An Overview of the United States Pharmacopeia's Recent Activities in the Areas of Vaccines, Virology, and Biological Standardization

By TINA S. MORRIS

The United States Pharmacopeia (USP) is a not-for-profit nongovernmental organization founded in 1820 that develops public standards for drug substances and products; these standards are enforceable by FDA and have been adopted by many nations around the world. USP General Chapters provide industrial and academic researchers alike with crucial guidance, particularly in areas where there is a regulatory void.[1] A good recent example is the proposed USP general information chapter <1043> on *Ancillary Materials for Cell, Gene, and Tissue-Engineered Products*, which has been official since April 1, 2005, in *USP 28–NF 23 (First Supplement)*. Rapid advances in vaccine research and manufacturing continue to challenge the regulatory environment and necessitate the availability of expert guidance in step with state-of-the-art technology. In an effort to meet these challenges, USP's Council of Experts Committee for Vaccines, Virology, and Immunology has recently initiated two new General Chapter initiatives that will provide information and guidance for researchers and manufacturers in the vaccine and virology fields and beyond. Chapter <1235> *Vaccines and Vaccine Test Methods* will focus on the analytical requirements for the different types of vaccines currently in manufacture and development. Chapter <1237> *Virology Test Methods* will discuss modern diagnostic virology techniques as applicable to vaccine and biologics manufacturing. The development and scope of <1235> are discussed in a *Stimuli* article that will appear for public comment in issue 31(5) of *Pharmacopeial Forum*, USP's journal of standards development and compendial review. These efforts tie in with other important General Chapter activities such as the current revision of General Chapter <111> *Design and Analysis of Biological Assays*, as well as a new general information chapter <1033> *Validation of Biological Assays*, which are led by ad hoc advisory panels and working groups. This article reviews these and related USP activities in the areas of vaccines, virology, and biological standardization.

Introduction

The USP was founded by pharmacists and physicians to standardize pharmaceutical formulations. Since its inception, USP's mission has been the promotion of public health by disseminating authoritative standards and information developed by its volunteers for medicines, other healthcare technologies, and related practices used to maintain and improve health and to promote optimal healthcare delivery.[2] The Federal Food, Drug, and Cosmetic Act of 1938 indicated that *USP* and *National Formulary* (NF) quality standards for pharmaceuticals are enforceable by FDA.

The USP standards-setting process for pharmaceuticals, biologics, and biotechnology products relies heavily on volunteers who populate the forty expert committees of the Council of Experts responsible for the content of USP-NF. The process also relies on the input of USP staff and the cooperation of manufacturers, regulators, and other interested parties. USP volunteers are experts in their given field and come from academia, industry, and government agencies. These volunteers are directly involved in setting USP policies and standards.

Overall, the USP standards-setting process (Fig. 1) can be summarized as follows:

1. Industry or other interested parties work with USP scientific liaisons to draft a proposed new monograph or General Chapter.

2. The responsible USP expert committee (EC), working with

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the scientific liaisons, reviews the material for monographs or General Chapters, comments, and approves for publication in USP's *Pharmacopeial Forum (PF)* as proposals.

3. The USP staff scientific liaison reviews and edits these proposals.

4. The monograph or General Chapter is published in *PF* for public review.

5. Public comments are received and processed by the liaison; the scientific liaisons decides if republication in *PF* is necessary for monographs and forwards comments received on draft General Chapters to the responsible EC for consideration.

6. The EC members review comments and submit their response(s) to the liaison.

7. The liaison analyzes comments and the EC's response. If significant revisions to the proposal are needed, the revised proposal is republished in *PF* for public review. If no further revisions are needed, the proposal is submitted to the appropriate USP governing body for balloting, and, if approved, the monograph or General Chapter becomes official within *USP–NF*.

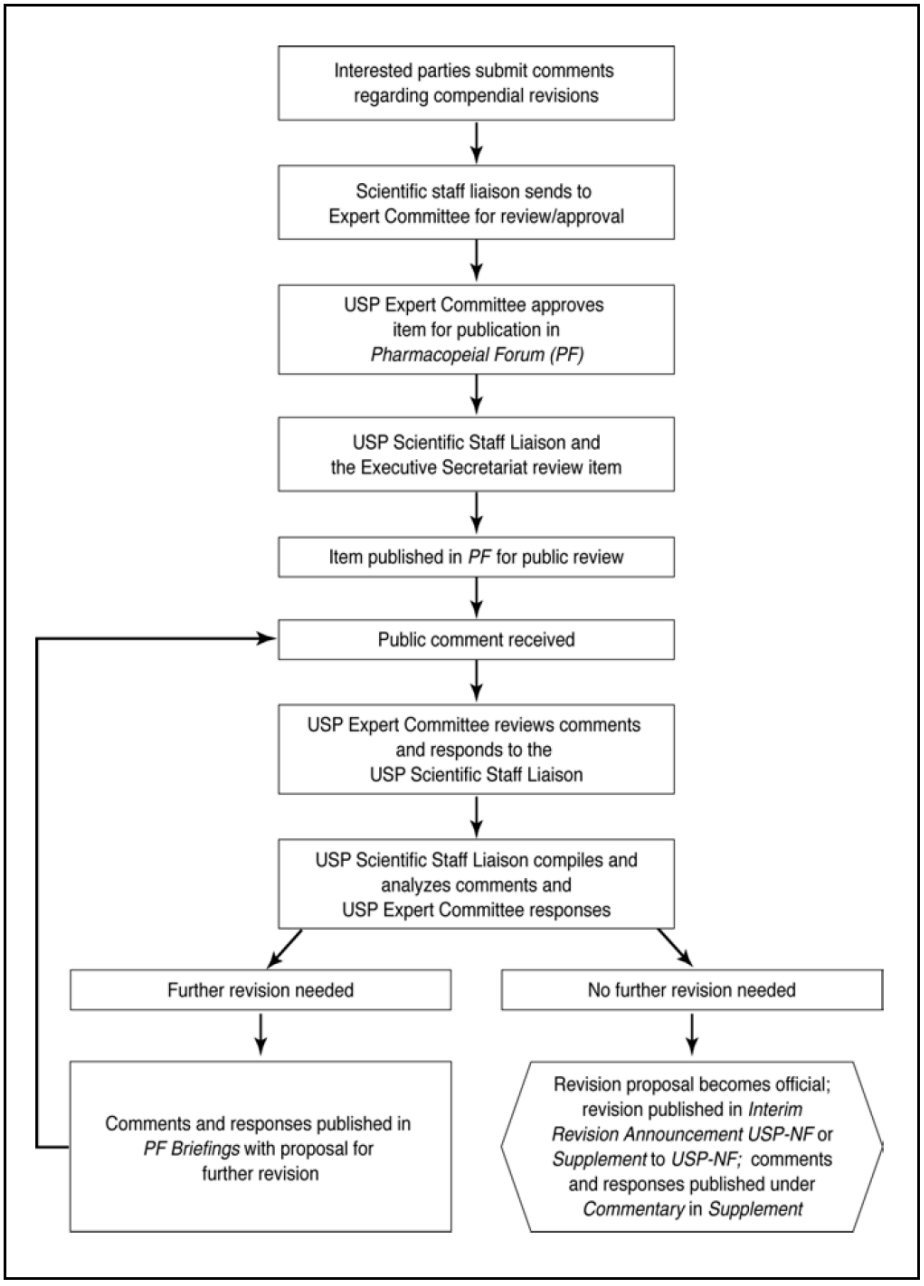

Figure 1. Public review and comment process for standards development.

USP Involvement in Biologics and Biotechnology

USP is becoming increasingly active in developing standards and information for the biologics/biotechnology industry. These activities are spearheaded primarily by four Expert Committees that comprise the Complex Actives Division of USP:

1. Biologics and Biotechnology: Proteins and Polysaccharides (BBPP)

2. Biologics and Biotechnology: Blood and Blood Products (BBBBP)

3. Biologics and Biotechnology: Gene Therapy, Cell Therapy, and Tissue Engineering (BBCGT)

4. Biologics and Biotechnology: Vaccines and Virology (BBVV)

Since their inception, these expert committees have been very active in their disciplines. On several occasions the committees have provided general test and information chapters that not only represent state-of-the-art technologies and methods but also helped fill regulatory gaps in these areas. A recent example is general information chapter <1043> *Ancillary Materials for Cell, Gene, and Tissue-Engineered Products* (official in *USP 28–NF 23, 1st Supplement*) authored by the BBCGT committee, which provides very important information for the use of many materials of biological origin such as fetal bovine serum and others currently used in tissue culture. This general information chapter divides ancillary materials into various categories based on the potential risks to both the manufacturing process and patients, and recommends a qualification program for

each category. Also presented are recommendations concerning the assessment of the level of ancillary materials remaining in finished product and their removal. In order to provide additional guidance about ancillary material qualification, the responsible EC has initiated a pilot program to develop monographs for ancillary materials. Each monograph in this program contains a minimal set of chemical, biochemical, and biological attributes for specific ancillary materials. As an aid to the analyst, these monographs will specify the use of appropriate reference standards to help demonstrate monograph compliance. As part of a pilot program, monographs for fetal bovine serum, interleukin-4, transferrin, trypsin, and protein A, with associated official USP Reference Standards, are being developed.

Vaccines and Virology

The Expert Committee on Vaccines and Virology (BBVV) develops new and revises existing vaccine monographs, vaccine excipient monographs, and vaccine general test and information chapters.[3] The committee also interacts with other expert committees on cross-cutting issues. Vaccines in *USP–NF* traditionally have referenced 21 *CFR* 600–680 and resulted in the so-called "short monograph" that, in addition to a product description, also included the following items: Packaging and Storage, Expiration Date, and Labeling. In 1997, FDA decided to remove from *CFR* items related to specific vaccines and to replace them with private licensing agreements. As a result, and to fill the gap, USP decided to initiate the development of vaccine monographs containing public standards. Approximately 16 of the abbreviated monographs are currently listed in *USP*. The BBVV committee has begun working on updating all these monographs to full length. New monographs developed by the committee are full length as well.

Two new monographs have recently been completed: *Anthrax Vaccine Adsorbed* became official with *USP 27, First Supplement. Anthrax Vaccine Adsorbed* is defined as a sterile, milky-white suspension made from cell-free filtrates of microaerophilic cultures of an avirulent, nonencapsulated strain of *Bacillus anthracis*. The following tests for the vaccine are specified in the monograph: *Identification*, 83-kDa antigen content, total protein, aluminum content, safety, sterility, pH, NaCl content, formaldehyde content, benzethonium Cl content, and relative potency.

BCG Live became official in *USP 28*. The vaccine is not a traditional vaccine in the sense that it is used for cancer therapy. *BCG Live* is a freeze-dried preparation of attenuated live bacteria derived from a culture of bacillus Calmette–Guérin (*Mycobacterium bovis*, var. BCG) and is used intravesically in the treatment of carcinoma *in situ* and papilloma tumors of the urinary bladder. Tests for the vaccine specified in the monograph are *Identification*, virulent mycobacteria, safety, sterility, skin reactivity, tuberculin sensitivity, residual moisture, viability, and potency. Vaccine monographs currently under development by the Expert Committee include Hepatitis A Adsorbed, Hepatitis B recombinant, and Hepatitis A and Hepatitis B combination vaccine.

The BBVV committee also has authored a guideline for submitting new vaccine monographs and revision requests to USP.[4] The guideline applies to new vaccines, as well as those that already have a "short monograph" in *USP–NF*. It is designed to assist industry and other organizations in developing monographs for their new vaccines, including those produced by biotechnological processes. It is useful at the development stage to ensure that analytical procedures for purity, quality, and potency are developed and validated as an integral part of vaccine manufacture. Although the number of manufacturers is limited, vaccines usually are marketed globally. This makes it advantageous to have common international standards for each product. As a means of working toward uniform global standards, USP strongly encourages all vaccine manufacturers to submit Requests for Revision for vaccine monographs as firms develop their products. The European Pharmacopoeia recently has published several vaccine monographs. Specifications in these monographs do not always conform to those contained in the biological license approved by FDA.

Although USP is a national pharmacopeia, its standards are recognized in numerous countries. Monographs in USP may help U.S. manufacturers minimize international regulatory burdens during global distribution of their products. Section 502(g) of the Federal Food, Drug, and Cosmetic Act of 1938 specifies that a drug is adulterated if it is recognized in *USP–NF* and does not adhere to the monograph standards therein (or so state on its labeling). Thus, establishing a USP monograph for a vaccine and labeling vaccines as "USP" help ensure the quality of products both domestically and in international commerce and allow end-product testing to a single public standard of quality.[2]

A major focus of current committee activity is the generation of two general information chapters, chapter <1235> *Vaccines and Vaccine Test Methods* and chapter <1237> *Virology Test Methods*. A Stimuli article designed as a preview of chapter <1235> will appear in *PF* 31(5). The chapter will contain general information about vaccines, their history, development, and production. Different types of vaccines, both traditional and novel, will be described. The chapter also will contain an extensive and detailed test section that describes both general vaccine test methods as well as specific tests that are unique to the different types of vaccines currently licensed or under development.

An outline for chapter <1237> has been developed, and a first draft for publication in *PF* is planned for early 2006. The chapter will cover different aspects of viral testing in modern laboratories. Although this is primarily a topic for the BBVV committee, many aspects of the chapter will affect other committees as well, for example, the discussion of viral clearance testing, which affects all biotechnology-derived products made in mammalian cell lines. Viral testing also is of crucial importance to the blood and blood products area — as it is for the gene and cell therapy field that uses viral vectors — therefore, input will be sought from these experts as well. For both chapters currently under development, the committee is very keen to

receive early and involved discussion from the public, which will allow the committee to provide the best and most useful information to end users in the scientific community.

In addition to and in connection with these chapters, the BBVV committee has recently initiated chapter <1125> *Nucleic Acid-Based Techniques*, which is being addressed by an ad hoc advisory panel of industry, government, and academic experts from the area of nucleic acid testing. A chapter outline has been finalized, and a first draft of the chapter is targeted for completion by the end of 2005. The chapter will be comprehensive and method-driven, with a strong focus on nucleic acid amplification techniques. It is the goal of the expert panel to tie this chapter closely to <1235> and <1237>, but it also links to other relevant, cross-functional chapters discussed later in this article. In line with these efforts, the committee plans to develop physical standards to accompany <1125> as part of USP's overall effort to develop procedural reference standards for the analysis of biologics and biotechnology-derived articles.

In addition to the chapters described above, the BBVV committee is planning to initiate a general information chapter on immunological methods. This will also involve members from other committees and additional experts in the field.

Cross-Cutting Activities and Future Directions

Development, manufacturing, and testing technologies, especially in the area of biologics and biotechnology, are rapidly advancing, and many of the new technologies affect all areas of the field. To ensure that guidance information in *USP-NF* is current and meaningful to the entire industry, the Complex Actives Division Expert Committees collaborate very closely on General Chapters and general information chapters that are considered cross-cutting. A good example is <1047> *Biotechnology-derived Articles – Tests*, a chapter that has recently been successfully harmonized with the Japanese and the European Pharmacopeias. USP is currently amending this chapter to include USP procedural reference standards (PRS) that will allow the users to confirm system suitability for the different procedures. For example, the section of <1047> that covers amino acid analysis describes ten procedures for protein hydrolysis, eight amino acid analysis techniques, and a subsection on data calculations. A subsection also describes the importance and use of suitable PRS. These PRS include the twenty-plus amino acids that are commonly found in proteins and are used in the calibration and system suitability determination of the chromatographic instrumentation. PRS (typically norleucine) also are used as internal standards to correct for differences in sample application and changes in reagent stability and flow rates. Furthermore, a PRS in the form of a highly purified, relatively inexpensive protein with a well-characterized amino acid composition, such as bovine serum albumin (BSA), can be used to demonstrate the integrity of the entire procedure, train new analysts, evaluate new equipment, and certify new laboratories.

USP has successfully developed and is now distributing Reference Standards for the 20 most common amino acids that comprise proteins. Each amino acid is available as a distinct molecular entity reference standard. USP is currently evaluating candidates for hydroxyproline and norleucine as PRS to be used in amino acid analysis. USP will develop and test mixtures of the 20-plus amino acids for use in instrumental calibration and system suitability assessments. This testing will involve determining each mixture's suitability for use in all the amino acid analysis techniques specified in <1047> and the mixture's stability profiles. Furthermore, the development of a BSA PRS to support amino acid analysis methodologies is being explored. Evaluation of BSA PRS candidates also would include testing the material for use in other procedures. These could include use as a surrogate to calibrate total protein assays, as a molecular weight marker in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) procedures, as a tool to demonstrate the integrity of partial protease digestion during sample preparation for peptide mapping, and as a means to demonstrate system suitability solution of peptide mapping chromatographic instrumentation. In addition to these standards, USP is considering the development of other standards to support this General Chapter, including molecular weight and isoelectric focusing markers to calibrate SDS-PAGE and isoelectric focusing gels, respectively.

Other important current cross-cutting efforts are the revision of General Chapter <111> *Design and Analysis of Biological Assays*, as well as the preparation of two new chapters: <1084> *Glycoprotein and Glycan Analysis* and <1033> *Biological Assay Validation*.

Chapter <1084> is another general information chapter that will be supported by procedural reference standards and is being written with this concept in mind. The chapter is analogous and complementary to chapter <1047> *Biotechnology-derived Articles – Tests*. This new chapter will give the user standard protocols that cover all aspects of glycoprotein and glycan analysis.

Work on cutting-edge technology issues in all areas of biologics and biotechnology will be further enhanced and expedited once the new USP biologics and biotechnology laboratory becomes fully functional. The laboratory will be part of the new USP headquarters, slated for completion in 2007. The laboratory will contain molecular biology, microbiology, cell biology, and analytical biochemistry sections equipped with state-of-the art equipment, exemplified by separate PCR and flow-cytometry areas. The laboratory also will enhance USP's capabilities for engaging in international collaborative studies for establishing international biological reference materials. USP is already well recognized for its international leadership and participation in organizations such as the WHO Expert Committee on Biological Standardization (ECBS); the Pharmacopeial Discussion Group (PDG) that works on the harmonization of pharmacopeial general chapters and excipient monographs; and for its observer status at the Veterinary International Conference on Harmonization (VICH).

Conclusion

USP is making interesting and exciting progress in standardizing biologics and biotechnological therapeutic products, and the analytical methodologies used to characterize them. Interested parties are strongly encouraged to review *PF* and submit comments on any new item proposed. Readers may submit comments independent of their current affiliation. Furthermore, scientists can contact USP if they are interested in collaborating on any of the new reference standard programs described above or if they would like to suggest additional items or projects for USP to consider.

REFERENCES

1. USP distinguishes between General Chapters and general information chapters: "Articles recognized in these compendia must comply with the official standards and tests and assays in … General Chapters numbered below 1000. General [information] Chapters numbered above 1000 are considered interpretive and are intended to provide information on, give definition to, or describe a particular subject. They contain no official standards, tests, assays, or other mandatory requirements applicable to any pharmacopeial article *unless specifically referenced in a monograph or elsewhere in the pharmacopeia*" (emphasis added). *USP 28–NF 23*. Rockville, MD: United States Pharmacopeial Convention, Inc.; 2005:4–5.
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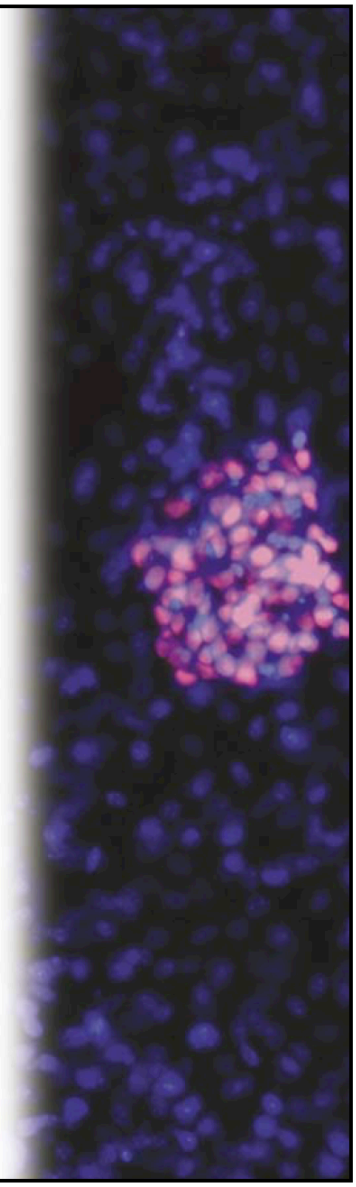

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