An Executive Summary

USP General Chapters and Reference Standards that Support the Development and Characterization of Biologics

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USP documentary and physical standards provide procedures and best practices for testing of biologics.

Introduction

The United States Pharmacopeia (USP) works with global biologics and biotechnology Expert Committees and Expert Panels to develop documentary standards (monographs and general chapters) and Reference Standards that support biopharmaceutical quality assessment. A scientific nonprofit organization, USP is not part of the government although its standards are enforceable by the US Food and Drug Administration and other government agencies. Today, USP has over 400 experts from industry, academia and other stakeholder groups who volunteer their time to develop USP standards; development of the standards also includes a 90-day public comment process after publication in USP's *Pharmacopeial Forum* (*PF*) for each new and revised standard. Approximately 75% of USP experts are in the US, but a growing number are from other countries. USP's Expert Committees and Panels also include representatives from the government, in particular the FDA. Established every five years, USP's Expert Committees and Panels for biologics cover a broad range of therapeutic products from peptides to larger recombinant proteins, to complex biologicals (e.g., blood derived proteins, vaccines, or cell therapy products). USP's General Chapter Biological Analysis Expert Committee develops chapters and associated Reference Standards that support the characterization of multiple types of biologics. What follows is a discussion of USP's latest chapters and Reference Standards to help biopharmaceutical companies more efficiently characterize biologics by adopting USP's previously validated documentary and reference standards.

Standards and Initiatives for Biologics

USP's long-term investment in biologics led to the development of a broad set of standards that include monographs, general chapters, and Reference Standards.

USP monographs are specifications for pharmaceutical articles in commerce (from release through product shelf life). Such monographs contain tests, assays, and acceptance criteria needed to demonstrate that the article meets required quality standards for identification, purity, and potency, and is labeled accordingly. Monographs bolster the safety net of products that are circulating worldwide by providing a public standard developed with input from industry, regulators and other stakeholders. Although they are based on US-approved specifications, USP monographs have been adopted by many countries worldwide.

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USP general chapters are critical because they are often cited in monographs. Chapters support monographs by centralizing methods and procedures that are commonly used. USP ensures that chapter methods are current and are reflective of the most up-to-date methods and common technologies used in industry. As chapters are modernized, several monographs are updated simultaneously in that chapter revision. Users should always be sure they are using the most up-to-date official chapter.

General chapters are numbered intentionally with the following number convention: test chapters, which are numbered below 1,000, contain validated methods that users can verify as suitable for their own particular purposes. Chapter methods are not required for a particular product until a monograph exists that cites it. If a monograph does not exist for a particular product and a user adopts a chapter method and cites it in their internal standard operating procedure, then it is critical that the currently official chapter is followed. This type of chapter is very helpful to individuals who want to get a jumpstart on method development and validation. Informational chapters that are numbered higher than 1,000 are for general knowledge purposes and contain best practices for a particular topic.

USP's Reference Standards are critical for assessing whether many of the methods work. They provide traceable standards to demonstrate the acceptability of procedures. Reference Standards are often used for system suitability (e.g., to ensure that the method is performing as expected before taking sample measurements and moving forward on the basis of those results). In other cases, Reference Standards are for a particular active pharmaceutical ingredient or impurity and may also be used for quantitative purposes or, for example, to identify a particular impurity by its retention time in a chromatographic procedure.

Recent Major Initiatives for Biologics

USP recently completed some major initiatives for biologics, including publishing several new general chapters.

Chapter <509>: Residual DNA Testing

The proposed Chapter <509>, which was published in *PF42(5),* contains a method for measuring residual host cell DNA in recombinant therapeutic products that are produced in either *Escherichia coli* (*E. coli*) or Chinese hamster ovary (CHO) cell lines. More cell substrates may be added in future revisions.

The chapter also contains a validated sample extraction procedure that is used before quantitative polymerase chain reaction (qPCR) detection. This procedure includes a Proteinase K digestion step combined with chaotropic salt (sodium iodine) extraction and isopropanol precipitation, though other sample extraction methods can also be used and combined with qPCR.

Highlights of the extraction method include:

- Specification of **primer and probe sequences** without locking users into a specific primer or probe;
- Inclusion of **cycling conditions**;
- Built-in flexibility about which **fluorescent dyes** and **instrumentation** can be used;
- **Negative controls** run in singlet (at least) and **positive extracted controls** run in triplicate

Draft system suitability requirements include:

- **Negative control solution:** The cycle threshold (C_t) corresponding to the *negative control solution*, if any, is NLT C_t of the lowest concentration *standard solution*.
- **Sensitivity:** The C_t corresponding to the lowest concentration of the *standard solution* is NMT 39.
- **Linearity:** The regression coefficient associated with the *standard solution* is NLT 0.98. The slope is between –3.1 and –3.8.

Proposed acceptance criteria include:

- Measurable samples must fall within **the standard curve**.
- **Accuracy:** The mean recovery of three replicates of positive control solution is between 50% and 150%. [Note: Correct for sample dilution, if needed.]
- **Relative standard deviation:** NMT 30% for three replicates of sample solution and NMT 30% for three replicates of positive control solution.

Limits for residual DNA will be defined in future product monographs, as opposed to in this chapter.

Chapter <1132>: Residual Host Cell Protein Measurement in Biopharmaceuticals

USP General Chapter <1132> focuses on best practices for the development and characterization of critical host cell protein (HCP) reagents, as well as on the development and validation of an HCP immunoassay method. Sandwich immunoassays with polyclonal antibodies are often the preferred HCP measurement method because they are very sensitive, quantitative, easy to run and automate, and low cost.

Chapter <1132> also discusses orthogonal techniques—such as electrophoresis, Western blots, mass spectrometry, and chromatographic and proteomic approaches—to determine if the immunoassay reagents and method are suitable for residual HCP detection, identification, and measurement.

Chapter <1132> also provides tips for demonstrating that the assay is measuring most of the HCPs that might be present in a sample.

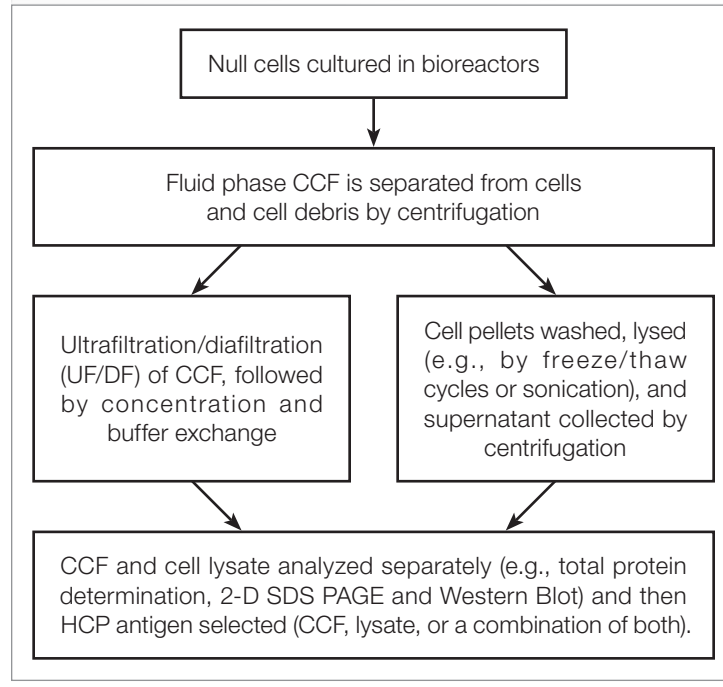
For example, a Western blot approach is usually taken to determine if the polyclonal antisera recognizes the majority of HCPs that may be present in a sample. However, Chapter <1132> also discusses an affinity column approach because this is a rapidly evolving area and is also suitable. Both

approaches are being used in the characterization of reagents.

The chapter also encourages users to consider preparing bulk quantities of the immunogen and assay standard so they last the product's lifetime because critical reagents developed later may differ in coverage when compared to the original reagents. Laboratories struggle with the best approach to prepare the HCP antigen/immunogen so the chapter provides best practices and a figure illustrating a common approach (see **Figure 1**, which is also in *USP-NF*)

Other issues discussed in the chapter include the standardization of reportable units (e.g., the use of ng of HCP/mg of product versus ng/ml) and options for what to do when a few or a single HCP co-purifies with the product and samples do not dilute linearly.

Figure 1: Example of Mammalian HCP Ag Preparation



Chapter <129>: Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies

Chapter <129> contains validated compendial procedures with established system suitability criteria for therapeutic monoclonal antibodies. Methods described include:

- Size-exclusion chromatography, which looks at higher and lower molecular weight species than the monoclonal antibody itself;
- Capillary SDS electrophoresis (under reducing and non-reducing conditions); and
- Oligosaccharide and sialic acid analysis.

Chapter <129> also contains an associated USP Monoclonal IgG System Suitability Reference Standard that is used to determine if the method's results are suitable before evaluating the sample results.

Chapter <129> does not contain product- or class-specific acceptance criteria, and thus cannot replace a monograph or dictate requirements for an individual monoclonal antibody. Quality attributes of monoclonal antibodies that are highly product specific would be addressed at the monograph level.

Chapter <507>: Protein Determination Procedures

Chapter <507> offers six validated methods with system suitability criteria and one Reference Standard.

- Method IA and IB—Ultraviolet Light Absorbance (280 nm) under denaturing or native conditions;
- Method II—Bicinchoninic Acid (BCA);
- Method III—Bradford Method;
- Method IV—Lowry Method;
- Method V—Amino Acid Analysis; and
- USP BSA for Protein Quantitation RS.

These core methods include ultraviolet as well as colorimetric methods, which are used to measure proteins. The chapter recognizes that amino acid analysis is also being used for total protein measurements.

The new Reference Standard might also be appropriate for system suitability purposes.

Summary

In collaboration with its Expert Committee and Panel members, USP has a long history of providing public standards that support the development, characterization, and release of therapeutics. USP believes that public standards for biologics are very important in mitigating quality risk, accelerating product development, and fostering the availability of quality medicines in support of global public health. New standards support both existing therapeutics as well as those in development. USP test chapters, which contain validated methods and associated Reference Standards, can be particularly helpful to test for and control quality at any stage in the product life cycle. Test chapters facilitate method adoption following verification that it is suitable for a new application.