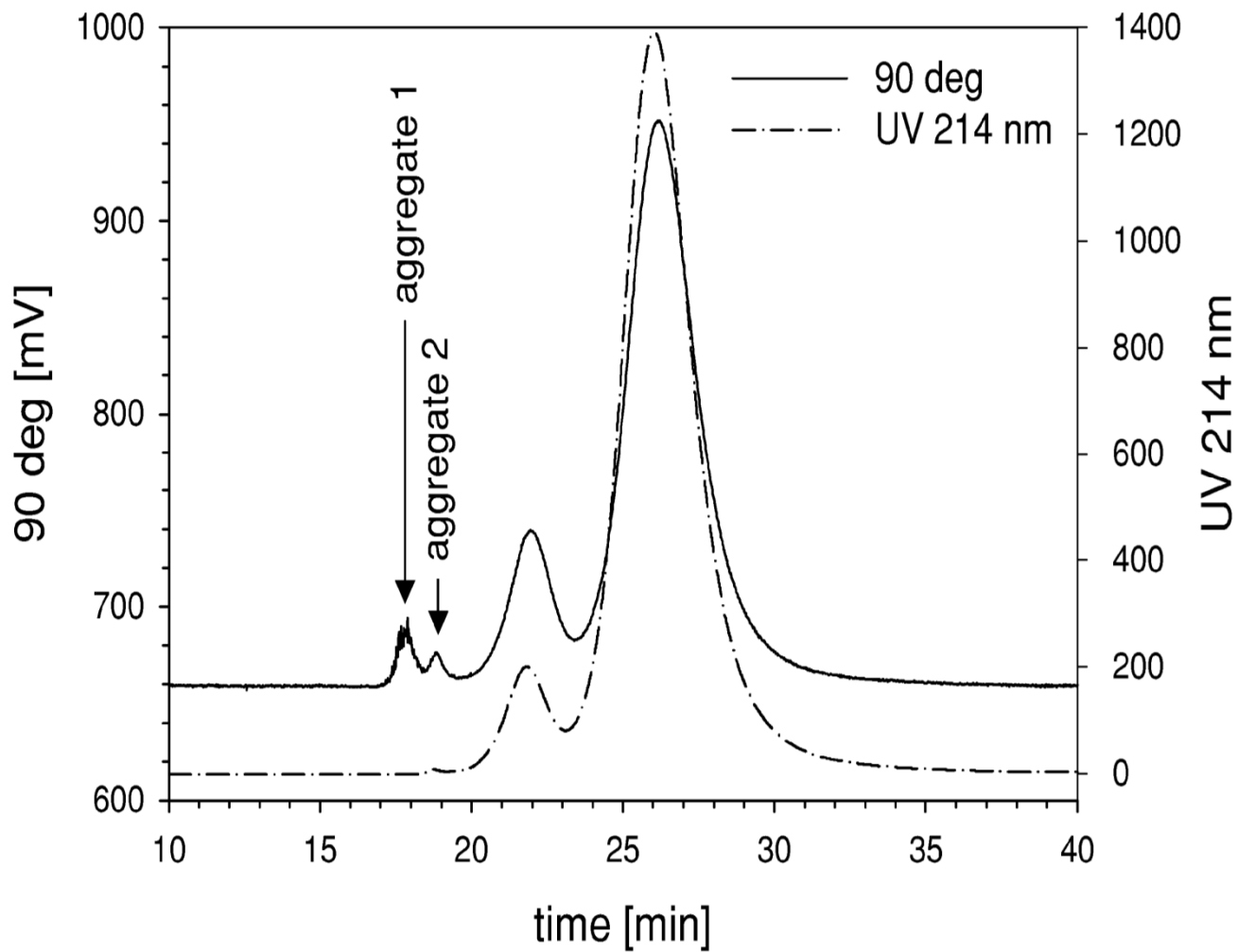
Method Validation & System Suitability

Method Validation

- FDA's guidances on method validation indicate that **“well characterized reference materials, with a documented purity, should be used throughout the validation study”** (ICH Q2B) but little additional detail is provided other than the following:
 - Ref Std are critical in validating specificity for an identity test but additional materials that are closely related and may be present are also recommended as negative controls (ICH Q2B)
 - Establishing accuracy of the analytical procedure by comparison to a reference material is recommended but **“accuracy may be inferred once precision, linearity and specificity are established”** (ICH Q2B)
- While a DS/DP Ref Std is useful in determining the validation characteristics of a method, production lots can be used for determining many, but not all, of the validation characteristics of an assay
- Other reference materials are necessary (i.e., impurity/purity “standards”)

System Suitability

- Verifies that the analytical method is performing as expected (i.e., expected resolution, reproducibility, sensitivity, recovery, etc) and therefore, that test results are meaningful
- Achieved through the use of a Ref Std
- USP <261> for chromatography defines some suitability tests
- For example for SEC, the Ref Std may be used to establish
 - Peak asymmetry
 - Theoretical plate
 - Retention times (resolution)
 - Recovery (e.g. total monomer peak)
 - Relative standard deviation
 - Consistency with historical profile of standard



From Ahrer et al., J of Chromatogr 1009 (2003)



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In Acceptance Criteria for Release

Identity

- Identity is established by comparison of a “*unique aspect*” of the reference product (i.e., drug substance/API) to that of a test article
- The test maybe qualitative or semi-qualitative in nature: expected N-terminal sequence; significant signal in Western blots or ELISA assays as compared to Ref Std; comparable to Ref Std Peptide map profile
- Where appropriate, a negative control Ref Std should be used as a system suitability standard. Materials with closely related structures may be used as this control but validation of specificity has been acceptable



“Purity/Impurity” Standards

- FDA recommends that the DS “Ref Std should be of high purity ... but “in some cases distinct reference materials for product-related substances, product-related impurities and process-related impurities, may be needed” (ICH Q6B)
- Typically, many standards are used for process related impurities where quantity in the test article is calibrated to the standard (e.g., insulin, anti foaming agents, antibiotics, DNA, host cell proteins, etc.)
- DS/DP Ref Std are used to assess product related variants (impurities or product related substances) and are used in establishing system suitability, and in the acceptance criteria used in the specifications
- Individual standards are not typically used to quantitate the actual amount of a specific product related variant. Instead “conforms to standard” (with objective limits), or “percent of total peak area” is used to define acceptable results. Percent relative to the standard is not typically used



Impurity Standards

- A reference material that produces a profile that is not representative of all expected product related variants (i.e., two bands in a PAGE profile or only monomer peak in SEC) may not reliably identify new degradents that could occur during manufacture or on stability
 - The use of impurity standards generated under appropriate stress conditions for assay validation, system suitability and or acceptance criteria, should be considered



Impurity Standards

- “If an appropriate impurity standard is unavailable, validation of analytical specificity may be demonstrated by use of another [orthogonal] method instead of a RS” (ICH Q2A)
- This occurs in situations where limitations in an analytical method raises doubts about the method’s ability to detect all relevant variants
- This validation should include “samples stored under relevant stress conditions light, heat humidity acid/base hydrolysis and oxidation” (ICH Q2A)
- Analysis should include “A comparison of results from the two assays” (ICH Q2A)
 - Examples; AUC to validate measurement of aggregate content, MFI to validate sub-visible particle content



Potency

- Potency standards are critical for determining the potency of a test article.
 - Used routinely for system suitability and
 - Often used routinely to calibrate the activity of the test article, but not always
- Results should be reported relative to the potency standard (e.g., percent of reference or relative units) unless justified
- The confidence in the accuracy of the established “conventional true value” of the potency standard should be within a well defined, appropriate range and supported by a statistical analysis. Potency testing according to the specification is unlikely to be sufficient for determining potency of the Ref Std with appropriate precision.



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Sources of DS/DP Reference Material



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

- “Applicants should established an appropriately characterized in-house **primary reference material**, prepared from lot(s) **representative of production and clinical materials**”
- “In house working reference material(s) used in the testing of production lots should be calibrated against this primary reference material”
- “Where an international or national standard is available and appropriate, reference materials should be calibrated against it”



Source Materials (In practice)

- Official potency standards, if available are typically used to calibrate potency of the primary standard but not always
 - Assigning potency values when replacing a standard by obtaining values from independent laboratories using their own method and then averaging the results has limited utility
- Manufacturers frequently do not establish a primary and secondary standard for physico-chemical properties or for potency when official standards are not available.
 - Current USP draft potency monograph indicates that the primary standard is simply ***the first*** standard used
- What is the purpose of a two-tiered system?
 - Lack of a two tiered system may lead to frequent qualification of new standards against the previous standard. A frequent change in the primary reference standard potentially introduces risk of product drift



1-Tiered versus 2-Tiered

A = primary reference standard representative of the clinical trial material; B through F - new primary standards replaced every 3 years (estimated from two submissions)

2-Tiered: A = B, A = C, A = D, A = E, A = F

1-Tiered: A = B = C = D = E = F but is A = F 15 years later?

- Which approach provides better assurance that over time the product continues to reflect the clinical trial material? Answer.
 - Product A is representative of the clinical trial material, if it is stored under conditions that prevent any decrease in product quality.
 - Error may be cumulative for 1-tiered approach and could allow for a drift in product characteristics over time
- Creation of new primary reference standard should be carefully evaluated and not performed every time a change in the manufacturing process alters the product's characteristics
- Improvements in product quality may support a change in the Ref Std



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Qualification of a Biotech Ref Std



FDA Guidance on Qualification of a Biotech Ref Std

- “An appropriately characterized in-house primary reference material, representative of **production and clinical materials**” (ICH Q6B)
- “Reference materials for biological products should be representative of the manufacturing process and should be as fully characterized as practical including:
 - physicochemical characteristics, structural characteristics, biological activity, and/or immunochemical activity”¹
- No specific recommendations are made on replacing a standard

¹Draft Guidance Analytical Procedures and Methods Validation (August 2000)



Qualification of the Primary Ref Std

(in practice)

- Ref Std frequently prepared from the commercial scale production process following scale up of the phase 3 process & may be pooled from multiple lots. Testing is typically robust. For example, testing one glycoprotein std included:
- Specification testing including, but not limited to
 - Activity/aggregation by SEC/ Identity/ Peptide Mapping/ RPHLC/ protein content/ IEF/Appearance/Endotoxin/ Sialic Acid content/ Oligosaccharide mapping/pH/SDS-PAGE
- Additional characterization including, but not limited to
 - Free thiol/ amino terminal sequencing/CD/MW by MALD-TOF MS/ Western blot/ receptor binding/ additional activity assays/process related impurities/AUC/ peptide mapping, LC/MS/monosaccharide compositional analysis/western blot analysis

Acceptance Criteria for the Primary Ref Std (in practice)

- The Ref Std is qualified if it has the product quality attributes that are representative of the clinical trial and production lots i.e., meets all specifications and is “**comparable**” (“**representative**” would be a better criterion) to the clinical trial material/commercial production lots
- Sometimes “qualified” if the standard used is “comparable to the Ref Std used in the clinical trial”
- Original applications include a protocol describing how future reference material lots will be evaluated. Of late, we have frequently requested withdrawal of these protocols.



Issues with Ref Std Qualification Protocols

- Typically, no information is provided in the protocol indicating how the reference standard is used or how the protocol ensures the method is suitable for its intended purpose(s)
 - It is impossible to perform a thorough evaluation of the qualification protocol if you don't know how the Ref Std is used
- The primary reference standard is typically qualified by drug substance specification testing, including the same sampling plan and acceptance criteria established in the specification. That is, any lot meeting specs can be used as a reference standard.
- Other applicants qualify the Ref Std by demonstrating that the new standard is **comparable** to the currently approved reference material. For example, if the absolute difference in attributes is no greater than a specified multiple of the production variability, typically ± 3 SDs.

Issues with Ref Std Qualification Protocols

- Using the same number of test samples as described in the specifications may not provide a high degree of confidence that the value obtained is an accurate measure of the true value
- Using acceptance criteria established in the specifications would allow for product characteristics in the new reference standard that are out of trend with the desired or expected attributes (Tightening to mean \pm X SDs does not help). DTP generally considers this insufficient.
- Is comparability the appropriate criteria when assay results are expressed relative to a reference standard?



Issues with Ref Std Qualification Protocols

- Additional physicochemical and biological testing is usually performed but without any acceptance criteria associated with these characterization tests
 - The utility of additional product characterization without defining acceptable results for these tests is of little value – why perform these tests if any result is acceptable?

My conclusion:

These approaches do not appear to provide assurance that critical product attributes will not drift over time due to the changing characteristics of the Ref Std and is particularly worrisome when acceptable results for a release test are expressed relative to a reference material



Potential Solutions

- A two-tiered Ref Std system should be employed to minimize repeated replacement of a primary standard
- Protocols should describe the intended purpose of the reference standard and how it is used in each assay
- The acceptance criteria should ensure that the expected product characteristics do not drift over time
- When results are expressed relative to the Ref Std:
 - Product attributes of the new standard should be qualitatively & quantitatively “equivalent” to the previous standard with a high degree of confidence
 - While the actual value of a new standard may differ from the old standard, this difference must be accounted for when normalizing results



Potential Solutions (cont.)

- The desired range of the true value of a reference material must be defined with a high degree of confidence as established by a statistical analysis
- For less precise assays it may be necessary to test many samples (30 has been proposed by FDA statisticians) of both the current and proposed standard to ensure a precise value is obtained
- Comparable may be an acceptable outcome for results of additional characterization of the Ref Std, if the attribute is not critical to its intended purpose

Recommendations on Ref Std Stability

- “Documentation of the storage conditions and formulation supportive of reference material(s) stability should also be provided” (ICH Q6B)

But:

- Ref Std should not be stored under conditions/times that are permissive for degradation. Long time storage at $\leq 70^{\circ}\text{C}$ and shorter term storage at higher temperatures during “in use” has been sufficient for most products
- Robust stability data (multiple samples to ensure adequate precision of the results) should support the storage conditions and established use interval of the working standards
- Analysis of historical results obtained using the Ref Std should be continuously monitored to ensure consistency of the data regarding the quality characteristics of the primary and working standards and its acceptability for use



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