Commentary

USPNF 2025 ISSUE 3

June 1, 2025, updated October 3, 2025

In accordance with USP's *Rules and Procedures of the Council of Experts ("Rules*"), and except as provided in Section 9.02 *Accelerated Revision Processes*, USP publishes proposed revisions to the *United States Pharmacopeia and the National Formulary* (*USP–NF*) for public review and comment in the *Pharmacopeial Forum (PF),* USP's free bimonthly journal for public notice and comment. After comments are considered and incorporated as the Expert Committee (EC) deems appropriate, the proposal may advance to official status or be re-published in *PF* for further notice and comment, in accordance with the Rules. In cases when proposals advance to official status, a summary of comments received and the appropriate Expert Committee's responses, as well as Expert Committee-initiated changes, are published in the Proposal Status/Commentary section of USPNF.com at the time the official revision is published.

The *Commentary* is not part of the official text and is not intended to be enforceable by regulatory authorities. Rather, it explains the basis of Expert Committees' responses to public comments on proposed revisions. If there is a difference or conflict between the contents of the *Commentary* and the official text, the official text prevails.

For further information, contact:
USP Executive Secretariat
United States Pharmacopeia
12601 Twinbrook Parkway
Rockville, MD 20852-1790 USA

Comments were received for the following when they were proposed in Pharmacopeial Forum:

General Chapters
<7> LABELING
<11> USP REFERENCE STANDARDS
<381> ELASTOMERIC COMPONENTS IN INJECTABLE PHARMACEUTICAL PRODUCT PACKAGING/DELIVERY SYSTEMS
<761> NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY
<921> WATER DETERMINATION
<1041> BIOLOGICS
<1047> GENE THERAPY PRODUCTS
<1119> BIOBURDEN MONITORING
<1119.1> BIOBURDEN TEST
<1245> COMPACTION SIMULATION
<1265> WRITTEN PRESCRIPTION DRUG INFORMATION -- GUIDELINES
<1761> NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY- THEORY AND PRACTICE
<1762> SOLID-STATE NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY—THEORY AND PRACTICE

Monographs
ACETYLCYSTEINE INJECTION
ALBENDAZOLE
ALBENDAZOLE TABLETS
APIXABAN
APIXABAN TABLETS
ASPIRIN AND DIPYRIDAMOLE EXTENDED-RELEASE CAPSULES
BIMATOPROST
CHLORZOXAZONE
CHLORZOXAZONE TABLETS
DESONIDE CREAM
DESONIDE LOTION
DESONIDE OINTMENT
DEXTRAN 40
DEXTRAN 70
DIBASIC CALCIUM PHOSPHATE DIHYDRATE
GLIPIZIDE EXTENDED-RELEASE TABLETS
HYDROCODONE BITARTRATE AND ACETAMINOPHEN ORAL SOLUTION
HYDROXYAMPHETAMINE HYDROBROMIDE
MOMETASONE FUROATE CREAM
PERPHENAZINE
TECHNETIUM TC 99M MEBROFENIN INJECTION
TRIPTORELIN PAMOATE

No comments were received for the following proposals:

Monographs
ARGATROBAN IN SODIUM CHLORIDE INJECTION
ATENOLOL AND CHLORTHALIDONE TABLETS
DONG QUAI ROOT
DONG QUAI ROOT POWDER
EPTIFIBATIDE
MOMETASONE FUROATE OINTMENT
MOMETASONE FUROATE TOPICAL SOLUTION

General Chapter

General Chapter/Section(s):	<7> LABELING
Expert Committee:	Nomenclature and Labeling 2020
No. of Commenters:	10

Labels and Labeling for Compounded Preparations
Comment Summary #1: The commenter indicated that some proposed text may be missing from the PF proposal.
Response: Comment not incorporated. The text in question did not appear in PF, but compounding topics will be addressed in a future revision.

Labels and Labeling for Compounded Preparations
Comment Summary #2: The commenter addresses missing text that was expected to be published in PF 50(3). Also addresses other concerns related to labeling for compounded preparations.
Response: Comment not incorporated. The text in question did not appear in PF. The compounded preparation labeling topic will be addressed in a future revision.
Comment Summary #3: The commenter stated in the Labels and Labeling for Compounded Preparations subsection, in the second bullet, the commenter requested revising the text to be more consistent with FDCA section 503A.
Response: Comment not incorporated. The NL EC finds the existing definitions to be sufficient at this time but may consider amending this text in a future revision.

Comment Summary #4: The commenter recommended to consider improving consistency of terms official article, official product, official drug product, drug product, and product.
Response: Comment partially incorporated. Edits to these terms in <7> will be incorporated as appropriate in accordance with the USP Style Guide.
Comment Summary #5: The commenter indicated that some proposed text may be missing from the PF proposal.
Response: Comment not incorporated. The text in question did not appear in PF. The compounding topics will be addressed in a future revision.
Comment Summary #6: The commenter requested to further combine compounded preparation labeling topics within <7> or indicate the location of labeling for compounded preparations in the Introduction of the chapter.
Response: Comment not incorporated. Will consider for a future revision.
Comment Summary #7: The commenter recommended in the first paragraph, first and second bullets, keep the term “proportion” to align with the text with Food, Drug, and Cosmetic Act (FDCA) section 502(e)(ii) and 21 CFR 201.10(c)(2).
Response: Comment incorporated.
Comment Summary #8: The commenter recommended in the Definitions subsection, to add the exception of identifying controlled substances on the shipping labeling. This aligns with the analogous text for human drugs in the DEFINITIONS section in the beginning of <7>.
Response: Comment incorporated.

[This section was updated on October 3, 2025 to update a section head]

Labels and Labeling for Drug Products and Compounded Preparations Expressed as Active Moiety in Name and Strength

Comment Summary #9: The commenter recommended adding the term "non-salt form" next to active moiety Labels and Labeling for Drug Products section.
Recommend reinstating text under subsection "Labeling" that was removed for this revision.
Response: Comment partially incorporated. 1) The EC considered this topic and decided that modifications to this subsection were not suitable at this time. 2) A sentence that was removed in PF has been returned for the final revision: "The names and strengths of both the active moiety and specific salt form (when applicable) are provided in the labeling."
Comment Summary #10: The commenter requested an additional format to the current formats that became official on September 1, 2023.
Response: Comment not incorporated. Expiration date formats in <7> became official in September 1, 2023, after a 3-year implementation period. The four-digit year format is also presented in the International Organization for Standardization standard date format (ISO 8601) and in FDA Guidances.
Comment Summary #11: The commenter stated, in the INTRODUCTION section, first paragraph that defines label and labeling, consider adding a statement to address when a lack of space prohibits fulfillment of a label requirement, the labeling components (e.g., other labeling including but not limited to a carton, tray, or overwrap labeling, prescribing information) must include the required text (see 21 CFR 201.10(h)(2)).
Response: Comment not incorporated. This specific topic has been a reference in the Labels and Labeling for Injectable Products section of General Chapter <7> for several years. The Expert Committee has deemed that it is sufficient, and it will continue to be a reference in the future.

Implementation Period
Comment Summary #12: The commenter recommended, should the proposal become effective, it will require a minimum of 12 months for new artwork to be updated and implemented. Finalization of the artwork will go through review by a cross-functional team which includes the medical/safety team prior to approval. It takes a minimum 6 months for FDA approval of the supplemental NDA after submission.
Therefore, if the proposal goes through as written, please consider a 24-month implementation timeline
Response: Comment not incorporated. General Chapter <7> will have an implementation period of 3 years.

Dietary Supplements
Comment Summary #13: The commenter asked for clarity about which calcium-containing products are targeted in the proposed revisions in <7>. Specifically, if OTC antacids are intended to be addressed in the revision.
Response: Comment acknowledged. OTC antacids are not addressed in this section. The NL EC sought to align the principal display panel of dietary supplements with similar prescribed products.

Expiration Date and Beyond-Use Date
Comment Summary #14: The commenter suggested, in the Expiration Date and Beyond-Use Date, Beyond-Use Date subsection, consider adding text to better explain how beyond-use dates (BUD) apply to commercial drug products. Recommendations include addition of text discussing the manipulation of a drug product or compounded preparation.
Response: Comment partially incorporated. Repackaging of commercial products is addressed in General Chapter <1178> Good Repackaging Practices. Recommendations regarding compounded preparations will be considered in a future revision. Additionally, the definition of beyond-use date is addressed in the Definitions section of the chapter. The definition of beyond-use date in this section has been aligned with the introduction.
Comment Summary #15: The commenter recommended, in the Expiration Date and Beyond-Use Date, Expiration Date subsection, in the sixth paragraph, delete redundant statements regarding OTC DPs are intended for sale without a prescription and replace “prescribed” with “labeled.”

Response: Comment incorporated. Redundant text deleted.

Amount of Active Moiety or Drug Substance per Dosage Unit
Comment Summary #16: The commenter recommended, in the Amount of Active Moiety or Drug Substance per Dosage Unit subsection, in the second paragraph, clarify the blister pack is unit-dose to blister pack.
Response: Comment incorporated.
Comment Summary #17: The commenter recommended, in the first sentence, consider deleting “therapeutically” or replacing the term with “physiologically or pharmacologically.” Therapeutic ordinarily means disease treatment, but not all drugs treat diseases—some just affect the structure or function of the body. This is supported by the active moiety definition in 21 CFR 314.3 that uses the terms physiological or pharmacological action.
Response: Comment not incorporated. The statement will remain the same in order to align with text in General Chapter <1121>.
Comment Summary #18: The commenter recommended adding text that addresses labeling of animal drugs in animal feeds in regard to active moiety or drug substance per dosage.
Response: Comment incorporated; text was added.

Compounded Preparations for Veterinary Use
Comment Summary #19: The commenter recommended adding and modifying text to align with FDA labeling for compounded preparations for veterinary use. [Commenter also requested title change from “Compounded Veterinary Preparations for Use” to “Compounded Preparations for Veterinary Use”]
Response: Comment incorporated. Edits were incorporated and specific text was added to align with FDA CVM language: "The labeling must indicate that ‘This is a compounded drug. Not an FDA approved or indexed drug’.”

Labels and Labeling of Liquids and Constituted Products
Comment Summary #20: The commenter requested a clarification on the exceptions in this case. It is not clear if the exceptions are:
1) all oral liquids (constituted or not), and solids intended to be constituted, or
2) constituted liquids and constituted solids
Request to allow more flexibility, e.g., remove the need for it to be "constituted" to have this flexibility in relation to labeling as per mL/per 5 mL, or, have a clear and extensive exceptions list which includes oral suspensions.
Response: Comment partially incorporated. The NL EC reviewed and decided to revise the second sentence in item #4 to provide more clarity. Text was modified in the last sentence of item #4 to read: “Alternatively, oral liquids or solids intended to be constituted to yield oral liquids can be labeled in terms of each 5-mL portion of the liquid or resulting liquid.”
Comment Summary #21: The commenter recommended removal of the mg/mL dosing for liquid oral dosing form that was proposed in PF 50(3). Also recommended addition of weight per packet to this section.
Response: Comment not incorporated. The mg/mL concentration is important for healthcare providers to understand the concentration of the drug product that will be administered to patients. Will consider addition for packets for future considerations.

Total Quantity and Total Volume for Injectable Drug Products Packaged in Single- and Multiple-Dose Containers and Pharmacy Bulk Packages
Comment Summary #22: The commenter recommended, in the subsection titled Total Quantity and Total Volume for Injectable Drug Products Packaged in Single- and Multiple-Dose Containers and Pharmacy Bulk Packages, clarify this subsection is specifically for drug products (DP) for human use.
Response: Comment incorporated.

Expiration Date Format

Comment Summary #23: The commenter requested an additional format to the current formats that became official on September 1, 2023.
Response: Comment not incorporated. Expiration date formats in <7> became official in September 1, 2023, after a 3-year implementation period. The four-digit year format is also presented in the International Organization for Standardization standard date format (ISO 8601) and in FDA Guidances.
Comment Summary #24: The commenter requested removal of hyphens and forward slashes from expiration date format.
Response: Comment not incorporated. Expiration date formats in <7> became official in September 1, 2023, after a 3-year implementation period. The four-digit year format is also presented in the International Organization for Standardization standard date format (ISO 8601) and in FDA Guidances.

Beyond-Use Date
Comment Summary #25: The commenter recommended, in the fourth paragraph [of the Definitions section], consider adding text to better explain how beyond-use dates (BUD) apply to commercial drug products.
Response: Comment partially incorporated. Repackaging of commercial products is addressed in General Chapter <1178> Good Repackaging Practices. Recommendations regarding compounded preparations will be considered in a future revision. Additionally, the definition of beyond-use date is addressed in the Definitions section of the chapter. The definition of beyond-use date in this section has been aligned with the introduction.

Use of Leading and Terminal Zeros
Comment Summary #26: The commenter suggested to consider improving the text to prevent misinterpretation that a terminal zero is needed. Recommended text update: “To help minimize the possibility of errors in drug dispensing and administration, quantities of active moiety or drug substance expressed in whole numbers must be shown without a decimal point that is followed by a terminal zero (e.g., express as 4 mg, not 4.0 mg).”
Response: Comment partially incorporated. Text in that sentence was modified for clarity to read: “To help minimize the possibility of errors in drug dispensing and administration, quantities of active moiety or drug substance expressed in whole numbers must be shown without a decimal point and terminal zero (e.g., express as 4 mg, not 4.0 mg).”

Dietary Supplement Labeling
Comment Summary #27: The commenter requested adding additional text to the proposed Calcium supplement text within the chapter to include a percent daily value for consumers. Also requested an implementation period for the updating of labels for calcium supplements.
Response: Comment not incorporated. Will consider for a future revision. An implementation period is being considered.

Labeling for Animal Drug Products
Comment Summary #28: The commenter recommended, in the Labeling for Animal Drug Products subsection, clarify that it is a requirement to label the species per Compliance Policy Guide (CPG) Sec. 607.100 - Adequate Directions for Use (Species Designation) - Animal Drugs and Veterinary Devices. Additionally, relocate the statement “This is a compounded preparation” to the subsection entitled Compounded Veterinary Preparations.
Response: Comment incorporated.

Alcohol
Comment Summary #29: The commenter requested adding Dehydrated Alcohol labeling requirements on alcohol-containing products, as it appears unclear which should be on the label based on text in General Notices 8.30 Alcohol Content.
Response: Comment not incorporated. The NL EC evaluated the comment but decided that no changes were needed for this section.

Units
Comment Summary #30: The commenter recommended, in the proposed Units subsection, consider deleting text that refers to prescribing.
Response: Comment not incorporated. The NL EC reviewed this comment and determined that this statement should remain because impacts to patient safety start with prescribing. When prescriptions are written incorrectly, there is potential for this error to cascade to the patient.

Products that Contain Vitamins and Minerals
Comment Summary #31: The commenter recommended similar equivalency statements and also indicated that some text was missing from the PF publication of <7>.
Response: Comment not incorporated. Will consider for a future revision. Calcium was published in the most recent revision and currently addresses products that only contain the single entity of calcium. The text in question regarding magnesium did not appear in PF but will be considered in a future revision.
Comment Summary #32: The commenter suggested, in the Products that Contain Vitamins and Minerals subsection, revise the subheader title to clarify that this section applies to only dietary supplements. As written, one can misinterpret this section to apply to drug products. "Dietary Supplements Products that Contain Vitamins and Minerals."
Response: Comment partially incorporated. The Dietary Supplements subheader will be raised one level, and the subsection title "Products that Contain Vitamins and Minerals" will be removed. Subsections below will be raised one level.

General Chapter/Section(s):	<11> USP REFERENCE STANDARDS
Expert Committee:	Council of Experts 2020
No. of Commenters:	3

Comment Summary #1: Commenter requested that the definition of digital Reference Standards (dRS) does not fully explain the nature and use of these types of standards, which can lead to confusion. Commenter proposed to revise the introduction section for a clearer definition (how dRS is obtained) and application of digital reference standards on how data are extracted and applied. Chapter should provide examples of USP digital reference standards (dRS).
Response: Partially incorporated. The definition was reworded for clarity, indicating that USP Reference Standards can be presented either in digital format or as physical materials. For details on how dRS are obtained, refer to the revisions to the section Establishment Approaches and Value Assignment, which contains an added paragraph addressing the requests in the comment. Examples of dRS are not included in the chapter to avoid perception of incompleteness, considering the broad nature of the chapter. Some examples of potential digital reference standards include digital files containing data from processed spectra obtained through different technologies such as NMR, IR, MS, UV, X-ray, and digital files containing photographic images.
Comment Summary #2: Commenter indicated that application of digital standards to FT-IR may be challenging and not applicable due to differences in instrumentation and the scans provided in a digital format. Differences due to polymorphism may affect the comparison to the standard digital data. The digital scan cannot be physically dissolved in the same solvent and reconstitute for comparison. Commenter suggested including wording to allow for these differences.
Response: Not incorporated. Including wording for specific application of digital standards to FT-IR is outside the general scope of the chapter. Differences in instrumentation and the scans format should not be an issue for dRS implementation, since the data contained in digital files for the spectra can be processed using the exact scan format and instrument parameters to make a suitable comparison. Differences due to polymorphism may be addressed by providing digital spectra for each polymorph. Metadata about crystallization conditions can be included in the digital file. Samples may be compared to multiple digital reference standards each for the

different polymorphs or be recrystallized under the specific conditions specified in the metadata for one of them.
Comment Summary #3: Commenter suggested changes in the definition of reference standards to avoid using the term “references” resulting in a circular argument. Commenter recommends that digital reference standards be separately defined with clear descriptions of the process to establish digital reference standards from datasets and the criteria for the usage of digital reference standards in compendial methods. Commenter suggested to clarify the section “USP Reference Standards for USP or NF” to indicate when and how data from physical reference standards can be qualified as digital reference standards.
Response: Comment incorporated. The definition was reworded, indicating that USP Reference Standards can be presented either in digital format or as physical materials. The process to establish digital reference standards from datasets is described in the revisions to the section Establishment Approaches and Value Assignment, which includes a new paragraph addressing the concern in the comment. To clarify the criteria for usage of dRS, the paragraph added to the section "Proper Use" was reworded, indicating the following: "Criteria for proper use of dRS in compendial procedures are detailed in the applicable *USP-NF* monographs and general chapters." To clarify that when data from physical reference standards can be qualified as USP Digital Reference Standards, the section USP Reference Standards for USP or NF was reworded to indicate the official adoption of each specific format of a USP Reference Standard for each particular compendial application, and it is decided based on validation data.

General Chapter/Sections:	<381> ELASTOMERIC COMPONENTS IN INJECTABLE PHARMACEUTICAL PRODUCT PACKAGING/DELIVERY SYSTEMS
Expert Committee(s):	General Chapters—Packaging and Distribution
No. of Commenters:	6

General
Comment Summary #1: The commenter recommends retaining Section 4.3 Functionality Tests to ensure comprehensive regulation of packaging and rubber manufacturers, aligning characterization methods with <382>, and addressing the impact of larger needles for animal health products to enhance safety and patient trust.
Response: Comment not incorporated. USP received FDA comments expressing concerns about differences in fragmentation tests between chapters <381> and <382>, particularly the increase in particle size threshold in <382>. In response, USP anticipates revising <382> to remove section 5.1 Fragmentation and retain the fragmentation test in section 4.3 of <381>. For more details, please refer to the official USP web posting (⟨382⟩ Elastomeric Component Functional Suitability in Parenteral Product Packaging/Delivery Systems | USP-NF).
Comment Summary #2: The commenter is suggesting retaining Section 4.3 in Chapter <381> to avoid the burden of updating global regulatory filings and internal testing documents, which currently reference Chapter <381>. They propose replacing the content of Section 4.3 with a reference to Chapter <382> to maintain consistency and prevent potential negative impacts on quality systems and drug supply.
Response: Comment not incorporated. USP received FDA comments expressing concerns about differences in fragmentation tests between chapters <381> and <382>, particularly the increase in particle size threshold in <382>. In response, USP anticipates revising <382> to remove section 5.1 Fragmentation and retain the fragmentation test in section 4.3 of <381>. For more details, please refer to the official USP web posting (⟨382⟩ Elastomeric Component Functional Suitability in Parenteral Product Packaging/Delivery Systems | USP-NF).

Physicochemical- 4.2.1 Appearance (Turbidity/opalescence)
Comment Summary #3: The commenter suggests revising the text “… either a visual or instrumental comparison…” to “…either a visual (Procedure A) or instrumental comparison (Procedure B)” to provide clarity regarding the specific procedures for visual and instrumental comparisons
Response: Comment incorporated as suggested for clarity.

Comment Summary #4: The commenter recommends revising the sentence “Dissolve 1.000 g of analytical grade hydrazine sulfate in particle-free water and dilute with particle-free water to 100.0 mL” to “Dissolve and dilute 1.000 g of analytical grade hydrazine sulfate with particle-free water up to a final volume of 100.0 mL” for improved clarity and precision.
Response: Comment incorporated as suggested for clarity.

Physicochemical- 4.2.3 Acidity or Alkalinity, Test Solution
Comment Summary #5: The commenter suggests revising the phrase “To 20 mL of Sample solution add 0.1 mL of…” to “To 20 mL of Sample solution, add 0.1 mL of…” for improved clarity.
Response: Comment incorporated as suggested for clarity.

Physicochemical- 4.2.2 Color
Comment Summary #6: The commenter recommends revising the text “Compare the liquids in diffuse daylight, viewing vertically against a white background” to “Tubes should be viewed downward against a white background. If the illumination angle is controlled to avoid specular reflection and provides diffuse illumination relative to the observer angle, vertical or 90° horizontal observations may be made” for improved clarity.
Response: Comment not incorporated. USP found the comment unclear, but we believe that adding a sketch or drawing in a future revision could potentially help clarify the intended changes.
Comment Summary #7: The commenter suggests revising the sentence “Pass Sample solution through an inert filter of 0.45-µm pore size, discarding the first few milliliters of filtrate” to “Pass Sample solution through a low binding, inert filter of 0.45-µm pore size, discarding the first few milliliters of filtrate” for improved clarity and specificity.
Response: Comment not incorporated. No edit was made as the Expert Committee USP could not determine what was meant by “low binding.”

Physicochemical- 4.2.4 Absorbance
Comment Summary #8: The commenter suggests including a specific volume to give the reader an idea of an amount sufficient to adequately purge the insertion needle.
Response: Comment not incorporated. A single volume is difficult to determine if it needs to cover all practical possibilities.

Physicochemical- 4.2.5 Reducing Substances, Procedure
Comment Summary #9: The commenter suggests widening the acceptance criteria for ethylene-oxide-sterilized rubber closures to account for the impact of residual ethylene oxide and ethylene glycol, acknowledging that the aeration of ethylene oxide degradants, such as ethylene chlorohydrin and ethylene glycol, from the elastomer is a slow process.
Response: Comment not incorporated. USP has reviewed the request for revision to the Reducing Substance section. Because this recommendation would create a more stringent requirement, it would first need to be published as a PF revision proposal for stakeholder feedback. As the current revision cycle is ending, the Packaging and Distribution Expert Committee (PD EC) has added this topic to the proposed 2025–2030 workplan.
Comment Summary #10: The commenter suggests adding a new test for residual ethylene glycol and its oligomers in Type II rubber. This would ensure that the levels of ethylene glycol and its oligomers in the rubber material, including those formed during the sterilization process, remain within safe limits.
Response: Comment not incorporated. USP has reviewed your request for revision to the Reducing Substance section. Because this would create an additional requirement, it would first need to be published as a PF revision proposal for stakeholder feedback. As the current revision cycle is ending, the Packaging and Distribution Expert Committee (PD EC) has added this topic to the proposed 2025–2030 workplan.

General Chapters/Section(s): <761> Nuclear magnetic Resonance Spectroscopy/ Multiple Sections

Expert Committee(s): General Chapters-Chemical Analysis Expert Committee
No. of Commenters: 6

Introduction

Comment Summary #1: The commenter stated that the discrete energy levels are not what makes NMR different. What makes NMR different from all other spectroscopic techniques is the relatively weak interaction between nuclear spins and irradiation source, making nuclear spins sensitive probes of local structures. In other spectroscopic techniques, the interactions between particles (atoms, electrons) and the irradiation source is the dominant interaction. So, the commenter suggested changing the third sentence as follows: "*However, NMR spectroscopy differs from other spectroscopic methods in that the characteristic frequencies arise from interactions between nuclear spins and an external magnetic field or with each other, and that a relatively weak interaction between nuclear spins and radiofrequency irradiation source making nuclear spins sensitive probes of the local structure*."

Response: Comment incorporated as suggested for clarity.

Comment Summary #2: The commenter suggested clarifying the following terminology: User Requirements Specification (URS), Design Qualification (DQ), and Installation Qualification (IQ). URS and DQ are unfamiliar terms in NMR instrument qualification. IQ is performed by the NMR instrument vendor, not the end user (the vendor is the authority on IQ). The end user does not require URS or DQ to perform NMR experiments.

Response: Comment not incorporated. The EC members agreed that these terms are new for NMR instruments users. However, GC<761> had referenced GC <1058> Analytical Instrument Qualification. It is the end user's responsibility to make sure their instrument is fit for their specific application. Please refer to GC<1058> for more background and details on this topic.

Comment Summary #3: There are two commenters who suggested removing the "on a day-to-day basis" in the following sentence: "…and as reference for continuous verification of the instrument's performance on a day-to-day basis…". One commenter recommended the following change: "…and as reference for continuous verification of the instrument's performance on a measurement-to-measurement basis (i.e., system suitability tests using a known signal (e.g., TMS/TMSP) in each measurement).

Response: Comment partially incorporated. As the frequency of instrument performance verification is based on risk assessment, the following change has been made in the text: "…and as reference for continuous verification of the instrument's performance on a regular basis (i.e., system suitability tests)."

Comment Summary #4: The commenter had a different opinion on the frequency of OQ of the instrument. The commenter stated that OQ has to be done if the spectrometer configuration (hardware and/or software) has changed. If there is no change, only PQ has to be done to verify instrument consistency, as described in USP<1058>. Moreover, as per USP<1058>, PQ frequency is typically defined by the user and could be at defined calendar intervals (weekly, monthly, etc.) or not (e.g., at each use, as part of SST). The commenter recommended changing the text as follows: "Based on a risk assessment and control strategy, OQ and PQ should be *performed initially*, *and may be* repeated, either at predefined ~~within predetermined~~ intervals, *and/or following certain events such as instrument repair, maintenance, or software update. Normally, OQ would need to be repeated only when the instrument configuration (hardware and/or software) has changed; otherwise only PQ is needed to verify consistent instrument performance.* ~~It is common practice to perform an annual OQ and repeat the PQ on an annual, quarterly, or monthly basis. "~~

Response: Comment partially incorporated. This chapter points out that OQ and PQ should be repeated within predefined intervals, based on a risk assessment and control strategy. To avoid unnecessary misunderstanding, the following change had been made in the text: "Based on a risk assessment and control strategy, OQ and PQ should be repeated within ~~predetermined~~ *predefined* intervals. ~~It is common practice to perform an annual OQ and repeat the PQ on an annual, quarterly, or monthly basis~~."

Comment Summary #5: The commenter agreed that requalification should be part of a change control but only for a significant modification of the system, e.g., an electronic cabinet change.

The commenter suggested changing the text as follows: “Requalification should be considered as a part of change control *in the event of major modification(s) to the system*.”
Response: Comment partially incorporated. The EC members have discussed the comment and concluded that it is hard to clearly define “major modification.” The definition of major modification should be up to the user and the specific method used. The EC decided to delete the sentence: “Requalification should be considered as a part of the change control.”

Operational Qualification
Comment Summary #6: The commenter suggested including “sucrose anomeric splitting” as an additional bullet point under this section. Sucrose anomeric splitting is a critical test for shimming quality and signal-to-noise ratio (s/n) assessment.
Response: Comment not incorporated. The proposed sucrose anomeric splitting is a specific test. The EC does not want to provide a specific test in this case so that the users can have more options.
Comment Summary #7: The commenter stated that “bias assessment” in NMR OQ is not suitable for the pharmaceutical industry. “Bias” may be better suited for performance qualification or system suitability testing. Bias may originate from improper handling of sample or from the NMR experimental setup, which should be part of method development and validation. As such, the commenter recommended deleting “bias” from the chapter.
Response: Comment not incorporated. Bias assessment does not need to be checked frequently. The bias assessment listed is just an example, and the user can implement this if applicable.
Comment Summary #8: The commenter requested the removal of the requirement for concentration linearity for OQ. The concentration linearity was never a required test for OQ. With the proposal to remove linearity assessment for method validation, concentration linearity is even less relevant for NMR instruments since NMR measurements are inherently quantitative, provided that full relaxation conditions are met.
Response: Comment partially incorporated. There must be evidence to show the instrument qualification of linearity. If users do not demonstrate this linearity in OQ, they need to show the linearity of the instrument during method validation in each analytical procedure. To avoid confusion, the bullet point “Concentration linearity” was revised into "Linearity." The concentration linearity section was moved to GC <1761> to provide flexibility for all users to adopt linearity in different ways.
Comment Summary #9: The commenter requested differentiating between the initial OQ and the periodic PQ and be consistent with GC<1058>. The suggested change of text is as follows: " ~~To continuously verify the operational capability of the instrument, the OQ process should be repeated annually or as justified by a risk assessment.~~ *Once the initial OQ (and IQ) has been performed, the instrument’s continued suitability for its intended use is demonstrated through continued PQ. Repetition of OQ may be necessary, subject to a risk assessment.”*
Response: Comment partially incorporated. As mentioned, this chapter claims that OQ and PQ should be repeated within predefined intervals, based on a risk assessment and control strategy. To avoid unnecessary confusion, the text was revised as follows: “To continuously verify the operational capability of the instrument, the OQ process should be repeated ~~annually~~ periodically, or as justified by a risk assessment.”

System Suitability Test
Comment Summary #10: The commenter stated that the current text under this section is misleading and can be interpreted in a way that suggests system suitability tests must be performed on a day-to-day basis on a separate sample [e.g., acetone (0.3% CHCl3) line shape]. The commenter noted that the suitability of the system has to be determined based on each spectrum individually. An SST based on standard test samples and qualification results is insufficient to ensure the quality of each spectrum. Although the proper function of the device should be checked through regular performance qualification and requalification, the SST should be part of the analysis of each spectrum. A known peak of a reference material (preferably of TMS/TMSP) or of a solvent can be used, and the peak shape, line width, and/or

signal-to-noise ratio should be used as the SST criteria. This SST procedure would ensure that the impact of shimming is considered appropriately for each spectrum.
Response: Comment not incorporated. The commenter's proposal was more on the data quality assessment rather than the SST considerations.

Figure 1 and Table 1
Comment Summary #11: The commenter suggested the addition of some acceptance criteria for line shape instead of just repeating the data in Figure 1. The proposed changes are as follows: Change the Table to give acceptance criteria instead of typical results. For example, the acceptance criteria for the non-spinning line shape used by vendors are: 50% <= 0.7 Hz; 0.55% <= 7 Hz; 0.11% <= 14 Hz.
Response: Comment not incorporated. In "Figure 1. 1H NMR spectrum of chloroform in acetone-d6 obtained at 400 MHz," the line shape varies according to magnetic fields and instrument vendors. Please consult provided specification from the manufacturers. The EC decided to delete Table 1 to avoid any confusion for the users.

Pulse Width
Comment Summary #12: The commenter suggested the addition of guidance for setting the pulse length specification. The pulse length specification can be chosen based on the manufacturer's specification. It should be considered that the width of the excitation profile (see Spectral uniformity below) depends inversely on the length of the 90° pulse. Therefore, the pulse length specification can also be justified based on the required uniform spectral range. The manufacturer's specification should be respected as the minimum allowed value in order to protect the probe.
Response: Comment not incorporated. In "Figure 1. 1H NMR spectrum of chloroform in acetone-d6 obtained at 400 MHz," the line shape varies according to magnetic fields and instrument vendors. Please consult the provided specification from the manufacturers.
The EC decided to delete Table 1 to avoid any confusion for the users.
Comment Summary #13: The commenter suggested the addition of alternative methods such as the indirect pulse calibration method based on the decp90 sequence (A. Bax., J. Magn. Reson. 52, 76-80 (1983)) and the single scan pulse calibration (P.S.C. Wu & G. Otting, J. Magn. Reson. 176, 115-119 (2005)).
Response: Comment not incorporated. Alternative methods other than compendial methods are always acceptable if they meet the requirements listed in General Notice 6.30. Alternative and Harmonized Methods and Procedures.

Concentration Linearity
Comment Summary #14: The commenter suggested including an alternative method which simulates varying concentrations by changing the flip angle of the excitation pulse. The method is outlined on the poster submitted together with this document (see comment 13).
Response: Comment not incorporated. See response for Comment #13.
Comment Summary #15: The commenter stated that NMR is inherently quantitative, provided that full relaxation conditions are met. The commenter suggested removing the Concentration linearity from the OQ section.
Response: Comment incorporated. See the response to Comment #8. The concentration linearity section was moved to GC <1761> to provide flexibility for all users to adopt linearity in different ways.

Method of Internal Calibration
Comment Summary #16: The commenter viewed Equation 6 as an unusual form of the qNMR equation.
Response: Comment not incorporated. In the chapter, m_i is defined as the mass of any component in a sample, m_r is defined as the weight of the reference. From potency point of view, the current equation presents the proper pathway for calculation.

Method of External Calibration

Comment Summary #17: The commenter suggested that Equation 7 should be explicitly mentioned or should introduce the PULCON formula (Wider G., Dreier L., J. Am. Chem. Soc., 2006, 128, 2571).
Response: Comment partially incorporated. The text was revised as follows: "The classical external calibration method consists of…separate NMR tubes. *However, all NMR datasets should be using the same acquisition and processing parameters*."
The following text was moved to GC <1761>: "Another alternative is to introduce a computer-generated signal …by introducing it into the NMR spectrum of a calibrant solution of known concentration."

Quantitative Applications

Comment Summary #18: The commenter suggested the addition of most commonly used standards/calibrants into <761>.
Response: Comment partially incorporated. The information of commonly used calibrants was added in the chapter GC<1761>

Sample Preparation
Comment Summary #19: The commenter stated that it is not always possible to have the same solvent when the sample matrix is complex. For example, H_2O/D_2O for the sample vs D_2O for the reference should be acceptable since tuning of the probe head is verified on both NMR tubes.
Response: Comment not incorporated. The chapter uses the word "commonly" in the chapter for more flexibility, if needed. The EC decided not to use a specific example in this case.

Data Acquisition
Comment Summary #20: The commenter recommended further clarifying the definition of NP as it relates to Equation 9. NP is "the number of data points to be acquired," but it is currently unclear whether these NP points are real or complex data points. Accuracy of Equation 9 requires NP to be specified as either real or complex data points. This should be clearly stated in the text.
Response: Comment incorporated. Both spectral width (SW) and acquisition time (AT) determine the total number of data points (real and imaginary) to be acquired (NP) and the overall digital resolution (DR).
Comment Summary #21: The commenter requested for more guidance for users regarding the sentence "The RF width (PW) must be optimized to ensure a uniform excitation profile across the entire SW. Shorter pulses at higher power have wider excitation profile, but probe limits must be respected to avoid damage. In extreme cases (e.g., 19F qNMR) a smaller flip angle may help to ensure uniform excitation.
Response: Comment incorporated. The following was added to the chapter text for additional clarity: *It is not necessary to use 90º pulse in all cases. Sometimes, a shorter pulse can be used to ensure uniform response over a large spectral window.*
Comment Summary #22: The commenter stated that the impact of a flip angle on accuracy is unclear.
Response: Comment incorporated. Further statement in the Data Acquisition section was added to clarify. For example, when a 90° flip angle is used, a 99% signal recovery level can be achieved by setting T_R to at least 5 times the longest T_1 value. A 90° pulse has the highest efficiency with respect to SNR per unit of measurement time.

Data Processing
Comment Summary #23: The commenter stated that data processing must be defined as part of the method description.
Response: Comment incorporated. The text was revised to reflect this change: "Additional operations on the FID (such as zero-filling or linear prediction) can be applied ~~as appropriate~~ *as defined by the analytical procedures*."

Repeatability
Comment Summary #24: The commenter stated the validation criteria listed in the chapter are challenging for polydisperse biomolecules (such as polysaccharides or conjugates). The commenter recommended presenting the following option to modify the criteria: For a complex mixture or polydisperse molecules (e.g., polysaccharides or conjugate vaccine antigens), these RSD limits could be higher.
Response: Comment not incorporated. Refer to General Notice 6.30. Alternative and Harmonized Methods and Procedures, which allows for alternative methods under specified circumstances.

Intermediate Precision
Comment Summary #25: The commenter stated the validation criteria listed in the chapter are challenging for polydisperse biomolecules (such as polysaccharides or conjugates). The commenter recommended presenting the following option to modify the criteria: For complex mixture or polydisperse molecules (e.g., polysaccharides or conjugate vaccine antigens), these RSD limits could be higher.
Response: Comment not incorporated. See Comment #24.

Working Range
Comment Summary #26: The commenter suggested removing linearity for the tests for the working range. Linearity criteria have been removed from the proposed new text; therefore, they need to be removed from the working range as well. It is welcomed that in this latest draft for <761> that linearity has been removed as an attribute required for the validation of a quantitative NMR method. The reference to linearity retains a degree of ambiguity in whether linearity would be needed or not for the validation of an NMR method.
Response: Comment not incorporated. The EC clarified in the chapter text by providing options for the linearity test in the OQ or procedure/method validation. Users should demonstrate that the method works, meeting all three criteria (accuracy, precision, and linearity) in the work range. Linearity cannot be ignored in practice. The EC decided to add the linearity section back in the chapter.

Robustness
Comment Summary #27: The commenter suggested making the text more specific to the NMR analysis. The following changes were proposed: “This can include *assessing the NMR solution stability under defined storage conditions*, varying ~~the analyte storage conditions~~ test solution concentrations, interpulse delay values, sample temperatures within the range of measurement, and possible interfering species, to list a few examples.”
Response: Comment incorporated as suggested.

Operational Qualification
Expert Committee-Initiated Change #1: A discrimination may be important for Qualitative or Quantitative purposes. If done, in the case of Qualitative purposes, the four characteristics described are important and probably sufficient. For Quantitative purposes, Linearity and Accuracy must be complemented by Precision. We don’t know if the writer’s background was ISO, where Accuracy testing includes Precision and Trueness. GMP guidelines address accuracy (trueness) and precision separately. Therefore, Precision for instrument qualification should be added, to be in line with GMP.
Expert Committee-Initiated Change #2: In case “Uniform peak response” is the test responsible for testing Precision, the nomenclature is not the one that GMP uses for general purposes, and we recommend describing the test as *“Precision (Uniform peak response)”* or *“Uniform peak response (Precision)”* to clarify the item. Besides, there is no acceptance criteria. On other hand, URS must be compared (and met) with the instrument functional specifications (FS) to assess the AIS suitability and to prepare the OQ tests and acceptance criteria during qualification. Therefore, the supplier must provide something (FS) equivalent or better than that stated by the URS. Then, the statement “Given that spectral uniformity specifications are

typically not provided by the instrument manufacturer, the acceptance criterion for the RSD for each spectral region should be described in the URS" does not sound appropriate.

Validation and Verification OF NMR Analytical Procedures
Expert Committee-Initiated Change #3: "For the validation of NMR analytical procedures, one of the two approaches can be adopted: the life cycle approach and the traditional approach." The statement seems to indicate that the user may choose a lifecycle approach or a traditional one, but only the first one ensures that the procedure remains fit for the intended purpose. ICH permits the selection of any of those approaches. The EC believes that the lifecycle approach is an improvement in the control mechanisms, but that doesn't exempt the user from ensuring fitness for purpose, for example, by using system suitability tests and other tools.
Expert Committee-Initiated Change #4: In the life cycle approach, the procedure life cycle comprises the analytical target profile (ATP), which involves three life cycle stages. We understand that the ATP does not involve three stages of the life cycle but the life cycle involves three stages, with the ATP design being the first step, which establishes the fitness for the purpose of a procedure though the lifecycle. The life cycle validation approach controls the NMR analytical procedure and ensures it remains fit for the intended purpose, and it is different from the instrument life cycle management.

Pulse Width
Expert Committee-Initiated Change #5: As chapter <761> is a mandatory standard, the EC suggested deleting the sentence: "Alternative methods would also be accepted."

General Chapter/Section(s):	<921> WATER DETERMINATION
Expert Committee:	General Chapters - Chemical Analysis 2020
No. of Commenters:	4

Comment Summary #1: The commenter requested that guidance be provided on the validation/verification parameters of the water determination test in the chapter.
Response: Comment not incorporated. Method validation and verification is not in the scope of this revision. The EC recommends referring to *GC<1225> Validation of Compendial Procedure*s and *GC<1226> Verification of Compendial Procedures* for more information. In addition, there are some resources available in Compendial Tools for this topic.
Comment Summary #2: The commenter requested correction of the formula in Method Ia and Ib to include the sample weight as follows: Result = SF/W*100
Response: Comment partially incorporated. The formula is correct for the water amount in milligrams. The following changes were made for clarification. 1. The sentence was revised to read: "Calculate the amount of water in the specimen taken, in milligrams...". 2. A separate formula for calculating the water content in percentage is added in the chapter. With these changes and additions, users can now calculate either the amount of water in milligrams or the percentage of water in the specimen.
Comment Summary #3: The commenter suggested the revision of the sentence related to endpoint determination as follows to include the manual KF titration apparatus: *The endpoint **may be** determined electrometrically with an apparatus employing an electrical circuit and a pair of platinum electrodes...*
Response: Comment incorporated. The revision was made per the comment's suggestion to include a manually operated KF titration apparatus.

Standardization of the Reagent
Expert Committee-Initiated Change #1: In order to be consistent with the symbols used under PROCEDURE, the formula to calculate the water equivalency factor, F, while using Purified Water or water standards was changed from "Result =" to "F =" as follows:
"F = W/V."

Expert Committee-Initiated Change #2: In order to be consistent with the symbols used under PROCEDURE, the formula to calculate the water equivalency factor, F, while using sodium tartrate dihydrate was changed from “Result =” to “F =” as follows:
“F = W/V (36.04/230.08).”

Procedure
Expert Committee-Initiated Change #3: The original official text to calculate the content of water was updated for clarification and completeness as follows:
“Calculate the amount of water in the specimen taken, in milligrams:
Result = SF
in which, S is the volume, in milliliters, of the Reagent consumed in the second titration, and F is the water equivalence factor of the Reagent.”
Comment Summary #4: The commenter suggested adding some text to cover all modern auto-titrators that include titrators that utilize a change of potential and user defined volume drift as stop criteria. First, the commenter proposed the addition of "or potential" in the sentence:"...a slight excess of the reagent increases the flow of current or potential..." Second, the commenter mentioned that in the case where the apparatus has the ability to compensate for drifts, the endpoint detection time of 30 s is not needed.
Response: Comment partially incorporated. The recent revision does not account for titrators that utilize a change of potential to indicate titration endpoint and user-defined volume drift stop criteria. To ensure the method is inclusive of all modern auto-titrators, it is proposed to include "potential" and clarification for compensation for drift and the possibility of waving the 30 s, when the endpoint could be reached faster with reliability. The following changes were made in the chapter text. First, the sentence was revised as proposed. Second, the sentence "However, the endpoint detection time may be significantly reduced for substances that rapidly and completely dissolved in the reagent" was deleted. Then, the following sentence was revised for more clarity: "In cases where the instrument has the ability to compensate for drift (e.g., ambient moisture uptake), the 30 s requirement for the endpoint detection may be waived."

General Chapter/Section(s):	<1041> BIOLOGICS
Expert Committee:	Biologics Monographs 2 – Proteins
No. of Commenters:	1

Comment Summary #1: The commenter suggested including the following statement at the end of the first paragraph: "It should be noted that the term "biologics" does not refer to recombinant therapeutic protein products or animal-derived products administered by the Center for Drug Evaluation and Research" to clarify that the definition for biologics does not apply to biologics licensed under the PHS Act and administered by the Center for Drug Evaluation Research (CDER). The commenter also indicated that a reference to another USP chapter could be included if relevant.
Response: Comment not incorporated. The suggested revision to the text is outside of the scope of the revision and would require publication in PF to propose the suggested changes.

General Chapter/Section(s):	<1047> GENE THERAPY PRODUCTS
Expert Committee:	Biologics Monographs 5 - Advanced Therapies
No. of Commenters:	2

General Comments
Comment Summary #1: The commenter indicated that the General Chapter does not make definitive statements on the sterility test method for cell and gene therapy products, but no action requested.
Response: Comment not incorporated. No action was requested.
Comment Summary #2: The commenter suggested adding references to key statements as is done in review articles.

Response: Comment not incorporated. A list of relevant references is already provided at the end of the chapter.
Comment Summary #3: The commenter requested the citation of the USP chapter <1071> and test chapters <72> and <73> in the chapter. Chapters <72> and <73> were written to test short-life products such as cell and gene therapies.
Response: Comment not incorporated. These chapters were not official at the time of the last major revision to <1047> and are beyond the scope of the minor revisions proposed. The requested citations of additional USP chapters may be considered in a future revision to <1047>.

Introduction
Comment Summary #5: The commenter suggested clarifying the scope of the chapter to align with FDA's definition of human gene therapy products: "all products that mediate their effects by transcription or translation of transferred genetic material, or by specifically altering host (human) genetic sequences."
Response: Comment not incorporated. The proposed definition is not the formal definition of gene therapy, as originally stated by FDA in Federal Register Notice: Application of Current Statutory Authorities to Human Somatic Cell Therapy Products and Gene Therapy Products (58 FR 53248, October 14, 1993).

Table 1:
Comment Summary #6: The commenter requested that the term, "expressed via vector" should be further developed to explain that such nucleic acid would have to be transcribed from the inserted sequence.
Response: Comment not incorporated. It is out of scope for this minor revision but could be considered in a future revision.
Comment Summary #7: The commenter requested the addition of the additional gene modulation strategies.
Response: Comment not incorporated. It is out of scope for this minor revision but can be considered in a future revision.
Comment Summary #8: The commenter suggested changing the title of Table 1 from "Examples of Gene Therapy Products" to "Examples of Gene and Related Viral Therapy Products" because not all of the examples in the table are of gene therapies.
Response: Comment not incorporated. The scope of this chapter is limited to gene therapy and does not include virus-containing products that are not regulated as gene therapy, such as Vaccinia virus.

Ancillary Materials
Comment Summary #9: The commenter suggested revising "suitability for use" to "suitability for **intended** use."
Response: Comment not incorporated. An assessment of suitability implies it is being assessed for its intended use. The Expert Committee did not agree that the proposed edits enhanced the understanding of the statement.

Characterization of Cell and Virus Banks Qualification
Comment Summary #10: The commenter restated the exact text from the chapter, "Cell and viral bank characterization is an important step toward obtaining a uniform final product with lot-to-lot consistency and freedom from adventitious agents. Testing to qualify the MCB or MVB is performed once and can be done on an aliquot of the banked material or on cell cultures derived from the cell bank."
Response: Comment not incorporated. No action was requested.
Comment Summary #11: The commenter suggested revising the text to, "Purity must be analyzed **to exclude bacterial, mycoplasma, fungal, and viral contamination** (other than vector sequences)."

Response: Comment not incorporated. The requested revisions are beyond the scope of the minor revisions proposed. Additionally, purity and safety can be and often are interconnected and are not contradictory as stated by the commentor.

Table 2

Comment Summary #12: The commenter requested the addition of the following text "Quantity of virus after virus culture and following process steps Specific activity and ratio of E/F of virus in fractions after column chromatography Quantity of host-cell DNA in fractions after column chromatography" Quantity of host-cell protein in fractions after column chromatography" for the case of virus purification additional IPCs determining E/F ratio and HCP.

Response: Not incorporated. The requested revision is out of scope for this minor revision but can be considered in a future revision.

Specifications

Comment Summary #13:

The commenter requested the following edits: "ensure the safety and ~~efficacy~~ **potency** of the product before use…"

Response: Comment not incorporated. The use of potency or efficacy depends on the context. Discussion of the use of potency or efficacy may be addressed in a future major revision.

Comment Summary #14: The commenter suggested revising the text as follows: "The procedures in a specification for the product are anchored by appropriate reference standards **or phase dependent reference material. Exceptionally also in the case of potency due to the complexity and potential labile features of the transgenic product, assay controls might be used (e.g., for ex-vivo gene therapy products)**."

Response: Comment not incorporated. Additional verbiage is not required, as use of the current term "appropriate reference standards" could encompass phase-appropriate reference materials or assay controls.

Comment Summary #15: The commenter suggested revising the text to, "In-process ~~release~~ specifications should be established for each control step" because the phrase "in-process release specifications" is not commonly used, and it might be a mix of two distinct concepts, in-process specifications and release specification.

Response: Not incorporated. While the term "release" is not required, a product with a defined in-process specification must meet that specification in order to be "released" for further manufacturing; thus, the use of the term "release" in this context is not incorrect.

Comment Summary #16: The commenter suggested the following edits, "Changing to a new reference standard (lot) should include many tests, all of which are run side by side with the existing reference standard **whenever possible**. **Alternative approaches to introduction of a new reference standard may be considered in the event side by side testing is not possible."**

Response: Not incorporated. The additional verbiage is not needed as the use of the term "should" rather than "must" implies flexibility to use alternative approaches.

Considerations for Validation

Comment Summary #17: The commenter suggested that the chapter could highlight the challenges of the CGT area while applying validation principles.

Response: Comment not incorporated. This comment is out of scope of the revision but may be considered in a future revision of the chapter.

Comment Summary #18: The commenter requested that a statement be added that "Removal of any transgenic DNA traces needs to be shown" as the residual virus may be inactivate, but the transgenic DNA would still be present.

Response: Comment not incorporated. This comment is out of the scope of this revision and may be considered in a future revision.

Design Considerations for Gene Vectors

Comment Summary #19: The commenter suggested the addition of a reference to the statement, “AAV, do not integrate at high efficiency but can be expressed long-term in nondividing cells such as neurons or hepatocytes.”
Response: Not incorporated. This comment can be considered in a future revision as it is out of scope of the minor revision.
Comment Summary #20: The commenter suggested incorporating newer versions such as episomes or other cDNA. The commenter also suggested to incorporate CMC aspects among the different viruses that can be integrated (e.g., storage temperature, viral stability immunogenicity, and safety).
Response: Comment not incorporated. This comment can be considered in a future revision as it is out of scope of the minor revision.

Formulation of Gene Therapy Products
Comment Summary #21: The commenter requested the following edits, “Formulation changes during clinical development **is a major change and requires a comparability program** supported with preclinical studies and stability data.”
Response: Not incorporated. This comment can be considered in a future revision as it is out of scope of the minor revision.
Comment Summary #22: The commenter requested specifying which virus is discussed in this sentence: “This virus has been documented to be stable for approximately 4 months in phosphate buffered saline at 4°.” The commenter suggested that the virus may be serotype AAV5 because it is a stable virus.
Response: Comment not incorporated. This comment can be considered in a future revision as it is out of scope of the minor revision.

Table 4 and Table 5
Comment Summary #23: The commenter requested including Sterility in the same category of testing in Tables 4 and 5 by placing the sterility tests under the category of Safety.
Response: Comment not incorporated. This comment is out of scope for the minor revision proposed, but this topic of how sterility should be categorized is anticipated to be discussed during the next major revision of <1047> as there are existing inconsistencies in FDA guidance documents and FDA regulations on how sterility can be categorized.

Analytical Methods
Comment Summary #24: The commenter quoted exact text from the chapter. No changes were requested.
Response: Comment not incorporated. No changes were requested.

Potency
Comment Summary #25: The commenter suggested the following edits to the text, “ [...] Issues of cost, facilities, validation, and ethics determine the practicality of an in vivo potency test. **The in-vivo potency assay might show the proof of concept. However, applying the 3Rs and the often missing robustness of such in-vivo assays lead to the usage of an in-vitro potency cell based assay for the control system** [...].” because regulators and related guidelines strongly recommend avoiding in-vivo potency assay as a release test.
Response: Comment not incorporated. This comment may be considered in a future revision as it is out of scope of the minor revision.
Comment Summary #26: The commenter suggested adding 1–2 sentences describing the matrix approach and often multiple MoAs situation. The commenter suggested adding this text, “**Matrix approach, its phase dependent development and bioassay bridging of the in-vitro cell based assay to the in-vivo situation are a recognized and often applied strategy among the industry**.”
Response: Comment not incorporated. This comment may be considered in a future revision as it is out of scope of the minor revision.
Comment Summary #27: The commenter suggested the following edits, “Potency is defined as the therapeutic activity of the drug product. Together with dose, potency defines the biological

activity of each lot (see Dose-Defining Assays) **The potency assay is a stability indicating quantitative functionality assay of the transgenic region (Payload).[...]**"
Response: Comment not incorporated. This comment may be considered in a future revision as it is out of scope of the minor revision.

General Chapter/Section(s): <1119> BIOBURDEN MONITORING
Expert Committee(s): General Chapters – Microbiology
No. of Commenters: 30

General
Comment Summary #1: The commenter indicated that the "Briefing" section of USP <1119> Bioburden Monitoring incorrectly stated that USP <61> Microbial Enumeration Test is "purposed for release and testing." The commenter clarified that while <61> may sometimes be used to support release, it is not inherently a release standard, as release testing is determined by the FDA under CGMP regulations. The commenter recommended removing such statements from future draft publications to prevent confusion and ensure USP chapters are not misapplied as batch release standards.
Response: Comment incorporated. The reference to "purposed for the testing and release" of nonsterile products was removed in the final document.
Comment Summary #2: The commenter suggested that nonsterile substances, including active substances, are clearly indicated as in scope for USP<61>. They proposed clarifying that this drug substance refers to biologics such as protein bulk drug substances.
Response: Comment partially incorporated. The revised document specifies that the scope includes "drug substances, components, raw materials, excipients, and water" and distinguishes between different types of substances. However, explicit reference to "biologics such as protein bulk drug substances" is not directly mentioned.
Comment Summary #3: Several comments address the scope of USP <61> and its application to nonsterile substances for pharmaceutical use. They suggest clarifying the distinction between nonsterile finished drug products and nonsterile substances for pharmaceutical use.
Response: Comment incorporated. The chapter was clarified to separate out nonsterile finished drug products and nonsterile substances for pharmaceutical use.
Comment Summary #4: The commenter indicated that the scope of the chapter includes any material subject to a test for bioburden that is not used for the release testing of finished products. They noted that Table 3 appears to include both release and non-release testing points, creating confusion about whether the intent of the chapter is to provide overall guidance for bioburden control or specifically for non-release testing. The commenter suggested simplifying the guidance to eliminate the creation of a separate bioburden section and reduce the risk of misalignment between similar methods.
Response: Comment partially incorporated. Bioburden testing may be used in release testing, and the chapter was adjusted to reflect this. The main driver for writing <1119> and <1119.1> was that <61> does not capture all bioburden tests, such as the pre-sterilization bioburden test.
Comment Summary #5: The commenter requested to add clarity to the scope of the chapter, specifically noting that the in-process bioburden test should be considered part of the overall final product disposition. The current wording states, "The scope of this chapter includes any material subject to a test for bioburden that is not used for the release testing of finished product."
Response: Comment incorporated. Bioburden testing may be used in release testing, and the chapter was adjusted to reflect this.
Comment Summary #6: The commenter requested clarification on whether performing method suitability in line with <1119.1> and proving the method is suitable for its intended use allows the method to be considered compendial, and if it is permissible to refer to <1119.1> for bioburden method description, despite the chapter number being greater than 1000.
Response: Comment incorporated. The document notes that clarifications may be required if <1119.1> is moved below 1000 in the future, leaving open the possibility of further adjustments.
Comment Summary #7: The commenter indicated support for the new guideline chapter addressing bioburden in processes, highlighting its uniqueness in a pharmacopoeia. They

emphasized the importance of distinguishing this from microbial enumeration and the microbiological quality of final non-sterile products, which are covered by chapters <61> and <1111>. The commenter appreciated the clarification provided in the chapter as beneficial for both pharmaceutical manufacturers and inspectors.
Response: Comment acknowledged.
Comment Summary #8: The commenter indicated that the stated scope of <1119> is “any material subject to a test for bioburden that is not used for the release testing of finished product.” They noted that this includes “in-process samples, drug substances, components, and water” and expressed concern that this scope amounts to CGMP guidance, which falls under the FDA’s jurisdiction.
Response: Comment incorporated. The Expert Committee maintained the chapter’s broader coverage of bioburden monitoring, recognizing that USP monographs and chapters often address quality control and manufacturing considerations that may align with the CGMP principles without replacing the FDA’s regulatory role.

Comment Summary #9: The commenter indicated that there is a distinction between a microbial count and bioburden test. They explained that bioburden refers to the number and type of microorganisms related to the method of sterilization, such as sterile filtration, chemical, or moist heat sterilization. This definition emphasizes the term "burden" in the context of sterilization methods.
Response: Comment not incorporated. USP <1117.1> provides a useful clarification by distinguishing between a microbial count and a bioburden test. While both measure the presence of microorganisms, a bioburden assessment includes quantifying the total microbial load and characterizing the types of microorganisms, particularly in relation to specific microbial control or sterilization processes.
Comment Summary #10: The commenter recommended including guidance on what action to take if the bioburden test exceeds the bioburden level. They indicated that adding this guidance would complete the lifecycle of the bioburden sample by addressing the handling of bioburden data, as the current chapter ends at the completion of the test and only provides guidance on the recommended bioburden levels.
Response: Comment not incorporated. For informational chapters, overarching requirements leaves granular out-of-limit/out-of-specification procedures to each manufacturer’s quality system and regulatory expectations, particularly for in-process rather than final release testing.
Comment Summary #11: The commenter indicated concerns regarding the applicability of the maximum acceptable limits in relation to the small sample size and low precision of the bioburden test. They suggested that the statistical limitations of the methods should be discussed in section <1119> and emphasized that the bioburden test is not suitable for in-process monitoring and control, as it serves primarily as a confirmational test. The commenter highlighted critical control points during processing, such as storage temperature and bioburden-reduction filtration, which should be justified during process validation steps.
Response: Comment not incorporated. The chapter allows firms to adopt different limits based on sound statistics, and it permits the option for firms to use in-process sampling data as part of the batch-release process.
Comment Summary #12: The commenter suggested that the term "bioburden" should be more broadly defined beyond microbial count, as the burden may be increased by the type of microorganisms detected. They provided examples such as spore-forming bacteria, which may have a more negative impact on the sterility assurance of a terminally sterilized drug product due to the moist heat resistance of spores, and small bacteria and mycoplasma, which may pass through a 0.22 µm retention filter used for sterile filtration.
Response: Comment partially incorporated. The final <1119> reinforces that monitoring bioburden includes assessing which microbes are present, not just how many. Language such as “the number and the nature of the recovered organisms” is deliberately broad, acknowledging that spore-formers or tiny bacteria can significantly affect your sterility assurance level or microbial control strategy.

Comment Summary #13: The commenter suggested adding a note in the general chapter to clarify that bioburden analysis is not required for individual monographs that do not specify this requirement.
Response: Comment partially incorporated. The existing text in <1119> already clarifies that it applies to any material subject to a test for bioburden that is not used for the release testing of finished products. If an official monograph requires microbial enumeration, then <61> (and <1111>) typically govern it. Manufacturers may still perform bioburden monitoring under <1119> as part of their quality control strategy, based on risk and regulatory expectations.
Comment Summary #14: The commenter indicated that the limitations and lack of sensitivity of traditional plate count methods, as cited in USP <1223>, should be considered in the revision of USP <1119> and <1119.1>. They highlighted that traditional methods may only recover 0.1%–1% of the actual microbial cells present in a sample and that alternative methods with different signals have not correlated with increased user risk or higher likelihood of pathogens when there is an established safety record.
Response: Comment partially incorporated. Informational chapters provide overarching principles rather than detailed protocols. USP <1119> now includes a reference to <1223> for validation frameworks, supporting innovation and the adoption of new technologies.
Comment Summary #15: Several commenters recommended that the scope be expanded to include advanced microbiological methods for bioburden monitoring, such as solid phase and flow cytometry, PCR amplification, ATP measurement, and biofluorescent particle counting (BFPC). Additionally, they highlighted the need for a more comprehensive discussion on alternative methods, particularly for in-process monitoring, where regulatory hurdles may be lower than those for finished product testing.
Response: Comment incorporated. USP recognizes that many manufacturers are adopting rapid microbiological methods and that USP <61> alone is not sufficient for all scenarios. Therefore, the final text highlights the possibility of using alternative techniques (e.g., flow cytometry, digital detection) with proper method validation
Comment Summary #16: Commenters requested clarification on the scope of the chapter, specifically regarding the inclusion of sterile and nonsterile pharmaceutical products, biopharmaceuticals, and drug substances.
Response: Comment not incorporated. This chapter is meant for in-process monitoring or any bioburden tests that are not monograph release specifications.
Comment Summary #17: Several commenters suggested that throughout the document, the term “sample location” be replaced with “sample point” to better clarify the intent and accurately represent the bioburden sample types within the scope of the chapter.
Response: Comment partially incorporated. USP uses “location” in a broader sense for environmental and in-process sampling (such as surfaces or containers), while “point” can be used for a more precise port or line sampling approach.
Comment Summary #18: Several commenters suggested that throughout the document, the terms “limit,” “specification,” and “acceptance criteria” be replaced with the term “level(s).” They indicated that since the scope of the chapter is focused on bioburden monitoring rather than release testing, the term “level” would more accurately reflect this scope.
Response: Comment partially incorporated. Although monitoring may not equate to an official release test, the principle of having a defined upper boundary or limit remains. The chapter maintained consistent pharmacopeial phrasing to describe the maximum allowable microbial burden.
Comment Summary #19: The commenter indicated that certain statements in the chapter could be interpreted as requirements rather than guidance, potentially leading to their adoption as de facto standards in the absence of specific FDA guidance. They also noted that specifications may vary based on product and in-process sampling points, which fall under FDA’s regulatory authority.
Response: Comment not incorporated. Chapters numbered above <1000> are considered informational and intended as guidance, even if some wording appears prescriptive. The chapter does not establish enforceable requirements.
Comment Summary #20: The commenter suggested canceling <1119> and adding a brief section on investigations in <1119.1>. The commenter indicated that <1119> lacks guidance on

the investigative approach if the sample exceeds proposed limits, which could lead to compliance issues and potential rejection of products by FDA investigators and external auditors. The commenter expressed concerns about the inability of laboratory investigations to uncover sampling or testing errors and the implications of bioburden test results exceeding proposed limits.

Response: Comment not incorporated. Investigations are beyond the scope of the chapter. The bioburden limits are recommendations only, and the chapter is clear that alternative limits should be justified by the user. Currently, most firms have established their own justified limits and bioburden monitoring and testing compliant with CGMPs.

Comment Summary #21: The commenter recommended addressing the issue related to USP <61> and its alignment with the USP <1117.1> definition of a Bioburden Test. Specifically, they noted that the "Appendix" does not flow correctly because USP <61> appears to meet the definition of a Bioburden Test ("a test that quantifies bioburden in a sample").

Response: Comment not incorporated. The USP <1117.1> definition for a bioburden test includes an important note that differentiates it from the microbial enumeration test described in <61>. The figure and references in the final document continue to position <61> separately from "bioburden tests" to maintain the regulatory distinction between final product testing and in-process or intermediate sample testing.

Comment Summary #22: The commenter requested clarification on whether chapter <1119> would replace USP <61> as the guidance document for in-process monitoring and testing of bioburden and release testing of bulk drug substances.

Response: Comment not incorporated. If you are monitoring bioburden in-process (e.g., during production steps, in-process materials, and intermediates) and not using that test for official release acceptance, then <1119> can serve as the guidance document. In practice, many manufacturers will use both: <61> for final acceptance per <1111> and <1119> for in-process or intermediate checks. Therefore, <1119> does not displace <61> for release testing, but it does fill a gap for monitoring scenarios where <61> is not explicitly required.

Comment Summary #23: The commenter indicated that the high nutrient level of soybean–casein digest agar, its neutral pH, and the 30–35°C incubation temperature make it unsuitable for the enumeration of waterborne bacteria and environmental molds. The commenter also noted that literature suggests that only 0.1–1% of viable microorganisms can be isolated on conventional culture media, referencing USP <1223>. Additionally, the commenter mentioned that while the growth promotion and suitability testing challenge microorganisms found in <61> have a long history of use in compendial testing of non-sterile drug substances and drug products, they may not be suitable for in-process samples such as downstream processing materials in monoclonal antibody purification and pharmaceutical grade water. Therefore, the commenter suggested that other media and challenge organisms should be proposed in <1119>.

Response: Comment incorporated. The final version of <1119> includes a more explicit statement that users may adapt the standard total aerobic microbial count method, including choosing different media, temperatures, and microorganisms, if the anticipated bioburden will not be adequately recovered by typical TSA at 30°C–35°C.

Comment Summary #24: The commenter indicated that the bioburden limit of final sterilization items is related to achieving the sterility assurance level (SAL). They provided an example where the irradiation sterilization dose is established based on the bioburden on the product, referencing ISO 11137-2, method 1. They noted that to achieve an SAL of 10^{-6}, different bioburden levels require different sterilization doses, such as 17.6 kGy for a bioburden of 10 CFU and 21.2 kGy for a bioburden of 100 CFU. The commenter suggested that different sterilization parameters necessitate different bioburden limits.

Response: Comment not incorporated. USP <1119> acknowledges that different sterilization processes may require different bioburden limits to reliably achieve 10^{-6} SAL. Although it does not prescribe separate limits for every sterilization modality, <1119> offers a risk-based approach where acceptable pre-sterilization limits are determined based on how robustly the validated sterilization process can destroy or remove the bioburden.

Comment Summary #25: Several commenters requested clarification on plans to issue guidance on BET monitoring for materials tested outside of finished product release, including in-process samples, drug substances, and components.
Response: Comment not incorporated. USP recommends that the commenter make an inquiry to the USP contact for BET for further information.

Introduction
Comment Summary #26: The commenter suggested providing further clarification regarding the scope of the chapter, indicating that it could be interpreted as applying to materials used for classical non-sterile products. They noted that the context, particularly Table 2, implies it pertains to materials for manufacturing sterile products, such as biologics. Additionally, the commenter recommended relocating the first sentence to follow the sentences that clarify the scope to enhance the flow of the text.
Response: Comment partially incorporated. The text was revised to clarify the scope and reference the figure after the scope statement. The exact wording was adjusted to maintain consistent USP style and to cover in-process materials in non-sterile manufacturing.
Comment Summary #27: The commenter suggested that clarification is needed to specify that this chapter is intended for non-sterile drug substances (DS) going into sterile drug products (DP). Additionally, the commenter indicated that a definition for “purified drug substance” should be included either in <1119> or <1117.1> *Microbiological Chapters – Glossary*.
Response: Comment partially incorporated. The recommendation for a future change to section <1117.1> is noted, but it is out of scope for this revision.
Comment Summary #28: The commenter suggested that the instructions for performing testing of non-sterile product-related samples may lead to confusion or misunderstanding. They indicated that bioburden testing for these samples might be better suited with USP <61> testing. To enhance clarity, the commenter recommended including a statement that for non-sterile product dosage in-process samples and component testing, the use of either USP <1119.1> or USP <61> may be appropriate.
Response: Comment partially incorporated. The recommendation to clarify the testing options for non-sterile product dosage in-process samples has been acknowledged. It is noted that firms may choose bioburden testing, and USP <61> is applied per instruction from USP <1111>.
Comment Summary #29: The commenter suggested adding the terms "raw materials" and "excipients" to the scope of the chapter, noting that these materials may undergo bioburden monitoring, and that this addition would provide additional clarity, particularly since not all have monographs.
Response: Comment incorporated. The sentence was updated to: "The scope of this chapter includes any material intended for use in the manufacture of nonsterile, sterile, and low bioburden products that is subject to a test for bioburden as defined in Microbiological Chapters—Glossary <1117.1>; this includes, but is not limited to, in-process samples (e.g., sample stages upstream and before final bioburden reduction in the case of sterile products), drug substances, components, raw materials, and excipients and water."
Comment Summary #30: Several commenters requested the removal of "water" from this chapter, noting that it is already described in USP <1231>. One commenter also pointed out ambiguity regarding the type of water referenced (e.g., pharmaceutical water or water used for rinsing), which could lead to different testing interpretations under USP <1119>, USP <1119.1>, or USP <1231>. Additionally, concerns were raised about potential method suitability requirements for water under USP <1119.1> and the applicability of the testing methods outlined in USP <1231>.
Response: Comment not incorporated. The final text still broadly includes “water” under the scope of <1119> but defers Purified Water and WFI acceptance criteria to <1231>, leaving interpretation of other water uses (such as rinse water) to the manufacturer’s internal risk assessment.
Comment Summary #31: The commenter suggested that nonsterile substances, including active substances, are clearly indicated as in scope for USP<61>. They proposed clarifying that this drug substance refers to biologics such as protein bulk drug substance.

Response: Comment not incorporated. The chapter will be clarified to separate out nonsterile finished drug products and nonsterile substances for pharmaceutical use, which are destined for nonsterile finished drug products.
Comment Summary #32: The commenter requested clarification on the term "components" used in the Introduction section, asking whether it refers to primary packaging materials, formulation excipients, raw materials, or media components.
Response: Comment not incorporated. The term "component" is described in more detail in Table 3.
Comment Summary #33: The commenter suggested that the definition of bioburden should include both aerobic and anaerobic microorganisms, noting that *Clostridiae*, which are anaerobic, are prevalent in nature.
Response: Comment incorporated. A change was made in the revised version to clarify that bioburden may include anaerobic organisms (and other growth categories). The revised text now states: “Bioburden includes aerobic, facultative anaerobic, aerotolerant, and anaerobic microorganisms … In most cases, bioburden comprises mesophilic aerobic microorganisms …”
Comment Summary #34: The commenter recommended expanding the scope to include not only the modification of the method described in <1119.1> but also referencing Chapter <1223> for an alternative suitable, validated method: Validation of Alternative Microbiological Methods.
Response: Comment incorporated. Updated text to read: “In other cases, however, a different type of bioburden may be anticipated. This would require either modification of the method described in <1119.1> or the use of an alternative, suitably validated method…”
Comment Summary #35: The commenter suggested using alternative language for "executed upon" because this wording could be misinterpreted. The proposed wording aims to clarify that testing is being discussed.
Response: Comment incorporated. Sentence was edited as follows: “Bioburden monitoring is an important piece of the microbial contamination control strategy in the manufacture of nonsterile, low-bioburden, and sterile pharmaceuticals and is performed for a diverse range of sample types.”
Comment Summary #36: The commenter indicated that the reference to nonsterile and low-bioburden sample types in the statement "Bioburden monitoring is a critical activity in the manufacture of nonsterile, low-bioburden, and sterile pharmaceuticals executed upon a diverse range of sample types" implies relevance to the chapter, despite the briefing explaining that these types of samples are exempt.
Response: Comment not incorporated. While <61> (in conjunction with <1111>) addresses final-release testing of nonsterile products, <1119> specifically covers in-process or intermediate materials in both sterile and nonsterile manufacturing, and “low-bioburden” products. Even in a nonsterile production process, there may be points upstream (e.g., raw materials and intermediates) where bioburden limits and monitoring are necessary for quality control and contamination risk assessment. Therefore, mentioning “nonsterile and low-bioburden” remains relevant to the scope of the chapter.
Comment Summary #37: Several commenters recommended elaborating the current wording to provide an explicit reason for the need to perform an analysis of the bioburden of the item. By adding this clarifying statement, the reader is explicitly informed of the reason an analysis of bioburden would be needed and is directed to link this action to their microbiological contamination controls. Additionally, the proposed wording aligns with the European Commission Guidance Document “Annex 1: Manufacture of Sterile Medicinal Products, EudraLex- Volume 4-EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use, 2022.”
Response: Comment partially incorporated. Text was revised as follows: “Bioburden monitoring is an important piece of the microbial contamination control strategy in the manufacture of nonsterile, low-bioburden, and sterile pharmaceuticals … The purpose of bioburden monitoring is to ensure that the microbial load remains acceptable to mitigate the risk of microbial impurities, which may adversely impact product quality and stability.”
Comment Summary #38: The commenter indicated that bioburden is a point-in-time monitoring method that does not ensure a consistent microbial load. It is used to monitor and verify that the

test article, such as a pharmaceutical, raw material, or manufacturing supply, maintains a state of control or demonstrates decontamination.
Response: Comment incorporated.
Comment Summary #39: The commenter indicated that the intent of the statement in the introduction may not be clear to the reader and could be misinterpreted as bioburden monitoring following sterilization processes. They suggested providing clarifying wording to ensure it is understood that the intention was around assessing microbial load prior to sterilization. The proposed change was to specify that bioburden monitoring would be prior to the sterilization process to ensure the effectiveness of the sterilization processes.
Response: Comment partially incorporated. The sentence was changed to "Where necessary, bioburden monitoring can also be used to support the effectiveness of any microbial reduction process (e.g., sterile filtration, disinfection, or sterilization). However, high bioburden acceptance criteria should not be justified by the capacity of the sterilization or bioburden reducing step."
Comment Summary #40: The commenter indicated that bioburden monitoring is performed as part of terminal sterilization to verify the effectiveness of the selected sterilization modality, model, or challenge, rather than to demonstrate sterility assurance. The commenter suggested that bioburden testing should be used as supporting documentation for the selection of the sterilization process and not as a demonstration of sterility.
Response: Comment partially incorporated. While the final chapter does not explicitly state that “this test is not to demonstrate sterility,” it clarifies that bioburden monitoring is a supportive measure in verifying the microbial reduction process and should not be misconstrued as a standalone demonstration of sterility. The text was revised to: “Where necessary, bioburden monitoring can also be used to support the effectiveness of any microbial reduction process (e.g., sterile filtration, disinfection, or sterilization). However, high bioburden acceptance criteria should not be justified by the capacity of the sterilization or bioburden-reducing step.”
Comment Summary #41: The commenter recommended adding text to discuss the use of existing manufacturing process reviews and risk assessments as part of creating a bioburden monitoring program. Additionally, the commenter suggested including language around the use of process analytical technology. They noted that manufacturers of drugs and biologics often have detailed manufacturing process reviews and risk assessments focused on microbial contamination, which can be utilized in establishing a sampling and testing regime. They also highlighted the importance of accounting for newer technologies/platforms involving on-line or in-line testing versus grab samples.
Response: Comment partially incorporated. The final text maintains a general guidance approach, expecting manufacturers to incorporate or leverage existing process reviews, risk assessments, and any process analytical technology approaches as suitable for their operation.
Comment Summary #42: The commenter suggested that only critical factors related to microbial quality should be included in the risk assessment section. They proposed a change to focus specifically on the assessment of critical manufacturing inputs that impact microbial quality.
Response: Comment not incorporated. USP <1231> does not contain the relevant details that are found in USP <1119>.
Comment Summary #43: The commenter indicated that the term "volume" may imply that the bioburden sample is a pharmaceutical product, whereas bioburden can also apply to materials such as vials or other components from the manufacturing process. To address this concern, the terminology has been adjusted from "volumes" to "quantities" for clarity.
Response: Comment incorporated. The change has been made to ensure that the language accurately reflects the broader application of bioburden testing.
Comment Summary #44: The commenter indicated that the term "location" is confusing in the context of discussing points in the manufacturing process where a bioburden sample should be taken to monitor the state of control.
Response: Comment incorporated. Final text reads: “A bioburden program must include appropriate sample points, sample quantities, and test methods.”

Risk-based Bioburden Monitoring

Comment Summary #45: The commenter suggested that the term "locations" is confusing and recommended using language that reflects sampling occurring at a phase in the manufacturing process rather than a specific location. They proposed a change to use the term "process monitoring steps."

Response: Comment incorporated. The sentence has been updated to read: "The program also guides the determination of sampling points..."

Comment Summary #46: Several commenters suggested updating the sentence to clarify that the intent is about risk assessment rather than “management tools” in the section on Risk-Based Bioburden Monitoring.

Response: Comment incorporated. The sentence has been updated to remove “management tools” and now references “risk assessment” with “appropriate tools,” reflecting the commenters’ suggestion.

Comment Summary #47: Several commenters recommended removing the example of “classification status of cleanrooms” associated with the bullet for facility environmental conditions in the section on Risk-Based Bioburden Monitoring. Additionally, the commenter noted that reviewing trending data provides an opportunity to assess potentially problematic sampling areas, although this data may not always be available. They also suggested adding bullet points for additional items to be included in the risk assessment, specifically mentioning microorganisms expected to represent an increased risk to the manufacturing process and the review of historical data.

Response: Comment partially incorporated. The suggestion to remove “cleanroom classification” was not adopted because it is a relevant factor in a microbial contamination risk assessment. However, the new bullets were added to emphasize the importance of assessing the types of organisms most relevant to the process and leveraging trending/historical data.

Comment Summary #48: The commenter suggested that the volume of material can vary at different phases of the process and that this should be linked to sample quantities. They proposed adding a specific mention of the volume of material being processed or produced in the section on Risk-Based Bioburden Monitoring.

Response: Comment partially incorporated. A bullet point was added to address the "Quantity of material processed or produced."

Comment Summary #49: The commenter indicated that the section on Risk-Based Bioburden Monitoring does not list the growth-promoting properties of a material, while it does list inherent antimicrobial aspects of the material to be considered. The commenter suggested adding “inherent microbial conducive properties of the materials” to the list.

Response: Comment partially incorporated. While the principle behind the comment, considering whether a material is conducive to microbial proliferation is valid, the chapter’s final wording remains concise. The existing bullet points serve as high-level prompts for a more detailed, site-specific risk assessment.

Comment Summary #50: Several commenters suggested including 'Operator intervention / handling of product' in the list for the section on risk-based bioburden monitoring. This addition aims to enhance the clarity and comprehensiveness of the monitoring process by acknowledging the role of operator actions in product handling.

Response: Comment partially incorporated. The term “Operator intervention / handling of product” was not added verbatim; however, the final version includes “Manual interventions” in the bullet list.

Comment Summary #51: The commenter suggested removing the example of “classification status of cleanrooms” from the bullet point on environmental conditions within the facility. They indicated that some facilities following the Chapter guidance do not have classified cleanroom areas, and removing this example would eliminate possible confusion while still aligning with the bullet’s intent.

Response: Comment not incorporated. The final text retains the parenthetical example and does not remove “classification status of cleanrooms.” The bullet now reads: “Environmental conditions within the facility (e.g., classification status of cleanrooms) and facility cleaning and sanitization …”

Comment Summary #52: The commenter suggested that if the classification of the room is being considered, it would be logical to include the microbial control procedures as well.
Response: Comment incorporated. "Facility cleaning and sanitization" was added to the bullet list.
Comment Summary #53: The commenter requested additional clarification regarding the term "Storage Condition" in the context of Risk-Based Bioburden Monitoring, specifically whether it pertains to process storage (material/time/temperature) or material/sample storage.
Response: Comment incorporated. The clarification provided states that "Storage conditions (e.g., duration, temperature, container or vessel) for raw materials, intermediates, and materials subject to testing" addresses process storage (material/time/temperature) for items in the manufacturing chain before sampling.
Comment Summary #54: The commenter suggested being specific to the product in the context of the Risk-Based Bioburden Monitoring section, as it can be confused with the facility cleaning and disinfection program.
Response: Comment partially incorporated. A distinction was added by modifying the point to include "Environmental conditions within the facility (e.g., classification status of cleanrooms) and facility cleaning and sanitization" as a separate category from "Sanitization, decontamination, and other active microbial control processes (e.g., filtration, temperature, pH, osmolarity, water activity)."
Comment Summary #55: Several commenters suggested adding an extra bullet regarding final microbial control, specifically mentioning aseptically processed and terminally sterilized methods.
Response: Comment partially incorporated. A bullet point for final microbial control was added, but it uses "filtration for aseptic manufacturing" rather than explicitly including both "aseptically processed" and "terminally sterilized" as requested.
Comment Summary #56: The commenter suggested that the final state of microbial control for the product should be included in the risk assessment. They proposed adding a reference to final microbial control methods, such as aseptically processed or terminally sterilized.
Response: Comment incorporated. The risk assessment now includes a reference to "final microbial control," which aligns with the comment's suggestion.
Comment Summary #57: The commenter recommended that the bioburden risk assessment should be performed by a cross-functional team knowledgeable in the manufacturing process and microbiology. They also suggested that the risk assessment should be reviewed periodically and when any substantive changes occur to the manufacturing process. Additionally, the commenter proposed adding the formulation and intended use of the drug product to the manufacturing process.
Response: Comment partially incorporated. The bioburden risk assessment section has been expanded significantly to include related considerations such as materials, storage, and antimicrobial properties.
Comment Summary #58: The commenter recommended that the term “manufacturing process” be reworded to expand the scope for when a risk assessment should be reviewed. This change aims to clarify for the reader the elements assessed as part of the risk assessment when determining changes that would trigger a risk-assessment review, encouraging a more holistic approach.
Response: Comment incorporated. The revised sentence now reads: "The risk assessment should be reviewed periodically and when changes are made to any risk-assessment parameters or adverse bioburden trends detected."
Comment Summary #59: The commenter suggested that manufacturing personnel involvement in the risk assessment could cause a conflict of interest and influence the decision. They recommended that the bioburden risk assessment should be performed by the Microbiology department and Quality Assurance department to ensure that microbiology aspects and quality are maintained. Additionally, they noted that the microbiology SME involved should have knowledge of the manufacturing process.
Response: Comment not incorporated. The final text does not assign exclusive responsibility to QA or microbiology.

Comment Summary #60: The commenter suggested that adverse trends indicate a change, necessitating a reevaluation of the risk assessment. They proposed that the risk assessment should be reviewed periodically and whenever substantive changes occur in the manufacturing process or adverse bioburden trends are detected.
Response: Comment incorporated. The text has been updated to: "The risk assessment should be reviewed periodically and when changes are made to any risk-assessment parameters or adverse bioburden trends detected."
Comment Summary #61: The commenter suggested that the risk assessment should be reviewed periodically and also when any substantive changes occur to the manufacturing or cleaning/sanitization process. They emphasized the importance of including changes to the cleaning/sanitization process as a trigger for review.
Response: Comment partially incorporated. The text was changed to: "The risk assessment should be reviewed periodically and when changes are made to any risk-assessment parameters or adverse bioburden trends detected."
Comment Summary #62: The commenter suggested that changes to the raw materials of the product should be included in the risk-based bioburden monitoring section.
Response: Comment partially incorporated. Sentence updated to "… when changes are made to any risk-assessment parameters or adverse bioburden trends are detected."
Comment Summary #63: The commenter suggested allowing a bracketing approach for the risk assessments to cover products with a similar manufacturing process in the Bioburden Monitoring section.
Response: Comment not incorporated. The chapter presents recommendations but is not limited to those listed in the chapter.
Comment Summary #64: The commenter indicated that the chapter on Risk-Based Bioburden Monitoring should be specific in excluding particulates and other non-viable contamination.
Response: Comment incorporated. The word "microbial" was added in front of "contamination" to clarify that the chapter's scope excludes particulates and non-viable contamination.

Bioburden Sampling
Comment Summary #65: The commenter indicated that Table 1 highlights considerations for the selection of sampling points, sample quantity, location, and frequency, and that each selection should be accompanied by a documented rationale. The commenter also stated that Table 1 does not highlight sample quantity but refers to Table 2, which specifies the sample quantity.
Response: Comment partially incorporated. The text was modified to remove the redundant mention of "location," which was included as "sampling points."
Comment Summary #66: The commenter suggested that the section on Bioburden Sampling be updated to be consistent with other proposed changes for the selection of sampling for process monitoring steps, sample size, and frequency, with each selection accompanied by a documented rationale.
Response: Comment partially incorporated. The terminology has been updated to "sampling points," "sample quantity," and "frequency" to align with the suggested changes.
Comment Summary #67: The commenter suggested that the time duration between bioburden sample collection and testing should be provided to avoid false positive and false negative bioburden test results.
Response: Comment not incorporated. The chapter addresses this concern by requiring users to document storage conditions (e.g., temperature, location) and the allowable hold time before testing. Extended hold times must be justified through data (e.g., stability studies or sample-handling validations) to demonstrate that microbial counts remain representative over the chosen duration and storage conditions.
Comment Summary #68: The commenter requested that storage conditions of 2–8°C be provided after sample receipt if the test is not planned immediately.
Response: Comment incorporated.

Table 1

Comment Summary #69: The commenter suggested changing the title of Table 1 from “Considerations for Bioburden Sampling” to “Considerations for Bioburden Testing.” Additionally, the commenter recommended removing the “frequency of sampling” and “sample location” topics, stating that these are CGMP decisions made by the FDA and expressing concern that the current wording could justify inappropriate in-process bioburden sampling frequencies.

Response: Comment not incorporated. "Bioburden Sampling" more accurately describes the content of the table, which includes sampling points, frequency, and storage conditions, rather than the broader term "Testing." The decision to retain the topics of frequency and sampling points was made to ensure a robust bioburden monitoring strategy, with the assumption that users would interpret this guidance within the context of compliance requirements.

Comment Summary #70: The commenter recommended adding two additional references to Table 1. The first reference pertains to a publication that provides guidance on statistical-based microbiological sampling and the minimum sample volumes needed to detect low-level, non-homogenously distributed bioburden. The second reference is to the EU Annex 1, section 8.93, which discusses the procedures for taking bioburden samples from bulk products and prior to final sterile filtration.

Response: Comment not incorporated. The existing references are deemed sufficient for the intended scope.

Comment Summary #71: The commenter suggested adding to Table 1 a definition of how samples are to be taken at each location to avoid adventitious contamination.

Response: Comment not incorporated. Table 1 is intended to outline sampling locations rather than procedural methods for sample collection.

Comment Summary #72: The commenter suggested adding a new section to Table 1 that will let users understand which containers can be used during bioburden sampling. The proposed change includes specifying the use of sterile sampling systems to avoid cross-contamination and the use of pre-sterilized sampling accessories and containers. Additionally, the commenter noted that depyrogenated accessories should be used when a BET sample is needed to avoid endotoxin contributory factors.

Response: Comment not incorporated. The detail provided is beyond the intended scope of the general guidance chapter. This topic is more appropriate for site-specific standard operating procedures rather than inclusion in a general guidance chapter.

Guidance and Recommendations

Comment Summary #73: The commenter suggested referencing ISO 11737-2 in the section discussing sterilization.

Response: Comment not incorporated. The <1229> series covers sterilization and addresses the concerns raised by the commenter.

Comment Summary #74: The commenter recommended updating the list of relevant regulatory guidance by removing a specific reference, adding new references, and correcting an editorial duplication.

Response: Comment partially incorporated. The editorial duplication was corrected as suggested; however, the proposal to replace regulatory guidance references was not accepted. The EC decided not to expand the list with additional references.

Comment Summary #75: The commenter suggested identifying relevant regulatory guidance for the control and monitoring of bioburden, including sampling size and frequency, and providing examples of such guidance.

Response: Comment not incorporated. Existing guidances already cover all relevant aspects and only examples can be provided since regulations may change or vary based on local requirements.

Sample Location

Comment Summary #76: The commenter recommended removing or modifying the statement "Prior to filling a primary container for a nonsterile product." They indicated that this statement could lead users to incorrectly apply USP <1119.1> for final product release testing, as it may

be equivalent to a final product release test before bulk product is filled in a bottle and tested according to USP <61>.
Response: Comment incorporated. The statement was removed entirely from Table 1.
Comment Summary #77: The commenter suggested that the sample container, sample port location, and precautions for aseptic technique and gowning are relevant at all stages/steps mentioned in Table 1.
Response: Comment not incorporated. The existing text illustrates general principles and does not limit them to purification steps only.
Comment Summary #78: The commenter indicated that during different stages of biologics manufacturing, such as prepared media, buffers, and feed solutions, it is inferred that bioreactor media has an allowable level of bioburden. The commenter suggested that the preferred method for bioreactor media and other upstream samples should be methods adapted from USP<71>.
Response: Comment not incorporated. Table 1 references bioreactor media as an example of in-process sampling. The Expert Committee acknowledged that some manufacturing contexts, such as those involving ATMPs, present challenges for traditional bioburden testing; however, the chapter is intended to provide general guidance across a broad range of biologics manufacturing processes. As such, it does not prescribe specific methods or conditions, nor does it include references to USP <71> or specialized considerations for ATMPs, to maintain flexibility and applicability across different manufacturing scenarios.
Comment Summary #79: The commenter suggested that sampling practices considerations should be included in the risk assessment mentioned in Table 1.
Response: Comment incorporated. The sampling points have been expanded to include upstream steps before bioburden reduction.
Comment Summary #80: The commenter recommended clarifying the term "product" in the second bullet of Table 1, as the current language states "nonsterile product," which is not within the scope of this chapter. Additionally, the commenter suggested adding examples to the third bullet to assist the reader and prevent potential confusion by referencing material/product not within the scope of the chapter and to provide guidance on selecting manufacturing stages to be monitored for bioburden.
Response: Comment partially incorporated. The term "Sample location" was changed to "Sample points," reflecting part of the suggestion. However, the explicit clarification "low bioburden bulk product" was not included. The EC preferred to maintain broader applicability, as the existing examples sufficiently met their intended guidance purposes.
Comment Summary #81: The commenter suggested revising the term “immediately” in the guidance for sampling prior to bioburden reduction filtration, as it may lead to overinterpretation and confusion due to the variety of applications for in-line filtration. They recommended using an alternative phrase such as “process streams directly upstream of bioburden reduction filtration or sterilization” to better reflect the appropriate intent.
Response: Comment not incorporated. Risks with sampling are not considered microbial control risks within manufacturing. The same concept could be applied to the micro test and sample handling, which are out of scope.

Sample Storage Condition
Comment Summary #82: Several commenters requested editorial and clarity improvements to the wording of sample storage conditions, specifically revising "storage beyond 2°–8° at 24 h" to "storage beyond 24 h at 2°–8°" to better reflect intent and avoid misinterpretation.
Response: Comment incorporated. The wording was updated to "Storage beyond 24 h at 2°–8.”
Comment Summary #83: The commenter requested that 4 h at ambient conditions be added as a permissible hold time, consistent with USP <797>. They also inquired whether this hold time would be universally permissible or only applicable to materials without known antimicrobial activity, such as those with pH 4–9, isotonic solutions, or those with no or low levels of residual solvents.

Response: Comment not incorporated. Establishing a universal 4-h ambient hold time is not appropriate for this context. The hold time should be justified through a risk-based approach specific to the method and material characteristics.
Comment Summary #84: The commenter requested clarification that the justification and qualifications must be performed for liquid samples stored above 2°–8°C or for more than 24 h.
Response: Comment not incorporated. The requirement is intended to apply to all sample types (solid or liquid) and not just liquids, avoiding unnecessary specificity. The original statement requiring justification and qualification is considered sufficiently broad to cover all necessary cases, including liquid samples.
Comment Summary #85: The commenter suggested rewording the section on sample storage conditions to include context that ensures a reliable and representative microbial count. The proposed change was to define storage conditions that samples may be held at prior to testing to ensure a representative result for the analyte.
Response: Comment not incorporated. The intent is already implicit in requiring justification and qualification for extended storage.
Comment Summary #86: The commenter requested clarification on the types of samples that need to be stored at 2-8 degrees Celsius, noting that USP <1117> does not specify the sample types, which include in-process samples (liquid), water, rinse water, and swab samples.
Response: Comment not incorporated. The EC intended to keep the guidance general to allow flexibility in practices specific to user processes and internal procedures. Explicitly naming sample types could limit or overly prescribe practices beyond the scope intended by this general guidance document.
Comment Summary #87: Several commenters requested an editorial clarification to define the storage conditions for samples prior to testing. They suggested specifying that storage conditions, including temperature and location, must be defined. They also recommended that storage beyond 24 hours at 2°–8°C would be permissible with appropriate justification and qualification, referencing Microbiological Best Laboratory Practices <1117>.
Response: Comment incorporated. The storage conditions have been updated to specify "24 hours at 2°–8°C."

Bioburden Test Method
Comment Summary #88: The commenter indicated that the term "total aerobic microbial count" used in the bioburden test described in <1119.1> could be confusing to stakeholders, as it is a term typically used in USP <61>.
Response: Comment not incorporated. The phrase “total aerobic microbial count” is retained in <1119.1> to ensure consistency with terminology used in other compendial chapters. This term appropriately describes the microbial population targeted by the bioburden test and supports clear communication of the scope of testing across different USP chapters.
Comment Summary #89: The commenter indicated that the bioburden test method section mentions user requirements but does not explain where these types of user requirements reside.
Response: Comment not incorporated. This is up to each individual firm.
Comment Summary #90: The commenter suggested that the specific user requirement document should be optional, as this information will already be included in the risk assessment or method validation documentation.
Response: Comment not incorporated. The text does not specify that the user requirements should be a distinct individual document and allows for these requirements to be stated in other documents.
Comment Summary #91: The commenter suggested simplifying the bioburden testing method by eliminating the creation of a separate bioburden section <1119.1> and using USP <61> for all testing. This change would reduce the risk of misalignment between two similar methods. Additionally, the commenter proposed adding clarification that only Total Aerobic Microbial Count (TAMC) is required.
Response: Comment partially incorporated. The clarification emphasizing TAMC was addressed, maintaining emphasis on TAMC while still allowing flexibility. However, the

reference to <1119.1> remains to retain method flexibility within the scope of bioburden monitoring.

Table 2

Comment Summary #92: The commenter noted that Table 2 does not include sample quantity for bioburden sampling, even though it is mentioned in Table 1.
Response: Comment incorporated. A row for test quantity has been added to Table 2 for consistency with Table 1.
Comment Summary #93: The commenter suggested that the language in Table 2 should be consistent and less confusing. They indicated that the bioburden method can include components such as stoppers, and therefore, the language needs to be broad enough for the reader to understand that it may not just be the pharmaceutical product being tested.
Response: Comment partially incorporated. The final wording allows for dilutions and adjustments but avoids specifying pooling, as pooling may not be applicable in all bioburden testing scenarios. The committee kept the language broad to ensure clarity and applicability across different contexts.

Purpose

Comment Summary #94: Several commenters indicated that excluding TYMC from the bioburden test is not in line with USP <61> for testing non-sterile products. They acknowledged that it might be acceptable in some specific conditions to only test for TAMC but proposed emphasizing the risk related to other organisms that might be present within the bioburden, such as aerotolerant anaerobe organisms in mammalian cell culture steps.
Response: Comment partially incorporated. Instead of explicitly adding TYMC and aerotolerant anaerobes, the committee kept the requirement open-ended to allow for different organisms based on risk assessment.
Comment Summary #95: The commenter requested clarification on how the anticipated bioburden is defined in Table 2. They noted that the purpose row states that if the nutrient culture media and incubation conditions detailed in <1119.1> are insufficient to recover the anticipated bioburden, appropriate changes should be stated and justified. However, there is no explanation in the table on how the anticipated bioburden is defined, questioning whether it is based on Environmental Monitoring (EM) results or process experience from development.
Response: Comment partially incorporated. While Table 2 was not explicitly revised to include this clarification, the broader chapter context addresses the factors that contribute to defining anticipated bioburden, including Environmental Monitoring results and process development experience.
Comment Summary #96: The commenter suggested aligning the wording in the text and the table of the Bioburden Test Method to make the intent clearer. They indicated that aligning the language would remove any potential reader misunderstanding due to assuming word choice was intentionally designed to convey the level of requirement, specifically the use of “may” versus “should.”
Response: Comment incorporated. The sentence was updated to: "If the method detailed in <1119.1> cannot detect the anticipated bioburden, different nutrient culture media, incubation conditions, and growth promotion should be stated and justified."

Acceptance Criteria

Comment Summary #97: The commenter recommended aligning the wording in Table 2 to the Section Assessment of Bioburden Monitoring to make the statement clearer and create a link for the reader due to the alignment of the wording.
Response: Comment partially incorporated. Minor editorial changes were made, such as changing "amount of sample" to "sample quantity." However, the primary comment regarding changing "Acceptance Criteria" to "Bioburden Level" was not incorporated. The EC kept the original terminology ("Acceptance Criteria") to ensure consistency and clarity.

Test Method Type
Comment Summary #98: The commenter recommended including a provision for direct inoculation-based microbiological testing in Table 2. They noted that for cell-based solutions requiring presence/absence microbial contamination, direct inoculation to TSB for mesophilic aerobic microbial detection is a typical approach. This method, although qualitative, is subject to interference testing using methodology derived from USP <71>.
Response: Comment not incorporated. The chapter retains its quantitative method focus without provision for qualitative direct inoculation.

Suitability Requirements
Comment Summary #99: The commenter requested clarification on the suitability requirements in Table 2, specifically asking for an expansion on the consideration of chemical properties of the sample, including pH and antimicrobial properties.
Response: Comment not incorporated. This topic is already addressed in other chapters, which provide guidance on the impact of chemical properties on microbial testing. Expanding it here would be redundant.

Identification Requirements
Comment Summary #100: The commenter suggested that the significance of microorganisms recovered should be evaluated based on the use of the product, the nature of the product, and the downstream processing and its impact on final product quality. They indicated that the impact of microbial contamination varies significantly based on the species of microorganisms present and recommended alignment with USP<1111>.
Response: Comment not incorporated. This will be covered in a future new chapter on “objectionable microorganisms.”

Inherent Microbial Load
Comment Summary #101: The commenter suggested including the potential for serial dilution as an option in addition to testing smaller test quantities in Table 2 regarding inherent microbial load.
Response: Comment incorporated. Resolved by adding dilutions, maintaining quantities, and considering volumes.

Assessment of Bioburden Monitoring
Comment Summary #102: The commenter indicated that the statement regarding cleaned equipment surfaces being equal to the specification of the final rinse water and the evaluation of recovered microorganisms for nonsterile products is only relevant to low bioburden or nonsterile products. They noted that this chapter is not intended to be applicable for these products.
Response: Comment incorporated. Revised text in Table 3 now reads: "Cleaned equipment rinse samples: Equal to the specification of the final rinse water. For nonsterile products, recovered microorganisms should be evaluated to determine if they are objectionable in case no further downstream controls are in place (see Bioburden Control of Nonsterile Drug Substances and Products <1115>)."
Comment Summary #103: Several commenters suggested providing guidance on determining acceptable microbial loads for raw materials and in-process products. They recommended including a statement that bioburden levels should be set through a risk assessment, considering factors such as the potential for harboring or proliferating microorganisms and subsequent bio-reduction or sterilization processes.
Response: Comment partially incorporated. The footnote for Table 3 has been adjusted to capture that a risk-based rationale is expected when setting bioburden levels.
Comment Summary #104: The commenter recommended that the impact of microbial contamination should be considered based on the species of microorganisms present in the test user requirements. They emphasized that it is not sufficient to simply monitor numbers, as pre-sterilization samples containing Gram-negative bacteria may lead to endotoxin contamination in the final product. The commenter suggested aligning with USP<1111> and proposed that an assessment of recovered species should be completed to determine if they represent a loss of

control, risk to product quality, or patient risk. This assessment should include species-level identification and a risk assessment of the impact on product quality due to the organisms present.
Response: Comment not incorporated. There are no defined expectations for how 'an assessment of recovered species' is achieved. This could be based upon set limits or the selection of representative colonies. The identification of microorganisms at normal levels under standard manufacturing conditions can provide valuable insight into the baseline microbial flora and potential shifts in bioburden trends over time.
Comment Summary #105: The commenter requested clarification on the expectations for microorganism identification in the Assessment of Bioburden Monitoring section. Specifically, they asked how many colonies need to be identified and to what level (species/genus/etc.).
Response: Comment not incorporated. The request for clarification on microorganism identification is beyond the scope of this chapter.
Comment Summary #106: Several commenters suggested providing clarity for the handling of recovered organism data in the "Assessment of Bioburden Monitoring" section. Specifically, recommending changing the wording to indicate trending to align with the chapter scope of bioburden monitoring. The proposed change was to include the phrase “recovered organisms should be trended” to determine if they represent a loss of control, risk to product quality, or patient risk.
Response: Comment not incorporated. Some regulatory bodies already expect trending as part of a robust microbiological control program, making an explicit requirement redundant. The preferred broad wording allows manufacturers to determine their own trending strategies rather than mandating full trending for all recovered organisms.
Comment Summary #107: The commenter suggested that the requirement for identification, specifically the phrase “an assessment of recovered species must be completed,” should only apply when the control limit is exceeded. They proposed rewording the stipulation to: “In addition, an assessment of recovered species must be completed when exceeding the limit to…” and clarified that the limit refers to tests in Table 3.
Response: Comment not incorporated. Some regulators may expect routine identification in certain settings, such as aseptic processing, and prefer not to restrict identification to only out-of-specification events.
Comment Summary #108: The commenter indicated that the proposed text introduces an expectation to identify all recovered organisms. They argued that identifying microorganisms at normal levels and under standard manufacturing conditions does not add value to assessing process control.
Response: Comment not incorporated. As written, there are no defined expectations for how 'an assessment of recovered species' is achieved; this could be based upon set limits or the selection of representative colonies. The identification of microorganisms at normal levels under standard manufacturing conditions can provide valuable insight into the baseline microbial flora and potential shifts in bioburden trends over time.
Comment Summary #109: The commenter recommended removing the specificity of “control charts” and using more general language of “appropriate tools” in the section on the Assessment of Bioburden Monitoring. The commenter indicated that there are many different tools that can be used for bioburden trending, and control charts may not be the most appropriate based on bioburden data not being conducive for this tool.
Response: Comment incorporated. Sentence revised as follows: Trending analysis using appropriate tools should be conducted to evaluate the bioburden of the process and to identify the occurrence of adverse trends.
Comment Summary #110: The commenter suggested that there are situations with sampling when a gram stain may be appropriate and that the risk assessment should drive this. They proposed that the frequency and level of microbial identification be included in the risk assessment and trending analysis.
Response: Comment partially incorporated. The revised final text did not explicitly add wording to emphasize the frequency and level of microbial identification or specifically mention Gram staining. However, it maintained the broader requirement for trending analysis that could inherently cover microbial identification practices informed by risk assessment.

Table 3

Comment Summary #111: The commenter suggested that in-process bioburden monitoring for tablets at the coating and binder stage should be included in this chapter and noted the importance of evaluating hold time at each stage.

Response: Comment incorporated. The suggested change was added to Table 3.

Comment Summary #112: The commenter requested the addition of a new section on primary container components (e.g., rubber stoppers, vials, bottles, cartridges, or seals) for sterile finished drug products in Table 3. The commenter suggested that the test should be conducted before the washing/sterilization/depyrogenation process, with limits set per the bioburden test user requirements document and reported as CFU/unit(s) tested or CFU/cm². Additionally, the commenter recommended that recovered microorganisms should be identified and evaluated.

Response: Comment not incorporated. The chapter already addresses the concept of user requirements and risk-based sampling. Additional or more detailed CCS guidance, including new sub-sections on container closures, is beyond the scope of this general chapter.

Comment Summary #113: The commenter requested the deletion of Table 3 or, if not deleted, the inclusion of extensive additional caveats as footnotes. They indicated that they would not concur with its publication unless a footnote “b” is added, stating that "All limits must be established as per CGMP requirements, including but not limited to considerations relating to intended use of each product, nature of product, process stage, sample timing, process steps, equipment capability, sample method, and test method."

Response: Comment not incorporated. The existing text in the chapter and footnotes already indicates that these are recommended limits.

Comment Summary #114: The commenter requested clarification on whether objectionable evaluation is only for levels above defined limits. They suggested that if not, objectionable evaluation should be reserved for levels above defined specification or specified for end of process equipment, such as final pooling prior to bulk filtration or post excipient addition. They noted that upstream of these steps, there are multiple process steps to address concerns for levels attained below defined limits.

Response: Comment not incorporated. The compendial approach is that any recovered microorganism can be potentially objectionable depending on the product.

Comment Summary #115: The commenter indicated that the statement "For nonsterile products, recovered microorganisms should be evaluated to determine if they are objectionable" implies that an assessment is not needed for materials intended for sterile products. The commenter questioned if this was the intention.

Response: Comment not incorporated. Typically, any “objectionable” concerns in a sterile process are handled via a separate contamination control strategy.

Comment Summary #116: The commenter requested clarification on whether raw materials and excipients should be assessed for objectionable organisms, noting that limits may vary based on the intended product. Additionally, the commenter inquired about recommended limits for sterile small molecule drugs for suspension, which are more likely to be measured in grams than milliliters and are likely to be unfilterable.

Response: Comment not incorporated. Excipients and raw materials may be covered under broader “user requirements” or other monographs.

Comment Summary #117: The commenter suggested aligning the minimum amount of sample with USP <61> and considering 10ml as a minimum BB volume, performing bioburden prior to the final sterilization step on 100ml as per EMA/CHMP/CVMP/QWP/850374/2015. They proposed adding a statement to Table 3 indicating that alternative sample amounts to those mentioned may be acceptable when justified and supported by statistical analysis.

Response: Comment partially incorporated. The final chapter does mention that alternative volumes are permissible if justified, as noted in the footnotes and commentary throughout.

Comment Summary #118: The commenter suggested that alternative acceptance criteria for microbial process control should be allowed, including more flexible limits such as tests of solid intermediates/DS and CFU/g, to accommodate various process conditions, including bactericidal/static conditions, rather than just aqueous conditions.

Response: Comment not incorporated. A user can propose alternate forms (e.g., CFU/g) if validated and justified.

Comment Summary #119: The commenter suggested allowing medium pre-filtration samples as an alternative approach in Table 3.

Response: Comment not incorporated. The chapter already covers sampling at various steps (pre-filter, post-filter, etc.). It does not provide prescriptive references to “medium pre-filtration” specifically. Such details typically reside in a firm’s internal test plan.

Comment Summary #120: The commenter requested clarification on the scientific reasoning behind the proposed CFU limits for total aerobic count in Table 3. They also indicated that the varying units of measurement (x CFU/10 ml and x CFU/100 ml) could be confusing and suggested standardizing all specifications to x CFU/100 ml.

Response: Comment not incorporated. The chapter sets a range of limits and volumes to align with common industry practices and typical sample sizes for each material type. Adopting a single volume (e.g., always 100 mL) might conflict with certain in-process sampling norms or small-volume samples.

Comment Summary #121: The commenter requested that a note be included to clarify that the Total Aerobic Microbial Count encompasses bacteria, fungus, yeasts, and molds for better clarity.

Response: Comment partially incorporated. The chapter introduction was updated to state that “bioburden includes aerobic, facultative anaerobic, aerotolerant, and anaerobic microorganisms,” but it does not explicitly mention “molds” or “yeasts” in Table 3. The term “aerobic microbial” is considered sufficient.

Comment Summary #122: The commenter suggested eliminating Table 3 and allowing manufacturers to set risk-based bioburden limits. The commenter argued that the NMT 10 cfu/100 mL bioburden level is intended to prevent bacterial endotoxin contamination, but the number of bacteria equivalent to a pyrogenic dose is typically underestimated. They provided detailed information on the amount of endotoxin required to generate a pyrogenic reaction and the bacterial cells needed as a precursor.

Response: Comment not incorporated. Table 3 provides "recommended" (not mandatory) numeric targets. Removing it altogether would remove a key reference that many companies rely on as a baseline.

Comment Summary #123: The commenter indicated that the excessive EMEA bioburden level requirement for a sterilizing-grade filter, as stated in Table 3, is not risk-based. They referenced the book "Pharmaceutical Filtration – The Management of Organism Removal" by Theodore Melzer and Maik Jornitz, which discusses this issue. The commenter highlighted the inconsistency between the EMEA's acceptance level of NMT 10 CFU/100 mL and the FDA's 2004 Guidance for Industry – Aseptic Processing, which requires a retention validation of 107 CFU/cm^2 of filter surface with *Breundimonas diminuta*. They argued that the EMEA's requirement would necessitate an impractically large batch size to meet the filter validation challenge level.

Response: Comment not incorporated. The 10 CFU/100 mL is a broadly recognized benchmark.

Comment Summary #124: The commenter indicated that Table 3 recommends bioburden limits without justifying these limits based on a risk assessment, which is not described in <1119>. They questioned whether these limits represent regulatory requirements or industry practice and if their stringency is justified by risk analysis, suggesting that without evidence, the limits may appear arbitrary. The commenter proposed that the responsibility of setting bioburden limits should be assigned to individual manufacturers as part of their Contamination Control Strategy, in line with the recently revised EU Good Manufacturing Practice Annex 1 regulations.

Response: Comment not incorporated. The chapter provides recommended numerical limits for various sample types, deferring “risk-based” nuances (e.g., higher or lower limits) to the user’s internal validation or regulatory filing.

Comment Summary #125: The commenter suggested that Table 3 for the Recommended Bioburden Limits should include limits for Total Yeast and Mold Count (TYMC) in addition to the existing limits for Total Aerobic Microbial Count (TAMC).

Response: Comment not incorporated. Chapter <1119> focuses on total aerobic microbial count as the typical in-process measure, leaving mold/fungal counts addressed by user-defined risk assessments.
Comment Summary #126: The commenter indicated that they do not want the proposal to move forward. They suggested that the bioburden limits proposed must be risk-based and justified.
Response: Comment partially incorporated. Risk-based considerations have been addressed. The final chapter retains options for justified alternatives and outlines risk-based considerations for sampling and testing.
Comment Summary #127: The commenter requested clarification regarding the bioburden test requirements for container closure systems in Table 3 of <1119>, suggesting that these systems fall within the scope of <1111> for limits of components for pharmaceutical use. The commenter recommended elaborating on which types of samples are considered components and should therefore be tested according to <61>.
Response: Comment not incorporated. The purpose of <1119> is to support bioburden monitoring of in-process materials, components, and drug substances, rather than to serve as a final release testing chapter under <1111>.

Bioreactor—Pre-Inoculation
Comment Summary #128: The commenter suggested that the bioburden limits specified in Table 3 are too stringent. They indicated that a specification lower than 3 or 5 CFU per volume or quantities tested is not rational based on their experience and knowledge. The commenter noted that bioburden tests are performed under laminar flow hoods, which are not classified as real class A areas but rather support a class B area. They also mentioned that bioburden tests are not performed under isolators and that the consumables used are not compatible with sterile products. The commenter proposed that the lowest specification should be ≤3 or ≤5 CFU per volume tested, with any detected CFU identified to the species level and an investigation conducted to determine batch acceptability.
Response: Comment not incorporated. The decision was made to maintain the current bioburden limits to keep balanced regulatory expectations and industry best practices for extremely low bioburden in bioreactor pre-inoculation conditions.
Comment Summary #129: The commenter suggested that bioreactor pre-inoculation bioburden testing should be performed in an isolator to assure sterility during test execution. They noted that using a limit of <1 CFU/xx mL for bioburden testing could result in an out-of-specification event and associated deviation/investigation if any test contamination occurs. The commenter recommended that for these sample types, the sterility test method would be more appropriate.
Response: Comment not incorporated. The decision was made to maintain consistency in the enumerative method described in <1119.1> rather than introducing qualitative sterility test methodology (<71>).
Comment Summary #130: The commenter suggested that bioreactor samples, which are defined as bioburden samples, could complicate the bioburden test due to the presence of cells. They recommended including the compendial sterility test as an alternative, as it would cover both aerobic and anaerobic conditions.
Response: Comment incorporated. The final revised text allows for site-specific justification and validation of anaerobic/aerobic testing methods and sterility-based approaches, rather than explicitly specifying such methods within the document itself.
Comment Summary #131: The commenter recommended changing the limit for "Bioreactor—pre-inoculation" from "<1 CFU/100 mL" to "≤1 CFU/100 mL." The commenter indicated that the proposed limit of 0 CFU/100 mL does not consider the feasibility of testing in quality control (QC) for non-sterile manufacturing, where low-level microbial presence is more challenging to control. They assert that the control criteria should be "Not More Than 1 CFU/100 mL" to better reflect practical manufacturing conditions.
Response: Comment not incorporated. The proposed limits are recommendations rather than strict requirements. Manufacturers may implement alternative control strategies based on their specific processes and risk assessments.

Comment Summary #132: The commenter recommended removing the sampling point for the bioreactor pre-inoculation recommended bioburden limit of <1 CFU/100 mL. They suggested that contamination of the bioreactor is self-indicating and would be detected by process controls and end-of-production preharvest bioburden testing.
Response: Comment not incorporated. Including this sampling point aligns with industry best practices and regulatory expectations for proactive bioburden monitoring.

Chromatography Column
Comment Summary #133: The commenter suggested setting a limit of ≤100 CFU/10 mL for chromatography column or ultrafiltration pre-use storage effluent and pre-use equilibration effluent. They referenced a PDA article to support this limit, noting that the effluent does not contain product, which justifies a higher limit.
Response: Comment not incorporated. Raising these limits is potentially conflicting with overall microbial control strategy and risk management expectations.

In-Process Purification Samples
Comment Summary #134: The commenter suggested aligning the limit for in-process purification samples to be consistent with downstream specifications, such as those found in monographs or filed limits. They provided an example from the Insulin human monograph, which has a limit of 300 CFU/g, to support their request for a more flexible limit than the current ≤100 CFU/10 mL.
Response: Comment incorporated. The final revision includes the addition of a footnote that provides the requested flexibility, explicitly allowing for the justification of limits higher than 10 CFU/100 mL based on documented rationale.

Purified Drug Substance
Comment Summary #135: The commenter suggested that guidance should be provided for the pre-bioburden reducing filter for drug products, similar to the EU guidance. They indicated that the limit should align if the pre-bioburden reducing filter is implemented for additional assurance and not for specific defined reduction requirements. The commenter also noted that drug substance limits and processing capabilities can factor into this limit.
Response: Comment incorporated. A footnote was added to indicate that this level aligns with the filter bioburden level. Unless there is a validated bioburden reduction step between the purified drug substance (DS) and the sterile filtration train, the bioburden limit should align with the pre-filtration limit.
Comment Summary #136: The commenter requested a definition for “purified drug substance” to be included either in section <1119> or <1117.1>.
Response: Comment not incorporated. The definition is not specific to microbiology chapters and will not be added to the glossary.
Comment Summary #137: The commenter suggested that many of the proposed limits are based on a 1- or 10-mL sample size and recommended expressing the limit as 1 CFU/10 mL or 10 CFU/100 mL instead of using the symbol ≥. They cited statistical experts to support their recommendation, noting that a limit of 1 CFU/10 mL or 10 CFU/100 mL would have a relatively high probability of passing.
Response: Comment not incorporated. Sample size optimization in the associated method chapter (e.g., <1119.1>) leaves them to the user’s Method Suitability approach.
Comment Summary #138: The commenters recommended revising the bioburden limit for purified drug substances in Table 3 from ≤1 CFU/10 mL to ≤10 CFU/10 mL, citing concerns that the original limit is too restrictive given process capabilities and potential secondary contamination in non-ISO 5 environments. They also noted that the purified drug substance is an in-process sample taken before final bio-reduction filtration, distinguishing it from a sterile product. The proposed revision aligns with expectations for a low-bioburden drug substance at this stage.
Response: Comment partially incorporated. A general note has been included stating that any alternative acceptance criteria can be justified and documented by the user as needed.

Comment Summary #139: Several commenters provided differing recommendations regarding the bioburden limit for purified drug substances in Table 3. One commenter supported maintaining the ≤1 CFU/10 mL limit, aligning it with comparable monographs such as the Insulin Human monograph, unless alternative limits are filed in registrations or match the final drug substance specification. Another commenter recommended adjusting the limit to ≤10 CFU/10 mL, citing that the manufacturing process for monoclonal antibodies is a low-bioburden process and that this limit has been accepted by various regulatory agencies.
Response: Comment partially incorporated. A general note has been included stating that any alternative acceptance criteria can be justified and documented by the user as needed.
Comment Summary #140: The commenter recommended increasing the range for the purified DS recommended limit from <= 1 CFU/10 mL to <= 10 CFU/10 mL to account for false positives that could cause a non-sterile intermediate to fail.
Response: Comment partially incorporated. Any alternative acceptance criteria can be justified and documented by the user as needed.
Comment Summary #141: The commenter suggested adding examples to the existing footnote in Table 3 to allow higher limits for purified drug substances. Specifically, they recommended including examples such as drug substances stored frozen and bioburden reduction filtration in the drug product process prior to sterile filtration. They proposed that the footnote could state, "e.g., if validated bioburden reduction step exists before sterilizing filtration."
Response: Comment partially incorporated. A general note has been added indicating that any alternative acceptance criteria can be justified and documented by the user as needed.
Comment Summary #142: The commenter suggested that the specifications for purified drug substances and pre-bioburden reducing filters should be aligned, as these sample types are often the same and only undergo minor processes such as thawing or equilibration, which do not significantly impact the bioburden level. They recommended that the bioburden specification for both sample types be expressed as ≤ 10 CFU/100 mL.
Response: Comment partially incorporated. While the general alignment of specifications is acknowledged, it is noted that any alternative acceptance criteria can be justified and documented by the user as needed.
Comment Summary #143: The commenter indicated concerns regarding the purified drug substance and its release specifications in the pharmaceutical industry. They highlighted that the specific limit of ≤1 CFU/10 mL might be misinterpreted as a release limit rather than an in-process test limit. The commenter emphasized the need to consider varied user requirement risk factors, such as the lower bioburden risk for bulk purified material stored in deep freezers compared to liquid bulk drug substances. They also noted that the provision to refilter a drug substance when bioburden levels are exceeded is precluded for liquid purified bulk. The commenter argued that the proposed limit was likely derived from pre-sterilized drug product limits, which is not appropriate since the purified bulk is only one component of the formulated drug product. They suggested replacing the current limit with language similar to that used for pre-harvest bioreactor sampling, specifically “Per bioburden test user requirements document,” to avoid unjustified batch rejections for non-sterile operations.
Response: Comment partially incorporated. The general note indicates that any alternative acceptance criteria can be justified and documented by the user as needed.
Comment Summary #144: The commenter requested clarification on whether the limits mentioned above in Table 3 are exclusively related to biopharmaceuticals.
Response: Comment partially incorporated. The limits are not limited to a singular type of drug substance.

Ready-to-Sterilize Components (RTS)
Comment Summary #145: The commenter requested clarification on the RTS TAMC limit of ≤ 100 CFU/stated sample size tested in Table 3. They asked whether this limit applies to medical devices such as unfilled syringes or vials prior to gas or irradiation treatment. The commenter recommended providing an example of this type of component.

Response: Comment not incorporated. The chapter defers to a user's own risk assessment for specific product types (syringes, vials, etc.) and sterilization methods (gas, irradiation, etc.). Details or examples would belong in a firm's internal validation/user requirements.
Comment Summary #146: The commenter suggested clarifying what "components" refer to in the context of ready-to-sterilize components. They indicated that if these components are container closures to be sterilized for aseptic filling in the manufacturing of sterile products, the Total Aerobic Microbial Count (TAMC) limit should be based on the mode of sterilization. They provided examples such as irradiation and gas sterilization, where the limits are prescribed in ISO guidance based on the sterilization method.
Response: Comment not incorporated. Mode-specific details, whether it is steam, gas, or irradiation, are addressed through internal protocols and relevant ISO or regulatory standards.
Comment Summary #147: The commenter requested the removal of the line item for "Ready-to-sterilize components (RTS)" from Table 3. The commenter indicated that there are various sterilization processes, each with different bioburden monitoring requirements and levels, and that specific guidance is provided elsewhere based on the sterilization process.
Response: Comment not incorporated. Table 3 aims to include all key in-process materials (e.g., purified drug substance, RTS components, etc.) such that users see a comprehensive list of recommended bioburden limits.
Comment Summary #148: The commenter requested clarification on the sample size required for ready-to-sterilize (RTS) components, specifically regarding the "100 CFU/per stated sample size tested" requirement.
Response: Comment not incorporated. USP keeps the requirement flexible so that each manufacturer can define a sample size appropriate to their component design and process. Users must document the rationale under their own risk-based sampling plans.
Comment Summary #149: The commenter suggested allowing a limit of NMT 1000 CFU for ready-to-sterilize components (RTS) based on a log 3 reduction during the sterilization process.
Response: Comment not incorporated. The chapter keeps a single universal limit to ensure baseline control and to avoid confusion with varied approaches (e.g., steam vs. radiation). The text allows manufacturers to justify different acceptance criteria, if needed, in their internal "user requirements" documentation.
Comment Summary #150: The commenter suggested that the specification for ready-to-sterilize material, which is currently set at "less than or equal to 100 CFU per tested samples," should be reconsidered. They questioned why the limit is set for combined samples rather than individual units and proposed setting a per unit limit or a combined limit, particularly if the system can robustly reduce bioburden to more than 100 CFU per unit.
Response: Comment partially incorporated. The final revised document maintained the original wording: "Ready-to-sterilize components (RTS) ≤100 CFU/per stated sample size tested." However, a clarifying footnote was added to address the intent of the comment.

Pre-Terminal Sterilization (Moist Heat or Heat)
Comment Summary #151: The commenters requested clarification on the term "heat" in "Pre-Terminal Sterilization (moist heat or heat)" in Table 3, noting that it is unclear what methods are covered beyond moist heat and dry heat. One commenter suggested explicitly defining the intended scope, while another recommended removing "or heat" altogether to align with EMA 2019 sterilization terminology.
Response: Comment not incorporated. The original wording ("moist heat or heat") has been retained, as the term is considered adequately understood within the industry context. The suggested clarification or alignment with EMA terminology was not implemented.
Comment Summary #152: The commenter indicated that the specification for pre-terminal sterilization (moist heat or heat) of ≤100 CFU/100 mL does not make sense. They argued that the specification should be related to a container rather than a volume, as each container behaves like a mini autoclave. Additionally, they noted that this specification is not justified for overkill sterilization or depyrogenation, which provides extremely low Sterility Assurance Levels (SALs) commonly encountered in the pharmaceutical industry.
Response: Comment not incorporated. The sample is taken before the material enters the final container or may need multiple containers to sample. The chapter's focus is on uniform, general

bioburden limits that can be adapted to a range of processes and materials. Maintaining a volumetric approach ensures consistency across different scenarios.
Comment Summary #153: The commenter requested clarification on the rationale behind setting the bioburden limit at 100 CFU/100 mL for pre-terminal samples in individual packages. They questioned what the limit would be for medical devices subjected to chemical sterilization or for filled containers with a volume of less than 100 mL.
Response: Comment not incorporated. Container-specific limits and different sterilization methods (e.g., chemical sterilization for devices) are considered beyond the scope and better addressed in user-specific standard operating procedures.

Pre-bioburden reducing filter for drug product
Comment Summary #154: The commenter suggested that the bioburden reduction filtration limit should be based on a risk assessment. They noted that in some situations, based on product-specific risk assessment, a limit of 10 CFU/100 ml may be appropriate.
Response: Comment partially incorporated. The final wording explicitly states that higher limits are permissible if supported by a documented demonstration of filter capability and risk-based justification.
Comment Summary #155: The commenter recommended aligning the terminology and criteria for the pre-bioburden reducing filter for drug products with the EMA/CFHMP/CVMP/QWP/850374/2015 guidelines. Specifically, they suggested setting a bioburden limit of ≤10 CFU/100 mL, with an allowance for higher bioburden levels if due to starting materials with inherent microbial contamination, provided it is demonstrated that the filter can achieve a bioburden of NMT 10 CFU/100 mL prior to the last filtration.
Response: Comment partially incorporated. The revised text now explicitly clarifies when a higher bioburden might be acceptable, such as in cases of inherent microbial contamination in starting materials and clearly states the requirement for demonstrating the filter's capability to reduce the bioburden to NMT 10 CFU/100 mL before the final filtration.
Comment Summary #156: Several commenters requested clarification on why the pre-bioburden reducing filter specification is the same as the pre-sterilization filter specification, both being ≤10 CFU/100 mL. The commenter also inquired whether this specification applies to all filters in a given process that reduce bioburden or just the filter used before the sterilization filter.
Response: Comment incorporated. The final revision includes the addition of a footnote that provides the flexibility requested, explicitly allowing justification of limits higher than 10 CFU/100 mL based on documented rationale.
Comment Summary #157: The commenter recommended that the pre-bioburden reducing filter for drug products should have a limit of ≤ 10 CFU/10 mL, aligning with the recommended DS limit since there is no further processing upstream of the bioburden reduction filter.
Response: Comment incorporated. The final revision includes the addition of a footnote that provides the flexibility requested, explicitly allowing justification of limits higher than 10 CFU/100 mL based on documented rationale.
Comment Summary #158: The commenter suggested aligning the limit of the pre-bioburden reducing filter for the drug product with the limit of the pre-terminal sterilization sample, as the sample of the pre-bioburden reducing filter is similar.
Response: Comment incorporated. The final revision includes the addition of a footnote that provides the flexibility requested, explicitly allowing justification of limits higher than 10 CFU/100 mL based on documented rationale.
Comment Summary #159: The commenter indicated that the proposed requirement of NMT 10 CFU/100 mL for samples before the bioburden reducing filter (sample prior to any filtration) seems unreasonably stringent for the production of sterile products. They noted that the proposed bioburden limit for samples before any filtration is the same as for WFI, despite the use of non-sterile materials for compounding and the presence of sterilizing filters after the bioburden reduction filter. The commenter questioned the necessity of this stringent limit given the additional sterilizing steps in place.
Response: Comment incorporated. The final revision includes a footnote that provides the requested flexibility, explicitly allowing for the justification of limits higher than 10 CFU/100 mL based on documented rationale.

Comment Summary #160: The commenter suggested that the pre-bioburden reducing filter specification should be set at 100 CFU/100 mL, as the current specification is too stringent. They noted that the pre-bioburden removal sterilizing filter specification and the pre-bioburden reducing filter specification should not be the same and that the current specification is more stringent than the pre-terminal sample specification, which is collected next to this step. The commenter requested an evaluation of this issue.
Response: Comment incorporated. The final revision includes the addition of a footnote that provides the requested flexibility, explicitly allowing for the justification of limits higher than 10 CFU/100 mL based on documented rationale.
Comment Summary #161: Several commenters recommended changing the name of the sample type in Table 3 to clarify the point in the process where the sample is collected. Additionally, the commenter suggested updating the recommended bioburden level to align with the expectation for reduction in microbial levels between samples taken prior to a bio-reduction step and samples taken after the step. The proposed changes were to rename the sample type to "Bulk solution prior to primary filtration" and to establish the bioburden level based on process capabilities.
Response: Comment not incorporated. The suggested wording "Pre-bioburden reducing filter" was retained rather than adopting the specific wording "Bulk" or "Bulk sample" suggested by the commenters. The recommendation to rename as "Bulk" or "Bulk solution" was not explicitly adopted. However, additional clarifying text was added to ensure users understand the intent and applicability clearly.

Pre-Sterilizing Filter for Drug Product
Comment Summary #162: Several commenters recommended revising the sample type name for "Presterilizing filter for drug product" in Table 3 to "Bulk solution prior to final filtration" or "Bulk solution prior to final sterile filtration." They stated that the revised language would improve clarity regarding where the sample is taken and align with terminology used in industry guidance documents, such as Annex 1.
Response: Comment not incorporated. The original terminology has been retained, as the authors determined it is sufficiently clear for users to understand the intent without additional clarification.

Purified Water/Water for Injection
Comment Summary #163: The commenter indicated that the reference for the sample type, Water for Injection, was incomplete, specifically noting the citation “Per Water for Pharmaceutical purposes <1231>”.
Response: Comment incorporated. The reference has been corrected.
Comment Summary #164: The commenter indicated that the inclusion of Purified water and Water for injection in Table 3 could lead to confusion due to the specific requirements for recovering nutritionally deprived species in more specialized agars. They believe that this inclusion does not add value and may cause potential confusion regarding the suitability of nutrient media.
Response: Comment not incorporated. Keeping Purified water and Water for injection in the table does not override or conflict with specialized requirements for enumerating nutritionally deprived organisms. Instead, it reinforces that Purified water and Water for injection are also subject to bioburden monitoring.
Comment Summary #165: The commenter suggested removing the rows for "Purified water" and "Water for injection" from Table 3.
Response: Comment not incorporated. Chapters typically give comprehensive coverage of all relevant sample types, including purified water (PW) and water for injection (WFI).

Cleaned Equipment
Comment Summary #166: The commenter suggested that the identification of microorganisms should only be performed on the last rinse of cleaned equipment rinse samples. Additionally, the commenter recommended adding that a risk assessment can be used to define when the identification of microorganisms should be performed, such as at the Action limit or another level

of bioburden. The commenter also requested clarification on the identification level needed, such as species, genus, or group.
Response: Comment partially incorporated. The broader wording about downstream control has been addressed, but the specific suggestions regarding last rinse identification and explicit risk assessment criteria were not adopted.
Comment Summary #167: The commenter requested clarification in Table 3 regarding the evaluation of recovered microorganisms in cleaned equipment rinse samples for nonsterile products. They suggested adding verbiage to specify that this determination is only necessary if no further processing, rinsing, or adequate downstream controls are in place.
Response: Comment incorporated. The updated text now reads: "Cleaned equipment rinse samples: Equal to the specification of the final rinse water. For nonsterile products, recovered microorganisms should be evaluated to determine if they are objectionable in case no further downstream controls are in place."
Comment Summary #168: The commenter indicated that the statement “recovered microorganisms should be evaluated to determine if they are objectionable” in Table 3 appears to add a requirement that is historically applicable to finished products, not clean equipment testing. The commenter suggested removing the statement regarding the evaluation of objectionable organisms.
Response: Comment not incorporated. The statement is applicable to situations with no further downstream controls.
Comment Summary #169: The commenter suggested that for manufacturing non-sterile Drug Substance, recovered microorganisms above the action limit should be evaluated to determine if insanitary conditions are present. The commenter indicated that the evaluation of insanitary conditions is only relevant when action limits are exceeded.
Response: Comment not incorporated. This is a decision point for the user’s procedural controls.
Comment Summary #170: The commenter requested clarification on the bioburden limit for cleaned equipment rinse samples, specifically how the final rinse water specification applies when surface sampling is performed using the contact plate or swab technique. They provided an example of converting a water limit (e.g., 100 CFU/mL for purified water) to a surface area equivalent (e.g., 50,000 CFU/cm²).
Response: Comment not incorporated. The application of surface conversion factors is highly dependent on user-defined justifications and procedural SOPs. Establishing a standardized conversion in the table may not account for variations in sampling methodologies, surface materials, and recovery efficiency. Users should apply limits based on their validated procedures.

Primary Container Components
Comment Summary #171: The commenter requested clarification on the necessity of determining if recovered microorganisms are objectionable, given that the primary containers would be sterilized. They questioned why objectionable microorganisms would be a concern under these conditions.
Response: Comment not incorporated. Some microorganisms may produce heat-stable toxins or cause other contamination concerns, such as spore formers that might survive. Thus, even when a final sterilization step is planned, USP recommends monitoring for objectionable organisms.
Comment Summary #172: The commenter requested that the sample size be included or a reference to where the sample size would be proposed, and the actual limits be included in Table 3. The commenter indicated that this addition would align with the bioburden test user requirements document and ensure clarity regarding the evaluation of recovered microorganisms in nonsterile products.
Response: Comment not incorporated. The decision was made not to add prescriptive sample-size requirements because the chapter covers many manufacturing scenarios, leaving it to each manufacturer to justify sampling plans based on risk.

Appendix

Comment Summary #173: The commenters recommended revising the flowchart in the Appendix to clarify the reference to <1117.1>. One commenter suggested changing "Bioburden Test as defined in <1117.1>" to "Is bioburden testing conducted as defined in <1117.1>?" to improve clarity and address concerns that bioburden testing might no longer be required under the existing wording. Others noted that test methods per <61> should take precedence over <1117.1> when specified acceptance criteria exist and that <1117.1> is used as a decision point but may require additional guidance.

Response: Comment partially incorporated. The reference to <1117.1> has been removed.

Comment Summary #174: The commenter suggested adopting an updated version of Figure 1 in the Appendix. The updated figure aims to provide more clarification for readers on how to utilize USP guidance. By streamlining the figure, it will give readers more direction on which Chapter will provide the necessary guidance.

Response: Comment incorporated. Updated Figure 1 as per recommendation.

Comment Summary #175: The commenter requested that the Appendix provide an exact definition of Bioburden Tests as defined in <1117.1>. The commenter indicated that the current definition is not clear and that microbiologists need to be able to clearly choose which method is needed.

Response: Comment partially incorporated. The reference to <1117.1> has been removed to address the clarity issue, but an exact definition has not been added.

Comment Summary #176: The commenter indicated that Figure 1 in the Appendix of <1119> has the first decision point to follow the method but not the specification in an existing USP monograph. They noted that Table 3 states a limit for a purified drug substance of ≤ 1 CFU/10 mL, which is too stringent for all types of dosage forms. The commenter explained that a purified drug substance is more likely to be a solid than a solution or suspension; therefore, the CFU limit would be expressed per gram weight. They also mentioned that drug substance and product microbiological specifications in USP monographs retain the specifications first approved by the FDA for the innovator's submission, which are frequently not risk-based or in compliance with USP <1111>. Additionally, the decision tree recommends the official test methods in <61> and the proposed methods in <1119.1>, which is an informational chapter. The commenter suggested that recommending two different tests for a single quality attribute is not a USP norm and should be strictly avoided.

Response: Comment not incorporated. The issue is already covered in the footnote.

Comment Summary #177: The commenter indicated that the Introduction references Figure 1 in the Appendix to clarify when chapters <1119> and <1119.1> apply. They expressed concern that the flowchart does not adequately define the scope of the chapter, particularly noting that the definition of Bioburden test in <1117.1> lacks direction. The commenter pointed out that the current wording could be interpreted as applicable to traditional non-sterile product manufacture, despite the content, particularly Table 2, suggesting that it is intended for materials and processes used in sterile product manufacturing, such as in biologics. They proposed that it should be explicitly stated that this chapter applies to in-process bioburden for sterile products and that the scope of <1119> should be clearly differentiated from chapter <61>, which does not pertain to in-process bioburden testing for sterile products.

Response: Comment partially incorporated. The chapter was clarified to specify its relevance to nonsterile processes to address concerns about scope and applicability.

General Chapter/Section(s): <1119.1> BIOBURDEN TEST
Expert Committee(s): General Chapters – Microbiology
No. of Commenters: 22

General

Comment Summary #1: The commenter indicated support for the new guideline chapter, finding it very useful as it is the only chapter in a pharmacopoeia that addresses and clarifies the situation of "bioburden" in process. The commenter mentioned that for in-process bioburden, a

Total Aerobic Microbial Count (TAMC) test is often sufficient and well described in the new chapter.
Response: Comment acknowledged.
Comment Summary #2: The commenter indicated that it is unclear how the methods described in USP <1119.1> are functionally different from those outlined in USP <61> Microbiological Examination of Nonsterile Products. They noted that some growth parameters in Table 1 of each chapter are slightly different and questioned whether this was intentional.
Response: Comment not incorporated. This chapter is considered a test chapter and clarifying information is located in the parent chapter <1119>. For now, this chapter is >1000 and is not considered applicable and therefore provides recommendations.
Comment Summary #3: Several commenters recommended avoiding the use of the terms “limit,” “specification,” and “acceptance criteria” throughout the document. They indicated that these terms are typically associated with release testing, which is not within the scope of this chapter, and their use could lead to misinterpretation by readers.
Response: Comment not incorporated. Since the chapter includes conditions where release testing occurs or a firm decides to apply bioburden testing as part of release testing, the term "limit" should still apply.
Comment Summary #4: The commenter suggested including another medium for fungi, such as Sabouraud dextrose agar (SDA), in the chapter.
Response: Comment not incorporated. Method suitability includes recovery of yeast and mold on SCDA.
Comment Summary #5: The commenter indicated that the chapter does not provide significant new content regarding method suitability compared to the globally harmonized chapter USP<61>. They expressed concern about the risk of misalignment with the globally harmonized chapter and suggested omitting the draft.
Response: Comment not incorporated. The companion chapter provides enough distinct guidance beyond USP <61> to justify its inclusion.
Comment Summary #6: The commenter requested more information and/or reference to existing guidelines regarding the suitability of the counting method in the presence of product, specifically for primary container closures and ready-to-sterilize components. The commenter sought clarification on what constitutes an appropriate buffer solution.
Response: Comment not incorporated. The direct mention of container closures/components was removed, and the recommendation was rolled into the broader guidance for solid samples.
Comment Summary #7: The commenter suggested that it would be beneficial to state that the method described is not inconsistent with the concept of a modified version of <61> methodologies but provides a more direct link to a generic method for use in products and processes outside of release testing of non-sterile finished drug products.
Response: Comment not incorporated. The briefing statement is provided as explanatory and is not adopted as final text.
Comment Summary #8: The commenter indicated that USP <1119.1> is defined in the briefing section as an equivalent to USP <61> for bioburden testing. They questioned why this chapter is categorized as an informational chapter (>1000) rather than a general test chapter (<1000).
Response: Comment not incorporated. USP will consider a future revision to move this chapter to <1000.

Introduction
Comment Summary #9: The commenter suggested clarifying the temperature range for the enumeration of mesophilic bacteria and fungi by adding "and at 30-35°C" at the end of the sentence. They noted that while the mesophilic temperature range is from 20 to 45°C, some molds, such as *Ramularia plurivora* and *Cladosporium* spp., may not be recovered at 30-35°C even if they are mesophilic.
Response: Comment not incorporated. The parent chapter explains how different media might be used depending on the purpose of the test, context of use, and anticipated bioburden. SCDA is a sufficiently broad nutrient culture medium recovering aerobes and facultative anaerobes.

Comment Summary #10: The commenter suggested including anaerobic bacteria in the introduction, noting that some anaerobic strains such as *Clostridia* are found everywhere. They proposed changing the text to "aerobic and anaerobic conditions."
Response: Comment not incorporated. Enumerating strictly anaerobic organisms is beyond the scope of this chapter.

Enumeration Methods
Comment Summary #11: The commenter suggested that the specific user requirements document should be optional, as this information will already be included in the risk assessment or method validation documentation.
Response: Comment not incorporated. The specific user requirements document is recommended as per chapter <1119>.

Growth Promotion Test, Negative Controls, and Suitability of the Counting Method
General Considerations
Comment Summary #12: The commenter indicated that the phrase “The ability of the test to detect microorganisms in the sample must be established via method suitability” creates ambiguity regarding whether method suitability or validation is required. The commenter suggested revising this section to clarify if validation or method suitability is only necessary when changes occur.
Response: Comment not incorporated. The current wording is aligned with <61>.

Preparation of Test Strains
Comment Summary #13: The commenter requested clarification on the term "master seed lot." They asked whether it refers to a culture obtained from a strain collection.
Response: Comment not incorporated. The concept of a “master seed lot” remains as originally stated, implying that labs should treat it as their primary, validated culture source.

Table 1
Comment Summary #14: The commenter suggested that *Staphylococcus aureus* should be mentioned as *Staphylococcus aureus* subsp. *aureus*, similar to the updates made for formerly *Pseudomonas aeruginosa* and *Bacillus subtilis*.
Response: Comment incorporated.
Comment Summary #15: The commenter recommended separating the column for suitability and bioburden testing into two distinct columns in Table 1. They indicated that the incubation time for the routine test should not be less than that for method suitability. With the current table, it is possible that the user incubates for 5 days for suitability (≤5 days) and less than 5 days for the routine test (≤5 days). The proposed change suggests having one column for suitability with ≤ X days and another for the routine test with ≥ X days.
Response: Comment partially incorporated. The bioburden test has been removed from the column.
Comment Summary #16: The commenter indicated that the use of "prep" in Table 1 is inappropriate and should be explicitly called out.
Response: Comment not incorporated. The existing wording is sufficient.
Comment Summary #17: The commenter recommended updating Table 1 to include or reference USP <61> parameters or explicitly indicate not to use the USP <61> parameters for *Candida albicans* and *Aspergillus brasiliensis* sections. USP <61> Table 1 suggests using Sabouraud Dextrose Agar (SDA) or Sabouraud Dextrose Broth (SDB) at 20–25° C for 2–3 days for *Candida* and SDA or Potato Dextrose Agar at 20–25° C for 5–7 days, or until good sporulation is achieved for *Aspergillus* as preferred test strain preparation.
Response: Comment not incorporated. This chapter is intended to present a single, unified approach (using soybean–casein digest medium at 30°–35 °C) that can be applied broadly for bioburden testing.

Comment Summary #18: The commenter requested the alignment of table headers with USP<61> in the last column of Table 1.
Response: Comment incorporated.
Comment Summary #19: Several commenters indicated that using the term “formerly *A. niger*” suggests that *Aspergillus brasiliensis* and *Aspergillus niger* are interchangeable, which is misleading. They clarified that while *A. brasiliensis* was once described under the species *A. niger, A. niger* continues to exist as a separate species. *A. brasiliensis* was established as a new species.
Response: Comment incorporated.
Comment Summary #20: The commenter suggested that the incubation temperature and days for the “Preparation of Test Strain” specific for *C. albicans* and *A. brasiliensis* should be the same as described in <61>.
Response: Comment not incorporated. Guidance in <1119.1> is focused on bioburden testing rather than finished product release and remains appropriate for its intended scope and purpose. Bringing it into alignment with <61> was unnecessary.
Comment Summary #21: The commenter suggested adding Sabouraud Dextrose Agar at 20–25°C, NMT 5 days as another option under the column of “Suitability of Counting Method in the Test Matrix and Bioburden Testing” for Yeast and Mold, noting that the only agar listed is soybean–casein digest agar used as media.
Response: Comment not incorporated. The parent chapter explains how different cultures might be used depending upon the purpose of the test, context of use, and anticipated bioburden. SCDA is a sufficiently broad nutrient culture medium.
Comment Summary #22: The commenter suggested that the incubation days in Table 1 for *C. albicans* and *A. brasiliensis* are too long, recommending a maximum of 3 days for *C. albicans* and 5 days for *A. brasiliensis*. They expressed concern that excessive incubation time could negatively impact the activity of the strains. Additionally, they proposed adding medium SDA and an incubation temperature range of 20–25°C for the preparation of these organisms.
Response: Comment not incorporated. The duration allows for much less than 7 days. The duration was chosen to provide broad options for incubation.
Comment Summary #23: The commenter recommended updating the column heading in Table 1 to remove the reference to Bioburden Testing. The commenter indicated that the table is specific to incubation conditions associated with growth promotion and method suitability testing.
Response: Comment incorporated. Change made as requested.
Comment Summary #24: The commenter recommended a temperature range of 30–35°C for bioburden testing. They suggested allowing different temperatures to be used if a company can demonstrate that the chosen temperature is capable of recovering bacteria, yeast, and mold. For example, they mentioned that a company might use 28°C for bioburden testing.
Response: Comment not incorporated. Justified changes to the instructions below may be used, as described in <1119>. This general statement indicates that users can modify parameters such as temperature if adequately justified and validated.

Growth Promotion of the Nutrient Culture Media
Comment Summary #25: The commenter suggested revising the first sentence of the "Growth Promotion of the Nutrient Culture Media" section to account for the potential impact of shipping conditions on the media by testing each batch and each shipment of ready-prepared nutrient culture medium.
Response: Comment not incorporated. The current guidelines are aligned with <61>, and more in-depth information on growth promotion can be found in <1117>.
Comment Summary #26: The commenter recommended simplifying the criteria used to define "a small number" in the Growth Promotion section. They noted inconsistencies, with some chapters referring to "small" as "Not more than 10 CFU" and others as "not more than 100 CFU." The commenter suggested removing the term "small number" and directly stating "Not more than 100 CFU" as the proposed inoculum level.
Response: Comment incorporated. The final text no longer uses the term "small number" and now states "not more than 100 CFU."

Comment Summary #27: Several commenters recommended updating the guidance to remove references to “portions” to avoid directing the reader to prepare multiple microorganism strains on a single plate. The commenter indicated that with an acceptable recovery percentage of 50–200% of the inoculum, there is a risk of creating “too numerous to count” conditions if the upper limit of 100 CFU is approached, and multiple organisms are inoculated on a single plate.
Response: Comment incorporated. The change was made as recommended.
Comment Summary #28: Several commenters requested clarification on the term "comparable" in the sentence, "For freshly prepared inoculum, microbial growth and quantity must be comparable to growth obtained with a previously qualified lot of nutrient media." Specifically, the commenter asked whether "comparable" means a factor of 2 or a more stringent measure.
Response: Comment not incorporated. The details regarding the term "comparable" are covered in section <1117>.
Comment Summary #29: Several commenters recommended deleting the text in the section "Growth Promotion of the Nutrient Culture Media." The commenter indicated that the chapter section, "Testing for Bioburden," describes Membrane Filtration, Pour Plate, and Surface Spread Methods, none of which use liquid nutrient culture media.
Response: Comment not incorporated. The Expert Committee chose to retain the statement to allow flexibility (e.g., for preparatory steps or alternative procedures) even though the primary test methods described focus on solid media.
Comment Summary #30: The commenter suggested updating the text to "Use standardized, stable suspensions or ready to use culture of test strains or prepare as stated below."
Response: Comment not incorporated. The final text maintains the reference to “standardized, stable suspensions” (plus the option to prepare from passages), leaving it at the user’s discretion to demonstrate suitability for other forms of prepared cultures.

Suitability of the Counting Method in the Presence of Product

Test Sample Preparation

Comment Summary #31: The commenter recommended adding a section or sub-section addressing the amount of product to be used in the "Suitability of the Counting Method in the Presence of Product" section. They suggested that this addition would provide additional guidance on sample quantity and referenced Chapter <61> for further detailed guidance. The commenter noted that while not all of Chapter <61> is applicable, some guidance would be relevant, particularly for products like powders.
Response: Comment not incorporated. The Expert Committee decided to retain the broad language, leaving the determination of sample quantity to the user’s discretion and method suitability studies.
Comment Summary #32: The commenter requested clarification on whether the guidance stating, “For freshly prepared inoculum, microbial growth and quantity must be comparable to growth obtained with a previously qualified lot of nutrient culture medium,” necessitates a concurrent test of the previous approved lot with the new lot of media. The commenter suggested adding guidance if a concurrent test is required.
Response: Comment not incorporated. The Expert Committee leaves this procedural detail up to the user’s discretion or internal SOPs, with the expectation that the method by which they demonstrate comparability (concurrent or otherwise) is documented and justified.
Comment Summary #33: The commenter suggested that other diluents may be required depending on what is being tested, noting that some raw materials and excipients may be fluid samples.
Response: Comment not incorporated. The general wording allows users to select appropriate sample-preparation procedures.
Comment Summary #34: The commenter suggested that for cytotoxic/antimicrobial products, the product may need to be prepared in a diluent containing a neutralizing agent.
Response: Comment not incorporated. The committee retained the broader statement that if antimicrobial activity is present, a validated neutralization approach should be used, leaving it to

the user's discretion to determine the appropriate neutralizer for cytotoxic or otherwise inhibitory products.
Comment Summary #35: The commenter requested clarification on when it would be necessary to adjust the pH of a diluted sample, specifically if the pH should be adjusted when it falls outside the range of 6–8 (i.e., <5.5 or >8.4) or if it is necessary to overcome antimicrobial activity.
Response: Comment not incorporated. The text remains flexible, allowing each lab to make that determination based on method suitability, validation data, and the nature of the sample.
Comment Summary #36: The commenter suggested deleting the sentence "If necessary, adjust to a pH of 6–8". The commenter indicated that no adjustment seems necessary if the ability of the test to detect microorganisms has been demonstrated.
Response: Comment not incorporated. If a laboratory's own validation confirms no need for pH adjustment, that remains permissible under the "if necessary" clause. However, the committee chose to keep that statement for those circumstances where pH does impact microbial growth and must be controlled.
Comment Summary #37: Several commenters indicated that the section on method suitability does not provide details on method validation. They suggested that if there is a higher expectation of method validation for primary container closures and ready-to-sterilize components, an additional section on validation expectations should be outlined in this chapter.
Response: Comment not incorporated. Validation of recovery is found in <1227> and is out of scope for this chapter.
Comment Summary #38: The commenter suggested that raw materials and excipients may include this category in the Test Sample Preparation section.
Response: Comment partially incorporated. The final text includes raw materials and excipients within the general instructions for sample preparation.

Inoculation and Dilution
Comment Summary #39: The commenter suggested revising the first sentence to clarify that the microbial suspension is being added to two different containers.
Response: Comment incorporated.
Comment Summary #40: The commenter indicated that the text in the section "Inoculation and Dilution" stating, "Add a sufficient volume of microbial suspension (not more than 100 CFU) to the prepared sample and control…" conflicts with the statement in the previous section, "Test Sample Preparation," which states, "For a fluid test sample, no preparation is required." The commenter recommended clarifying that the inoculum can be added directly to the sample or sample preparation to avoid confusion.
Response: Comment incorporated. The change was made as requested to clarify that the inoculum can be added directly to the sample or sample preparation.
Comment Summary #41: The commenter suggested adding a note to the section to provide guidance on the washing cycle, specifically recommending not to exceed a washing cycle of 5 x 100 mL. This addition aims to ensure proper guidance for microbiologists during the test sample transfer and membrane filter rinsing process.
Response: Comment not incorporated. The final text leaves the choice of rinse volume(s) and procedure to users, who are expected to validate their approach as part of method suitability.
Comment Summary #42: The commenter suggested adding a new section for dilutions, indicating that sample should be dissolved and diluted.
Response: Comment not incorporated. The chapter continues to rely on general guidance for sample preparation, leaving it to the user's discretion (and reference to other chapters such as <61>, if desired) to handle dilution procedures appropriately.
Comment Summary #43: The commenter suggested that for raw materials and excipients, which are considered substances for pharmaceutical use, the method outlined in <61> should be used.
Response: Comment not incorporated. <61> is required only when a monograph specifies its use.

Comment Summary #44: The commenter suggested adding a new section on test sample preparation to provide proper guidance to microbiologists, similar to the section in <61>. The proposed change includes specifying the amount used for the test.
Response: Comment not incorporated. Sample amounts are already provided in <1119>.
Comment Summary #45: The commenter recommended adding clarification to the term “prepared sample” to help readers link back to the test material.
Response: Comment not incorporated. The surrounding text provides sufficient clarity.
Comment Summary #46: The commenter suggested that to demonstrate acceptable microbial recovery, the test should use the lowest possible dilution of the prepared sample. They noted that if the sample causes growth inhibition due to factors such as antimicrobial activity or poor solubility, the microbial inoculum should be added after neutralization, dilution, or filtration using a validated method. Additionally, the commenter recommended including a reference to general chapter 61, as it explains this topic in detail.
Response: Comment not incorporated. The text continues to rely on the user’s discretion and validation approach for handling inhibitory products.
Comment Summary #47: Several commenters requested further clarification on the term "a validated method" in the context of inoculation and dilution. They inquired whether the validation pertains to the addition of inoculum or to the processes of neutralization, dilution, or filtration. They also sought guidelines and acceptance criteria for this validation.
Response: Comment incorporated. The change was made as suggested, removing the phrase "using a validated method" to clarify the process.

Neutralization
Comment Summary #48: The commenter suggested changing the phrase “antimicrobial activity may be negated” to “antimicrobial activity may be neutralized” to align with USP <61>. Alternatively, they proposed using “antimicrobial activity may be reduced or eliminated” if the intention is to avoid repeating the word “neutralized” in the paragraph.
Response: Comment incorporated.
Comment Summary #49: The commenter indicated that the proposed neutralization method is incompatible with the limits set in USP <1119>. Specifically, if the required sensitivity is 10 CFU/100 mL, the sample cannot be diluted to overcome antimicrobial activity and still meet this limit.
Response: Comment not incorporated. A 100 mL sample can be diluted and tested by membrane filtration. Dilution is possible, but more filtration is required to meet the acceptance criteria.
Comment Summary #50: The commenter indicated that the current statement regarding the dilution factor for microbial growth is not aligned with USP<61> and Ph.Eur. 2.6.12. The commenter suggested that the test should be performed with the highest dilution factor compatible with microbial growth and the specific acceptance criterion to ensure alignment with the USP chapters for the bioburden test method.
Response: Comment incorporated.

Recovery of Microorganisms in the Presence of Product
Comment Summary #51: Several commenters suggested that the suitability testing for membrane filtration should be performed as a single test rather than in duplicate, aligning with the approach used for bioburden testing. They noted that in USP <61>, only the suitability testing for pour plate and surface spread methods is performed in duplicate, and as a globally harmonized chapter, USP <61> should be the leading document.
Response: Comment partially incorporated. Clarity was added by addressing the final interpretation: Take the arithmetic mean for pour-plate and spread-plate methods.
Comment Summary #52: The commenter requested instruction on spiking versus rinsing as found in <1227>, specifically mentioning the use of 3 x 100 mL and spiking the final rinse. They suggested either providing this instruction directly or pointing to the relevant chapter for guidance.
Response: Comment not incorporated. The topic is covered in <1227>.

Comment Summary #53: Several commenters indicated that the section "Recovery of Microorganisms in the Presence of Product" requires testing each microorganism in duplicate during method suitability, which applies to all methods including the filtration method. They noted that this requirement is a discrepancy with USP<61>, section "Suitability of the counting method / Recovery of Microorganisms in the presence of products," which does not include any duplicate requirement for the filtration method.
Response: Comment incorporated.

Membrane filtration
Comment Summary #54: The commenter requested clarification on whether the use of "should" indicates a recommendation or a requirement. The commenter noted that "should" suggests a recommendation, but the results section implies that replicate plates are needed, as indicated by the arithmetic mean.
Response: Comment not incorporated. In the USP style, “must” typically appears for absolute requirements; “should” can still be binding but allows for minor justified modifications if scientifically validated.
Comment Summary #55: The commenter requested clarification on the rationale for requiring duplicate tests for verifications, noting that duplicate plating is not described in <61>, which is intended for release testing.
Response: Comment not incorporated. The requirement for duplicate plating is included to enhance the consistency and accuracy of counts.
Comment Summary #56: The commenter suggested using membrane filters with a nominal pore size not greater than 0.45 µm for microbial-retaining efficiency, ensuring the filter material is appropriate for the test sample. They recommended using one membrane filter for each microorganism listed in Table 1. Additionally, the commenter proposed adjusting the incubation duration for bioburden testing to be greater than 5 days for *C. albicans* and *A. brasiliensis*, or alternatively, bacteria for greater than 3 days and bacteria, yeast, and molds for greater than 5 days.
Response: Comment incorporated. Table 1 has been updated to reflect the suggested changes in incubation duration for bioburden testing.
Comment Summary #57: The commenter recommended revising the fourth sentence in the section "Recovery of Microorganisms in the Presence of Product" to ensure that spiking the indicator microorganisms with the sample matrix before filtration better ensures there is no test sample matrix interference impacting the recovery of microorganisms. The proposed change suggested transferring a suitable quantity of the test sample spiked with indicator microorganisms to the membrane filter, filtering immediately, and rinsing the membrane filter with an appropriate volume of diluent.
Response: Comment not incorporated. The chapter already prescribes adding microorganisms to the sample during the earlier steps (under “Inoculation and Dilution”) before membrane filtration.
Comment Summary #58: The commenter recommended clarifying the statement to ensure readers do not misinterpret that a rinsing step is required in all cases, as this should be driven based on method suitability. Additionally, the commenter noted that the chapter’s scope includes water samples. The proposed change aims to clarify that a rinse step is not needed in all circumstances and directs the reader to method suitability to determine if a rinsing step is appropriate and the amount of diluent to use.
Response: Comment partially incorporated. The final text now makes it clear that the rinse step is contingent upon method suitability requirements, rather than being a blanket requirement for all circumstances.

Pour-Plate Method
Comment Summary #59: The commenter requested clarification on whether the test for recovery of microorganisms in the presence of the product should always be performed in duplicate for each challenge microorganism, regardless of the method used. They noted that the Membrane Filtration section mentions using one membrane filter for each microorganism listed

in Table 1, which could be interpreted as not requiring duplicate tests when using the membrane filtration method, contrary to the prior paragraph that specifies performing separate tests in duplicate for each microorganism.
Response: Comment partially incorporated. The language requiring “two Petri dishes” (duplicates) does not extend to membrane filtration, presumably because membrane filtration already processes an entire sample (or a large, specified test volume) in a single pass. Thus, no change was made to unify these into a single “duplicate required” statement for all methods.
Comment Summary #60: Several commenters suggested aligning the section "Recovery of Microorganisms in the Presence of Product" with USP <61>. Specifically, the commenter proposed performing the pour-plate method in duplicate using Petri dishes 9 cm in diameter.
Response: Comment not incorporated. The text already states, "Use two Petri dishes for each of the microorganisms listed in Table 1."
Comment Summary #61: The commenter suggested updating the statement to clarify that the recommended guidance is not applicable to ready-to-use media.
Response: Comment incorporated.
Comment Summary #62: The commenter suggested revising the second sentence in the pour-plate method and surface-spread method sections to ensure that the volume of medium is proportional to the volume of the plate. The proposed change is to add the sentence: “If larger Petri dishes are used, increase the agar medium proportionately.”
Response: Comment incorporated.
Comment Summary #63: The commenter indicated that the temperatures in the "Recovery of Microorganisms in the Presence of Product" section are listed without specifying the unit of measurement (°C or °F). The commenter suggested adding °C to the temperatures indicated in this chapter, as it seems to be the correct unit.
Response: Comment not incorporated. The USP style guide does not require the addition of °C to the temperatures indicated as this is the default unit.

Surface-spread method
Comment Summary #64: Several commenters requested alignment with USP <61> for the section on the recovery of microorganisms in the presence of product. Specifically, the commenter suggested performing the surface-spread method in duplicate for Petri dishes 9 cm in diameter.
Response: Comment incorporated.

Results and Interpretation
Comment Summary #65: The commenter suggested that for the spread plate method, the maximum volume should be specified and should be less than 1.0 mL. They proposed that the volume spread should be a measured volume of not less than 0.1 mL but not more than 1.0 mL.
Response: Comment incorporated. A change was made to clarify the maximum volume that should be spread per 9 cm plate.
Comment Summary #66: Several commenters indicated that for routine testing using pour plate and spread plate methods, two plates are used, and the results are averaged using the arithmetic mean. However, for membrane filtration, only one test is performed using a single membrane, which does not require an arithmetic mean due to its ability to analyze larger volumes and its sensitivity.
Response: Comment incorporated.
Comment Summary #67: The commenter requested clarification on calculating the number of CFU in the original inoculum, emphasizing that the number must be below 100 CFU. They suggested taking the arithmetic mean of the counts per test nutrient culture medium and ensuring the mean count of the test organisms does not differ by a factor greater than 2 compared with the control.
Response: Comment not incorporated. The final language focuses on comparing the test result to the control by a factor of 2.
Comment Summary #68: The commenter suggested adding "if applicable" to the section on Results and Interpretation, noting that membrane filtration does not require duplicate plating.

Response: Comment incorporated. The revised text now states: "Take the arithmetic mean (for pour-plate and spread-plate methods) of the counts per nutrient culture medium."

Testing for Bioburden

Comment Summary #69: The commenter suggested that the section should require a minimum incubation duration of 5 days, instead of the current 3–5 days, to align with Table 1 for GPT and Suitability tests that require growth of yeasts and molds in 5 days. This change would ensure the detection of such microorganisms.

Response: Comment incorporated.

Comment Summary #70: The commenter recommended alternate wording to clarify the intent and provide additional clarity to the reader, ensuring that the test parameters are suitable for supporting the respective bioburden level.

Response: Comment not incorporated. The original language is concise and already encompasses the notion that sample size must be appropriate for the intended bioburden specification.

Comment Summary #71: The commenter suggested that revised wording will provide additional clarity to the reader for ensuring the test parameters are suitable for supporting the respective bioburden level. The proposed change was to include a reference to Table 3 USP <1119> for guidance on the amount of sample tested.

Response: Comment not incorporated. The final text states that the amount of sample tested is sufficient, without referencing Table 3.

Comment Summary #72: The commenter requested clarification on the phrase "the amount of sample tested must be sufficient to determine compliance" in the section on testing for bioburden. Specifically, the commenter asked for a definition of what constitutes a sufficient amount.

Response: Comment not incorporated. The Expert Committee intended to preserve flexibility and require manufacturers to determine the appropriate sample size based on their specific product and process validation.

Comment Summary #73: The commenter recommended changing the incubation time for the membrane filtration, pour-plate method, and surface-spread method from “30°-35° for 3-5 days” to “30°-35° for NLT 5 days.” The commenter indicated that the time for the routine test must not be less than the time for method suitability, which specifies an incubation time of ≤5 days for yeast and mold.

Response: Comment incorporated.

Membrane Filtration

Comment Summary #74: The commenter suggested that the section on the Test for Bioburden, Membrane Filtration should specify the number of membrane filters needed per sample. Additionally, they requested clarification on whether the final result, in the case of duplicates, is the arithmetic mean of both individual results.

Response: Comment partially incorporated. The section has been updated to clarify the distinction between membrane filtration and pour plate/spread plate duplicates.

Comment Summary #75: The commenter indicated that the section on Membrane Filtration states, “Transfer the appropriate amount to a single membrane filter, …” but does not clarify that membrane filtration is executed on a single membrane filter agar.

Response: Comment partially incorporated. The text has been clarified to distinguish between membrane filtration and pour plate/spread plate duplicates.

Comment Summary #76: The commenter recommended changing the term "wash" to "rinse" in the phrase “Transfer the appropriate amount to a membrane filter, filter immediately, and rinse the filter” to maintain consistency with previous sections.

Response: Comment incorporated. The final text standardizes on “rinse,” as the commenter recommended.

Comment Summary #77: The commenter recommended updating the current text to provide additional guidance and clarification on when it is suitable to "wash the filter." This update aims to clarify for the reader that a rinse step is not needed in all circumstances and directs the

reader back to method suitability to determine if a rinsing step is appropriate and, if appropriate, the amount to rinse the filter.
Response: Comment incorporated. A change was made such that the final text explicitly states a rinse is not always mandatory but must be determined by the validated method parameters.
Comment Summary #78: The commenter suggested adding the phrase "with the verified volume of qualified rinsing agent" to the Membrane Filtration section.
Response: Comment partially incorporated. The test was revised to "Transfer the appropriate test amount to a membrane filter, filter immediately and where required, rinse with the appropriate volume of diluent based on method suitability."
Comment Summary #79: The commenter indicated that the specified incubation time of 3–5 days for membrane filtration, pour plate method, and surface spread method may not be sufficient for recovering slow-growing organisms. They suggested that it should be possible to extend the incubation period to ensure all bioburden is recovered.
Response: Comment incorporated.
Comment Summary #80: The commenter recommended being more general in the wording for the section on Testing for Bioburden, as multiple media and incubation conditions might be required, referencing USP <1119>. The proposed change suggested transferring the membrane filter(s) to the surface of the required agar media and incubating under the appropriate conditions and then calculating the number of CFU per quantity of sample.
Response: Comment not incorporated. The final text still references SCDA specifically for routine bioburden testing, though users remain free (via method suitability and the broader guidance in <1119>) to use additional or alternative media as warranted by their product and microbial-risk profile.
Comment Summary #81: The commenter requested clarification on the use of the membrane filtration method for routine tests, suggesting that it should be one membrane filter per media, in line with USP <61>. They noted that since the proposed method specifies only one media (soybean–casein digest agar), there should also be only one membrane specified. Additionally, they pointed out a typo in the sentence "Transfer the membrane filters to the surface of soybean–casein digest agar and incubate at 30°–35° for 3–5 days," recommending that "filters" be corrected to "filter" to avoid confusion.
Response: Comment incorporated. The final text generally references a single membrane filter for routine enumeration, consistent with USP <61>. The typo has been corrected to specify "filter" instead of "filters."
Comment Summary #82: The commenter recommended that the suitability test for yeast and mold incubation duration should be a minimum of 5 days, as the current allowance is up to 5 days.
Response: Comment incorporated.
Comment Summary #83: The commenter requested clarification on which media to use for testing the rinse water of equipment cleaning, specifically when the final rinse is purified water and water for injection. The commenter suggested that if R2A media is recommended, it should be explicitly mentioned in the guidelines.
Response: Comment not incorporated. USP relies on method suitability and references to more specialized chapters (e.g., <1231>) for water-specific testing. If R2A or another medium is necessary for rinse water testing, <1119.1> does not prohibit or prevent this, it simply does not provide each specialized medium in its text.
Comment Summary #84: The commenter recommended changing the membrane filters from plural to singular format to align with the rest of the details around membrane filtration, which were stated in singular.
Response: Comment incorporated. An editorial revision was made to remove the inconsistent plural reference. The final official text in USP uses singular "membrane filter" throughout, aligning with the chapter's overall approach (one filter per test).
Comment Summary #85: The commenter suggested expressing the final result in the total volume analyzed and dividing it in CFU/mL.
Response: Comment not incorporated. Although the chapter text does not further elaborate on "total volume analyzed" specifically, it does explicitly permit reporting CFU in relation to volume, weight, or a discrete unit. The final text's flexible wording already supports this approach.

Pour-Plate Method and Surface-Spread Method
Comment Summary #86: The commenter suggested that the incubation period for the suitability test for yeast and mold should be a minimum of 5 days, as the current allowance is up to 5 days.
Response: Comment incorporated.
Comment Summary #87: The commenter requested guidance on using the Pour-Plate Method and Surface-Spread Method for bioburden samples that are not filterable due to low solubility, viscosity, or suspensions. They noted that the sensitivity of these methods is insufficient to meet the recommended limits in <1119> without increasing the sample volume plated or summing plates. The commenter highlighted that reporting the arithmetic mean of the counts would yield a sensitivity of <1 cfu/mL, which exceeds most limits in <1119>. They also mentioned that even with plating a larger volume (5 mL), the sensitivity remains <20 CFU/100 mL and expressed concern that the surface spread method might not be suitable due to the volume spread limit of NMT 1 mL.
Response: Comment not incorporated. <1119.1> does not provide step-by-step instructions for every scenario; it gives a general framework. It is up to each user to design a plating scheme (volume, number of plates, etc.) in alignment with their product's risk profile and acceptance limits, and to confirm suitability via a properly validated approach.

General Chapter/Section(s):	<1245> COMPACTION SIMULATION
Expert Committee:	General Chapters - Physical Analysis 2020
No. of Commenters:	2

1. General
Comment Summary #1: The commenter, noting that they think that only a few users know the issues and there is no real need to know about them generally, suggested mentioning the Presster only as a historical note.
Response: Comment was not incorporated. The EC determined that it is useful to mention compaction emulator as it represents a different design approach for the compaction simulation.

2. Section 2. Introduction
Comment Summary #2: The commenter stated that it would be nice to mention air entrapment when mentioning SRS since they are always linked. The commenter noted further that it would be nice to see vacuumed dies used to put some numbers on the effect of air entrapment.
Response: Comment partially incorporated. The Expert Committee added the sentence "Air entrapment at high compression speed could also contribute to the SRS" in the last paragraph of the section.

3. Section 2. Compaction Physics
Comment Summary #3: The commenter requested that the punch head shape needs to be modelled. The commenter noted that the punch head shape has no effect when loaded into a simulator. The stiffness of the punch head can be programmed into the control along with the stiffness of the press. The commenter added that "Comparing BB to D would result in a significant change in decompression rate".
Response: Comment incorporated. The Expert Committee revised Section 3.1.2 to include the punch head shape in the list of geometrical features used in the calculation of punch movement profile.

4. Section 4. Benefits of Compaction Simulation
Comment Summary #4: The commenter suggested including a paragraph on split dies and Hiestand reasoning that the advanced users are measuring their API's properties with these devices, and it would help the industry move forward if others realized the value of this. The

commenter provided an example to demonstrate its utility. The commenter provided a suggestion for the paragraph.
Response: Comment partially incorporated. The expert Committee revised the section's paragraph on advancement on equipment design to add "A split-die design may facilitate the characterization of tableting behavior of problematic powders that otherwise cannot form intact tablets when a compaction simulator is used" at the end of that paragraph.

5. Section 6. Applications of Compaction Simulation
Comment Summary #5: The commenter suggested adding "press" before "speed" in the sentence "Table speed is also known factor in the ..." to clarify what "speed" is referring to.
Response: Comment incorporated. The Expert Committee revised the text as suggested**.**
Comment Summary #6: The commenter, noting that one decimal place is insufficient to distinguish between x-axis labels, suggested changing the Figure 2 x-axis label to two decimal places to avoid duplicate instances of the same label.
Response: Comment incorporated. The Expert Committee Revised the figure as suggested.
Comment Summary # 7: The commenter, noting that good portion of their customers are modelling the process recommended it should be an entry that the powder properties are used to build a material properties database for mathematical modelling and comparative performance prediction.
Response: Comment incorporated. The Expert Committee revised the bullet list at the end of section 6.3 to add: "Establishment of a database for compression properties of both drug substances and excipients to facilitate material-sparing formulation design and optimization."

6. Section 7. Other Considerations for Successful Compaction Simulation
Comment Summary #8: The commenter suggested adding a paragraph as follows: To enable simulation of any press or roller compactor, the punch position control must be fully programmable and, therefore, servo-controlled. To accurately control large forces and high speeds in a matter of a few milliseconds is technically very challenging, requiring a very stiff loading structure, and very high-speed control algorithms to make all the necessary compensations and corrections. It is important to use expert independent advice when assessing if a compaction simulator is capable of simulating production conditions.
Response: Comment partially incorporated. The following text was added at the end of Section 7: "Accurate simulation of any press or roller compactor requires punch position to be fully programmable and, therefore, servo-controlled. To control large forces at high speeds accurately, e.g., in a matter of a few milliseconds, is technically very challenging and requires a very stiff loading structure, and very high-speed control algorithms to make all the necessary compensation and corrections."

General Chapter/Section(s):	<1265> WRITTEN PRESCRIPTION DRUG INFORMATION -- GUIDELINES
Expert Committee:	Healthcare Safety and Quality
No. of Commenters:	1

Alternative term
Comment Summary #1: The commenter indicated concerns with the proposed updates based on use of the terms "medication guide" and "Patient Medication Information." The FDA currently has regulations pertaining to the development and review of "medication guides" found in 21 CFR 208 and recently published a proposed rule, Medication Guides: Patient Medication Information, intended to amend that regulation. Additional information on Patient Medication Information can be found on the following FDA website: https://www.fda.gov/drugs/fdas-labeling-resources-human-prescription-drugs/patient-medication-information-pmi. While some of the suggestions in the guideline align with the current and proposed regulations, many do not. This may cause a great deal of confusion for the impacted parties.

Response: Comment incorporated. The term Consumer Medication Information (CMI) will be used to avoid any confusion.
Comment Summary #2: The commenter indicated that the form of information which is referenced in the guideline, patient information developed by third party entities (not the FDA nor the applicant of the medication), is historically referred to as a Patient Information Leaflet or Consumer Medication Information (CMI). The FDA published a guidance on CMI entitled, Useful Written Consumer Medication Information (CMI), that provides recommendations on the development of CMI that are also based on the Keystone Action Plan (referenced in the prior Prescription Drug Information Guidelines). The commenter recommended that USP use an alternative term for "medication guide" and "Patient Medication Information" to help reduce any confusion with the current FDA regulations or the proposed amendments in the proposed rule.
Response: Comment incorporated. The term Consumer Medication Information (CMI) will be used to avoid any confusion.

General Chapter/Section(s):	<1761> NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY- THEORY AND PRACTICE / Multiple Sections
Expert Committee(s):	General Chapters-Chemical Analysis Expert Committee
No. of Commenters:	5

Principles of NMR
Comment Summary #1: The commenters noted that the "×" used in the equations within this section are solely for numeric multiplication. Throughout literature, NMR equations typically use "×" to represent vector multiplication and to describe the resulting product orientation perpendicular to the multiplying vectors. For clarity, the commenters recommended replacing all the "×" used to denote numeric multiplication with "•" or "*" to avoid confusion.
Response: Comment incorporated. The "×" used in the equations are deleted.
Comment Summary #2: The commenter requested to put Equations 4 through 6 and their accompanying text before Equation 1 and 2, since these contents seem to be a description of the origin of the NMR signal.
Response: Comment incorporated.
Comment Summary #3: The commenter noted that Equations 4 and 5 both use ρ to represent angular momentum. There is no use of ρ to represent angular momentum in literature.
Response: Comment incorporated. In context, the angular momentum, ρ, is quantized in units of $\hbar$, which is Planck's constant divided by 2π, multiplied by the nuclear spin quantum number, *I*.
Comment Summary #4: The commenter recommended that Equation 4 be revised to define

$$\sqrt{[I(I+1)]}.$$

magnitude as
Response: Comment incorporated.
Comment Summary #5: The commenter pointed out that Equation 6 should have a minus sign: ΔE = -h ω/(2 π) = -h ν
Response: Comment incorporated. A minus sign was added in the equation.
Comment Summary #6: The commenter indicated some errors in Table 1 and the accompanying text. The generally accepted convention (found in various trusted NMR and Quantum Mechanical textbooks) is to use the annotation N+ for +m energy level population (or "spin up", or |α> or m=+1/2), and N- for -m energy level population (or "spin down", or |β> or m=-1/2). So, for example, for 1H, 19F, 31P, and 13C, N+ corresponds to the +1/2 population ("spin up"), and N- corresponds to the -1/2 population ("spin down"). For all the nuclei in Table 1, except 15N, their γ values are positive. Therefore, N+ corresponds to the low energy state, and N- corresponds to the high energy state, which is exactly the opposite as written in the text. Therefore, the commenter suggested changing "high- and low-energy states, respectively" to "spin up" and "spin down" populations, respectively. This description would be more universal than low- and high-energy states that depend on whether γ is positive or negative.

Another minor typographical error is that Rx/RH for 1H should be 1 instead of 1*10^6.
The following suggested changes were proposed by the commenter. 1). g-factor should be γ (Greek letter gamma, the magnetogyric ratio as described in the text). Note that g-factor is something else completely, so not to get confused between γ and g-factor.
2). For 15N, γ is negative, so change the value to -2.73.
3). Typo error with the frequency column header: N0 MHz should be v0 (MHz), i.e., the Greek letter nu.
4). In quantum mechanics and NMR, the spin quantum number I is usually given as fractional numbers, not float numbers. So, change 0.5 to 1/2. 5). Change w0 rad/s to ω0 (rad/s), i.e., the Greek letter omega.
Response: Comment incorporated. Table 1 and accompanying text were revised to reflect the correct information.
Comment Summary #7: The commenter requested deleting the sentence "*Both upward and downward transitions occur, but more upward transitions occur than downward because N- is greater than N+*."
Response: Comment partially incorporated. The requested deletion was made. The text was revised to read as the following: "*For a spin I=1/2 nucleus, there are two values for individual spin quantum number (m)N: + 1/2 and -1/2 for nuclei aligned with and against the applied magnetic field. Outside of an applied field, these states are equal. As soon as they are placed in a magnetic field, transitions begin to occur to establish an equilibrium concentration, which has more nuclei in the lower m+ state, which has lower energy. When a radio frequency (RF) is applied to the spin system, energy is adsorbed by the nuclei in the lower m+ state and they transition to the m- state. resulting in the NMR signal. After removal of the RF, transitions continue to occur between the two states until the equilibrium state is re-established*."

NMR Definitions
Comment Summary #8: The commenter noted that Figure 2 was confusing and also noted inaccuracies in the terminologies and suggested the following changes: 1). Change "Pattern" to overlapping multiplets. 2). Change definition of Peaks to resolved or partially resolved resonance, non-discrete. 3). Change definition of Lines to QM-based observable coherences, discrete. 4). Remove Transitions altogether.
Response: Comment not incorporated. GC <1762> is an informational guidance chapter. This figure explains the NMR principle based on QM. All information is correct and used in NMR general chapters. These terminologies are used for the purpose of those NMR chapters, but not for the general NMR community. USP published stimuli article and detailed the reasons why terminology needs to be well defined in the USP NMR general chapter.
Comment Summary #9: The commenter pointed out that the equation for Larmor frequency is not correct, there should be minus sign: $v0 = -\gamma/(2\pi) B0$.
Response: Comment incorporated. A minus sign was added in the equation.

Resonance, Signal, Spectrum, Pattern, Peak, Line, and Transition
Comment Summary #10: The commenter stated that CW NMR should no longer be discussed in this context.
Response: Comment not incorporated. The Expert Committee finds the current context to be sufficient, as the commenter did not provide any justification or suggestion to support such a change.
Comment Summary #11: A few commenters suggested changing some of the definitions in this section to align with NMR textbooks.
Response: Comment not incorporated. All information is correct and used in NMR general chapters. These terminologies are used for the purpose of those NMR chapters, but not for the general NMR community. USP published stimuli article and detailed the reasons why terminology needs to be well defined in the USP NMR chapter.
Comment Summary #12: The document includes a thorough description of T1 and repetition time/delay, but it lacks a recommendation of what is expected for a qNMR method to be valid, e.g., the expected recovery needed for certain tasks.

Response: Comment not incorporated. The Expert Committee recommends the user to determine how to set up repetition time, as the intended use.

Chemical Shift

Comment Summary #13: Under Equation 8, the commenter suggested to delete the sentence "In practice the chemical shift is usually determined as the difference in chemical shifts of the analyte and reference peaks divided by the operating frequency of the instrument in MHz." The commenter stated that this is not how the chemical shift is determined in practice.

Response: Comment not incorporated. The current text in the general chapter is correct. Equation 8 is revised to reflect the correction information: δ = (VI − VR)/V0.

Comment Summary #14: The commenter suggested deleting "Historically" in the sentence: "Historically, a substance was added to a sample to establish a reference signal, relative to …".

Response: Comment incorporated. The sentence was revised to read as:" ~~Historically, a~~ ***Chemical shift can be referenced by adding a*** substance ~~was added~~ to a sample to establish a reference signal~~, relative to which all other chemical shifts were determined~~."

Comment Summary #15: The commenter noted that the discussion on TMSP (2,2,3,3-d4 sodium 3-(trimethylsilyl)-propionate) and DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate), did not include the inherent pH issues. TMSP is a weak acid and its behavior in water is affected by pH. TMSP will generate peak of -0.017 ppm, not 0 ppm. Deuterated DSS is a strong acid and should be encouraged for use as a 1H reference. The commenter recommended revising this discussion for clarity and accuracy.

Response: Comment not incorporated. The EC agreed the current text and discussion deliver the correct information and decided to retain as is.

Comment Summary #16: The commenter stated that the concepts of "external calibration" and "indirect reference" seem to be interchanged. The old method of external reference is performed with another NMR tube with TMS or DSS in it, but without changing the field lock. Modern users utilize autolock, which changes the lock after the second reference NMR tube is placed in the probe. Equation 9 describes the "indirect reference" method for non 1H nuclei. The ratio should be $\frac{\nu R-13C}{\nu R-1H}$.

The vR-13C is a hypothetic frequency and, thus, non-observable. Moreover, the introduction of a chi scale is relatively new, and it is the introduction of a "unified scale" where a single primary reference is the 1H resonance of TMS for all nuclei.

Response: Comment partially incorporated. Equation 9 is an IUPAC recommendation. For clarification purposes, the sentence "For nuclei other than ^{1}H, external chemical shift referencing can…" was placed in a separate paragraph.

Comment Summary #17: The commenter suggested to expand the conditions that might affect B0 as following: "for instance, using a different solvent, temperature, concentration, and pressure."

Response: Comment partially incorporated. The sentence was revised to read as "Therefore, corrections might be needed when data are collected under conditions might affect B0 (~~for instance, using a different deuterated solvent~~)."

Comment Summary #18: The commenter stated that "high" or "low" frequency has a potential for inconsistency. "Deshielded" and "shielded" are the preferred terms. The commenter suggested changing the last sentence in this section to the following: "Therefore, it is correct to refer to peaks at the left side of the spectrum as ~~high frequency~~ ***deshielded*** and those at the right side as ~~low frequency~~ ***shielded***."

Response: Comment incorporated. The chapter text revised as suggested.

Coupling

Comment Summary #19: The commenter suggested deleting the last paragraph under the Coupling section, as the section contains incorrect and incomplete information.

Response: Comment incorporated. The original text in last paragraph was deleted. The following text was added: “Quadrupolar nuclei (I > 1/2) that possess large quadrupolar coupling, such as 14N, often will not produce observable J-coupling with neighboring nuclei. This occurs because the large quadrupolar coupling causes fast relaxation among the energy levels of J-coupling, and therefore averaging out J-couplings with neighboring nuclei. J-couplings with quadrupolar nuclei that possess small quadrupolar coupling, such as 2H, are often observed.”

Relaxation

Comment Summary #20: The commenter pointed out that Table 2 and Table 5 cover the same topic and suggested merging the two tables.

Response: Comment incorporated. Table 2 was deleted. Table 5 was moved to the current location of Table 2.

Comment Summary #21: The commenter requested clarification of “loss of Mxy” in Table 2.

Response: See comment 20.

Comment Summary #22: The commenter noted that various references to “relaxation delays.” Relaxation delay refers to the varied time period used for T1/T2 measurement in the middle of a pulse sequence. The more accurate term would be “recycle delay”.

Response: Comment partially incorporated. It should be noted that most methods use a repetition time, T_R, to increase the time between pulses. In this chapter, we use the term T_R, to be the time between two successive excitation pulses. T_R is the sum of a delay and the acquisition time (AT).

Comment Summary #23: The commenter pointed out that some instrument suppliers do not use recycle time as a parameter. The term *d1* is usually called relaxation delay. T_R = *d1* + AT (acquisition time).

Response: Comment incorporated. This section now reads as follows: “*It should be noted that most methods use a repetition time, TR, to increase the time between pulses. In this chapter, we use the term TR to be the time between two successive excitation pulses. This TR is the sum of a delay and the acquisition time (AT).”*

Nuclear Overhauser Effect

Comment Summary #24: The commenter suggested adding that for nuclei with negative gyromagnetic ratio (e.g., 15N or 29Si), 1H decoupling will decrease the peak intensity due to negative NOE.

Response: Comment incorporated. The following text was added to address the comment: “*An NOE is commonly observed in H-decoupled C spectra and can result in an up to three-fold increase of the peak intensity in these spectra. For nuclei with negative gyromagnetic ratio (e.g. 15N or 29Si), 1H decoupling will decrease the peak intensity due to negative NOE*.”

NMR Spectrometers

Comment Summary #25: The commenter pointed that dispersion is a parameter that should be added in the text.

Response: Comment incorporated. Dispersion was added in the section of NMR SPECTROMETERS. To further clarify the parameter, dispersion, the current sentence is revised as: “As the performance of an NMR spectrometer, in terms of sensitivity and spectral resolution and dispersion, depends importantly on the field strength of its magnet, it is common to classify the instruments by the approximate resonance frequency of the ^{1}H resonance, e.g., 600 MHz, or by their field strength, e.g., 14.1 T.”

Probe

Comment Summary #26: The commenter questioned why two inverse probes were discussed in the text. The commenter stated that BB(F)O (instead of BBI) vs. TXI would cover the two most common probe geometries.

Response: Comment not incorporated. This comment fits for use of (only) one NMR vendor. The EC decided to retain the text as is.

Repetition Delay

Comment Summary #27: The commenter noted that the accuracy of qNMR does not depend on the flip angle. A 90° flip angle with Tr>5T1 is the most efficient choice with respect to SNR per unit of measurement time.
Response: Comment incorporated. The text was revised as following: "*For typical NMR experiments, a repetition time (T_R) is used. T_R is the sum of the relaxation delay and the AT. For less than 1% error, the T_R should allow for at least 99% recovery. The most efficient process is to use 90° pulse, provided this yields a uniform excitation. Shorter pulse may be used if this is necessary to achieve uniform excitation*."

Post-Acquisition Data Processing
Comment Summary #28: The commenter recommended adding some additional discussion/instruction. Phase correction is critical for data processing/quantitation; additional detail will be valuable to the reader. The commenter proposed the following text for the Committee's consideration: "*A properly designed pulse sequence program that keeps the need for the first order phase correction minimal should be adopted whenever possible. If the first order phase correction is zero, the first FID data point should be scaled 0.5 to balance the double counting during FT*."
Response: Comment not incorporated. The text is too specific for the old model of the NMR instrument and may not be applied in some scenarios. For example, this may not apply when using a digital filter.
Comment Summary #29: The commenter pointed out some errors in Equations 15 and 16. The recommendations are to change as follows. Equation 15: Correct exp-i ω t (with -i ω t in the superscript) to either exp(-i ω t) or e-i ω t; Equation 16: Correct exp-i ω t (with -i ω t in the superscript) to either exp(-i ω t) or e-i ω t.
Response: Comment incorporated. The equations were corrected per commenter's suggestion.

Integration
Comment Summary #30: The commenter suggested adding a schematic diagram of a peak with the 8-10 points and the representation of the FWHM for better explanation.
Response: Comment incorporated. Added Figure 7 for this purpose.

Table 4
Comment Summary #31: The commenter found it difficult to use and understand Table 4.
Response: Comment not incorporated. The information provided in Table 4 is correct and sufficient.

Quantitation-Experimental Design
Comment Summary #32: The commenter noted that Equation 17 is incorrect as there are wo many minus signs in the denominator. In addition, the commenter requested adding a definition of the recovery of magnetisation, q.
Response: Comment incorporated. The equation has been corrected. q has been defined as the recovery of magnetization.
Comment Summary #33: The commenter requested the provision of examples of shorter acquisition times in relation to the magnetic field in this section.
Response: Comment partially incorporated. The text was revised as follows: "*In quantitative methods for nuclei other than 1H, the possibility of differential NOEs can be avoided by using an inverse-gated 1H decoupling regime (that is, only turning on the decoupler during AT) with short acquisition times (e.g., 1 s) and longer recovery delays*."

Bias Assessment as an OQ Or PQ Test
Comment Summary #34: The commenter suggested spelling out CRM as this is the first time the acronym CRM is used.
Response: Comment incorporated.

Figure A1

Comment Summary #35: The commenter suggested simplifying the Ishikawa diagram in Figure A1 to encourage the use of qNMR for assay determinations.
Response: Comment not incorporated. Figure A1 is not only an example, but it shows all relevant factors for qNMR applications. The point of this complete view is to show the varied contributions to the uncertainty of the whole qNMR procedure. Simplifying it could introduce extra risks for best practice. More details have been included in Table A2, a heat map for risk assessment.

Reference
Comment Summary #36: The commenter pointed out that the weblink in reference (2) is a personal webpage not a controlled document. The document in the webpage itself refers to the two IUPAC articles for unified scale referencing. The commenter suggested referencing those two IUPAC articles directly.
Response: Comment incorporated.

Operational Qualification
Expert Committee-Initiated Change #1: A discrimination may be important for Qualitative or Quantitative purposes. In the case of Qualitative purposes, the four characteristics described are sufficient. For Quantitative purposes, Linearity and Accuracy must be complemented by Precision. The GMP guidelines address accuracy (trueness) and precision separately. This Chapter needs to add Precision to be in line with the GMP guidelines.
Expert Committee-Initiated Change #2: In case "Uniform peak response" is the test responsible for testing Precision, the nomenclature is not the one that GMP uses for general purposes, and we recommend describing the test as *"Precision (Uniform peak response)"* or either *"Uniform peak response (Precision)"* to clarify the item. On the other hand, URS must be compared (and met) with the instrument functional specifications (FS) to assess the AIS suitability and to prepare the OQ tests and acceptance criteria during qualification.

General Chapter/Section(s):	<1762> SOLID-STATE NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY—THEORY AND PRACTICE / Multiple Sections
Expert Committee(s):	General Chapters-Chemical Analysis Expert Committee
No. of Commenters:	9

General Comments
Comment Summary #1: The commenter suggested extending the text to include drug delivery systems using Solid-state NMR (ssNMR) in encapsulation analysis.
Response: Comment incorporated. A statement regarding the complex drug delivery systems was added in the first paragraph in the INTRODUCTION section with new references.
Comment Summary #2: The commenter suggested using "liquid state" instead of "solution state."
Response: Comment not incorporated. In the ssNMR technology, "solution state" is the common terminology used.
Comment Summary #3: The commenter recommended to use "SSNMR" instead of "ssNMR" in the chapter.
Response: Comment not incorporated. Both acronyms are used in the literature. However, USP has used "ssNMR" consistently throughout the chapter text.
Comment Summary #4: The commenter suggested including a statement of HR-MAS in the section of ^{1}H Solid-State NMR.
Response: Comment not incorporated. The Expert Committee (EC) agrees that it is a good suggestion; however, they think HR-MAS fits better in a solution NMR chapter.
Comment Summary #5: The commenter recommended using "peak" instead of "line" throughout the document.
Response: Comment not incorporated. "Line broadening" and "line width" are well accepted terminologies in NMR textbooks as well as literature. These terminologies are also aligned with other NMR chapters in USP-NF.

Comment Summary #6: The commenter recommended not using the terms “technologies,” “methodologies,” and “methods” interchangeably.
Response: Comment partially incorporated. To better align with the USP terminology, some “technologies” and all “methodologies” have been changed to “methods.”

Introduction
Comment Summary #7: The commenter recommended changing the first bullet point of “Unambiguous identification of polymorphs” to “Unambiguous identification of solid forms” or “Unambiguous identification of polymorphs and other solid forms.”
Response: Comment incorporated. The text was changed to “Unambiguous identification of polymorphs and other solid forms.”
Comment Summary #8: The commenter stated that solid-state NMR (ssNMR) has been around for a fairly long time and not an emerging technology.
Response: Comment partially incorporated. The emerging technology did not refer to ssNMR but ssNMR application for characterization of pharmaceutical solids forms. “Emerging” was replaced by “advanced” in the text.
Comment Summary #9: The commenter suggested to revise the second sentence in second paragraph as follows “*Unlike many other techniques used to characterize pharmaceuticals in the solid state, ssNMR can be used to study nondestructively and non-invasively both DSs and drug products, and it can provide quantitative information about different forms of DS without the need for standards.”*
Response: Comment partially incorporated. The term “standards” was changed to “calibration curve.” The “calibration curve” can convey the scientific meaning in this context better and more accurately than “standards.”

Differences Between Solution NMR and Solid-State NMR-Table 1
Comment Summary #10: The commenter recommended addressing the magnetic field as “NMR proton frequency.”
Response: Comment partially incorporated. The text was changed to “Magnetic field (^{1}H frequency).”
Comment Summary #11: The commenter stated that pulse delay can be up to hours depending on the sample.
Response: Comment partially incorporated. A note was added to Table 1 to explain that the values or example listed in the table are typically used for routine applications.
Comment Summary #12: The commenter stated that ^{1}H is now more common with advances in ultra-fast spinning speeds. Most bio-solids use ^{1}H detection.
Response: Comment partially incorporated. A note was added to Table 1 to explain that the values or example listed in the table are typically used for routine applications.
Comment Summary #13: The commenter requested clarification on samples for solution NMR. Soluble system can be formed by solids, liquids, and gases.
Response: Comment incorporated. The “soluble system” was changed to “sample solution.”
Comment Summary #14: One commenter requested changing the word “unnecessary” for spinning in solution NMR to “as needed.” Many 1D NMRs are still run with a spinning sample. The other commenter requested the addition of typical values for the spinning frequency.
Response: Comment incorporated. The text was revised to use “as needed” instead of “unnecessary.” Spinning frequency was added as requested (10–100Hz for solution NMR and 1-150Hz for ssNMR).
Comment Summary #15: The commenter suggested including ^{31}P as a common nucleus for solution NMR.
Response: Comment incorporated.

Differences Between Solution NMR and Solid-State NMR
Comment Summary #16: The commenter suggested including the designation “TCP” along with “TCH” because useful cross polarization can occur between any two nuclei with different magnetogyric ratios and natural abundances.

Response: Comment partially incorporated. A sentence (see bold below) was added as follows: "…which represents how quickly the magnetization is transferred from ^{1}H to ^{13}C, **although magnetization can be transferred between other nuclei pairs**."
Comment Summary #17: For the last sentence in the third paragraph, the commenter pointed out that the lattice does not become invisible to analytical techniques; rather, it can only exist and be observable when materials are crystalline.
Response: Comment incorporated. The sentence was revised as follows: "*This is because lattice properties only exist when materials are crystalline*."
Comment Summary #18: The commenter recommended changing "load into the rotor" in the third paragraph and "Must fit in the rotor" in Table 1 to "In ssNMR, filling the rotor may increase the sensitivity."
Response: Comment incorporated. The text in the third paragraph was revised as recommended. A footnote was added in Table 1 to allow for more flexibility.
Comment Summary #19: The commenter suggested revising the first sentence in the second paragraph as follows: "*Without applying special experimental techniques, spectra of solids are dominated by extensive line broadening, making direct interpretation of spectra difficult or impossible."*
Response: Comment incorporated. The sentence was revised as suggested.
Comment Summary #20: The commenter requested clarification on whether sample concentration or sample volume is referred to in the sentence: "When too much sample is placed…".
Response: Comment incorporated. The sentence was revised to "When the sample concentration in the NMR tube…"
Comment Summary #21: The commenter stated that the primary concern regarding high molecular weight samples is from fast, efficient T2 relaxation, not from incomplete averaging of dipolar/VSA interactions. The commenter requested revising the following sentence related to high molecular weight samples to the following: "Also*, if the molecular weight of the sample is too high, efficient T2 relaxation driven by rotational diffusion will lead to extensive line broadening.*"
Response: Comment incorporated. The sentence was revised as requested.
Comment Summary #22: The commenter suggested expanding the acronym CSA (chemical shift anisotropy) when it first appears in the text as well as defining the term as the chemical shift that depends on the orientation of a bod vector relative to the static field.
Response: Comment partially incorporated. When CSA fist appears, it should be written out in full. However, both CSA and dipolar interactions share a key physical characteristic, i.e., they are orientation-dependent interactions in ssNMR. Therefore, the text was revised as follows: "The *net result is that in solids, the anisotropy interactions, such as chemical shift anisotropy (CSA) and dipolar interactions, both of which depends on the orientation of the interaction with respect to the static magnetic field, are not averaged*."

Theory
Comment Summary #23: The commenter suggested changing "…samples of protein science…" to "…samples in protein science."
Response: Comment incorporated. Text changed as suggested.

Essential Line-Narrowing and Signal Enhancement Techniques for Solid-State NMR
Comment Summary #24: The commenters suggested several revisions regarding MAS and HPPD. 1. Replace "eliminate" with "reduce" to be more accurate. 2. Add a definition of magic angle. 3. Add that 54.7°. is the magic angle because of $3\cos 2(\theta) - 1 = 0$. 4. Revise the statement on the effects of MAS to be consistent within the text in this section.
Response: Comments incorporated. 1. Changed as suggested. 2. A definition was added in the text. 3. Added as suggested. 4. Revision made to maintain consistency within the text.
Figure 2
Comment Summary #25: The commenter suggested to add the following sentence at the end of the Figure 2 caption: "At faster spinning speeds (13 kHz), the sample can heat up, resulting in an increase in the rate of the phenyl ring flips in ibuprofen and incomplete averaging of the

chemical shift tensor due to the phenyl ring flip rate occurring on the same timescale as the MAS rate."
Response: Comment incorporated.

Magic-Angle Spinning
Comment Summary #26: The commenter suggested including the approximate dipolar coupling constants for $^{1}H/^{1}H$, $^{19}F/^{19}F$, $^{1}H/^{19}F$, $^{1}H/^{13}C$, $^{1}H/^{31}P$, and $^{1}H/^{15}N$ to show roughly how fast MAS would need to be to eliminate the interaction. The commenter recommended adding a table that has both dipolar and CSA values for different nuclei.
Response: Comment not incorporated. The information exceeds the scope and depth intended for a general chapter. Additionally, dipolar and CSA vary across different spin pairs and may not always be relevant to pharmaceutical applications.
Comment Summary #27: The commenter requested a clarification of the temperature symbol °.
Response: Comment not incorporated. Per USP-NF General Notices, temperatures in USP text are expressed in centigrade (Celsius) degrees.
Comment Summary #28: The commenter suggested adding VT ssNMR for safety concerns.
Response: Comment not incorporated. The context has adequately addressed the safety considerations related to sample temperatures.
Comment Summary #29: The commenter requested changing the spin frequency for fast or ultra-fast MAS from 150 kHZ to 190 kHZ as the current commercial instrument can achieve this limit.
Response: Comment incorporated.

High-Power ^{1}H Decoupling
Comment Summary #30: The commenter suggested revising the sentence, "The strong dipolar interactions also cause spin diffusion, or the energy-conserving transfer of magnetization between neighboring ^{1}H spins," as follows: The strong dipolar interactions also cause spin diffusion (the energy-conserving transfer of magnetization between neighboring) ^{1}H spins.
Response: Comment incorporated.
Comment Summary #31: The commenter suggested removing the extra "range" in the following: "… protons within a **range** of tens of nanometer range in most pharmaceutical solids."
Response: Comment incorporated.
Comment Summary #32: The commenter suggested the addition of the following sentences after "…for a given sample and acquisition condition.": "*In the past, continuous wave or CW decoupling was used in the solid state, but had limitations for being able to fully decouple the 1H nuclei from the low-abundant spins. The development of new decoupling techniques resulted in significantly improved decoupling and are now commonly used. These decoupling schemes include two-pulse phase modulation (TPPM).*"
Response: Comment incorporated. The commenter's suggestions can provide more background information about the high-power ^{1}H decoupling; therefore, the text added as suggested.

Cross-Polarization
Comment Summary #33: The commenter suggested using "magnetogyric" ratio instead of "gyromagnetic" to be consistent with IUPAC.
Response: Comment incorporated.
Comment Summary #34: The commenter suggested noting in this section, or in the later section on Relaxation, that the use of a flip-back pulse can aid signal averaging in cases where long relaxation times are problematic.
Response: Comment not incorporated. The challenge of flip-back pulses is that they only work for CW decoupling, and the advent of decoupling methods such as TPPM do not allow for flip back pulses.
Comment Summary #35: The commenter suggested including a statement that an optimal Hartman–Han match condition is modified by the spinning speed as follows: "*Residual*

molecular motion, or fast magic-angle spinning, can reduce some of the dipolar coupling between the spins, rendering CP less effective."
Response: Comment incorporated.

Additional Solid-State NMR Techniques
Sideband Suppression and Spectral Editing
Comment Summary #36: The commenter suggested the addition of the following text at the end of the first paragraph for more clarity: "but may not be suitable for removing large SSB manifolds such as those found with nuclei such as 207 Pb, where the spinning sidebands can extend for many orders of magnitude and may not be effectively averaged in a single experiment due to non-uniform excitation across the sideband manifold."
Response: Comment incorporated.

Techniques for Quadrupolar Nuclei
Comment Summary #37: The commenter requested clarifying if the "cubic environment" refers to a cubic crystal lattice.
Response: Comment incorporated. The text was changed to "…cubic crystal lattice environment…"
Comment Summary #38: The commenter recommended specifying the benefits of higher fields strength when observing quadrupolar nuclei.
Response: Comment incorporated. A sentence was added at the end of the first paragraph of this section: "*Unlike chemical shift anisotropy, quadrupolar interactions decrease with increasing field strength, which motivates higher fields for studying quadrupolar nuclei*."
Comment Summary #39: The commenter recommended that "Zeeman interaction" be further described or a reference be added.
Response: Comment not incorporated. "Zeeman interaction" was defined in a related USP GC <1761>.
Comment Summary #40: The commenter requested adding a discussion on the 90 pulse length. For quadrupolar nuclei in a non-symmetric environment, only the -1/2->1/2 transition is observed. This will have a lower 90 pulse length if all transitions are observed, such as that observed in solutions.
Response: Comment not incorporated. The comment was highly appreciated; however, this chapter is not intended to delve into such technical details.

TWO-DIMENSIONAL TECHNIQUES
Comment Summary #41: The commenter recommended stating that 2D ssNMR is practical for small molecules although the data collection process is long.
Response: Comment not incorporated. Experiments, e.g., HETCOR can be used for large molecules.
Comment Summary #42: The commenter requested to clarify the caption of Figure 3 on whether ibuprofen is isotope labeled or not.
Response: Comment incorporated. The caption of Figure 3 was revised to indicate ibuprofen was not labeled.
Comment Summary #43: The commenter requested revising "...characterizing the full CSA…" to "…charactering the CSA interaction…". In practice, "charactering the full CSA" means degerming the values of the irreducible elements of that tensor.
Response: Comment incorporated. Revised as suggested.
Comment Summary #44: The commenter suggested including a description of another frequently used ssNMR experiment (in addition to PASS, SUPER, and ROCSA). The commenter proposed the following text for consideration: "*Another experiment used is Rotational-echo double resonance (REDOR). REDOR is an ssNMR methodology, which provides intramolecular and intermolecular distance information by reintroducing heteronuclear dipolar coupling under magic angle spinning conditions*."
Response: Comment partially incorporated. The revised text now reads: "*Other experiments are often used to measure distances between either homonuclear or heteronuclear pairs of nuclei. For example, rotational-echo double resonance (REDOR) is an ssNMR method which*

provides intramolecular and intermolecular distance information by reintroducing heteronuclear dipolar coupling under magic angle spinning conditions."

Ultrafast Magic-Angle Spinning

Comment Summary #45: The commenter recommended adding text on the corresponding reduction in sensitivity due to the lower sample mass as well as the greatly reduced or eliminated ability to do cross polarization.

Response: Comment incorporated. The following sentence was added at the end of the first paragraph: "*One downside is that there is a corresponding reduction in sensitivity due to lower sample mass, which may negatively impact the ability to study dilute nuclei such as* ^{13}C*.*"

Comment Summary #46: The commenter stated that ultrafast MAS and regular 15 kHz on a 4 mm rotor are very similar. The pressure at the edge of the sample is high for any rotor spinning close to its design limit. In addition, the commenter requested correcting the term "progressive saturation" to "saturation recovery" in the previous text.

Response: Comment incorporated. The text was revised to read as "…both fast and ultrafast…" Moreover, "progressive saturation" was changed to "saturation recovery" as suggested in the previous section.

Comment Summary #47: The commenter suggested adding text on the effect of magnetic field strength on MAS and spinning sidebands either in this section or in the earlier section on MAS.

Response: Comment incorporated. The following sentence was added at the end of the second paragraph on MAS: "*Because CSA scales with field strength, faster spinning speeds are needed at higher static magnetic field strengths to obtain comparable spinning sideband patterns.*"

Dinamic Nuclear Polarization

Comment Summary #48: The commenter suggested revising "…the high theoretical gamma ratio of an electron…" to a more technical form as "…the high theoretical ratio of the gyromagnetic ratios of an electron…".

Response: Comment incorporated. Text revised as suggested.

Relaxation

Comment Summary #49: The commenter recommended removing the statement "…T1 and T2 are similar…" or clarify the meaning.

Response: Comment incorporated. The statement "Typically, relaxation time in solution for spin-lattice relaxation (T1) and spin-spin relaxation (T2) are very similar." Primarily refers to solution NMR. To avoid confusion, this sentence was removed.

Comment Summary #50: The commenter recommended the text "…which is not measured in solution NMR…" or clarifying that it is used in the context of relaxation dispersion experiments.

Response: Comment incorporated. The text was revised as follows: "…which is not **often** measured in solution NMR…"

Response: Comment incorporated. Text revised as suggested.

Spin–Spin Relaxation Time (T_2 and T_2^*)

Comment Summary #51: The commenter recommended not referring to T_2 and spin–spin relaxation of solids NMR since the decay of the NMR signal due to dipolar couplings is not relaxation. The commenter also pointed out that "spin-spin-lattice" is not the correct term.

Response: Comment partially incorporated. "Spin-spin-lattice relaxation" was revised as "spin–spin relaxation." The EC considered it necessary to retain the section as is to reflect the difference between T_2 and T_2^* in the solution and solid state.

Spectrometer

Comment Summary #52: The commenter suggested adding text on large-bore magnetics, which enable the design of probes that can handle much higher temperatures than narrow-bore NMR spectrometers.

Response: Comment incorporated. A sentence was added in the text: "*The wide bore may also offer an advantage of handling a broader temperature range for its better venting*."

Magic Angle Spinning Probes

Comment Summary #53: The commenter suggested revising the third sentence to "*The MAS* stator may be designed to accommodate rotors from less than 1 mm outside diameter to greater than 7 mm in diameter**."**

Response: Comment partially incorporated. The sentence was revised as follows: "*The MAS stator* ***at different sizes*** *holds rotors from less than 1 mm to greater than 7 mm in diameter*."

Comment Summary #54: The commenter suggested differentiating rotor diameters and rotor lengths. Both parameters are important for sensitivity and resolution because of the different sample masses and MAS speeds as well as coil lengths in different probe designs.

Response: Comment not incorporated. The rotor length information can be found in Figure 4.

Comment Summary #55: The commenter recommended mentioning that most modern ssNMR spectrometers use hollow cylindrical rotors. Both ends of the above rotors have removable caps, which greatly improves the ability to clean them thoroughly compared to rotors where the bottom was a part of the ceramic rotor and not removable.

Response: Comment not incorporated. The choice of rotor types primarily depends on the probe vendor.

Comment Summary #56: For HX probes, the commenter suggested stating that X is typically ^{15}N to ^{31}P because there are many variations outside of this range.

Response: Comment incorporated. The word "typically" was added in the sentence as suggested.

NMR Spectrometer Setup

Comment Summary #57: The commenter requested clarification on the following sentence: "Experimentally, this is performed by applying a 180° pulse, which, in a CP experiment, should result in a signal null." Measuring ^{1}H with a single-pulse experiment is faster and does not require the CP condition to be set. For the X nucleus, the null occurs at 90 when using CP followed by an RF pulse to measure ^{13}C 90.

Response: Comment not incorporated. The context includes necessary information of the CP setup. The comment was highly appreciated; however, these technical details are beyond the scope and intent of this general chapter.

Comment Summary #58: The commenters suggested discussing the spectrometer setup in the order in which it is typically done and note the standards used for each step. The commenter recommended adding a typical setup procedure in the text.

Response: Comment not incorporated. The purpose of this chapter is not to provide guidance about how to set up a spectrometer but rather to describe the steps that should be followed in any order. The instrument manufacturers can provide the setup to the users.

Table 2

Comment Summary #59: The commenter suggested reorganizing some paragraphs. For example, the MA adjustment and the COO resonance are helpful for glycine or 2MGA, which is the best method for achieving sharp resonance. The commenter would advise against stating adamantane as a sensitivity standard, as it has a couple of concerning properties.

Response: Comment not incorporated. While the Expert Committee values these comments, the purpose of this chapter is not to provide a specific operational list for an individual spectrometer but rather which topics should be of concern. The purpose of the current text is not to identify a specific procedure but highlight the potential topics that might be of concern.

Comment Summary #60: The commenter stated that shimming can also be done on ^{1}H using a suitable compound such as silicon rubber or a silicon-based polymer such as DOWSIL™ 9506 Powder.

Response: Comment incorporated. Silicon rubber was added in the table for ^{1}H.

T***able 3***

Comment Summary #61: The commenter suggested changing "liquid ammonium" to "liquid ammonia."

Response: Comment incorporated.

Comment Summary #62: The commenter requested the addition of the sources for the chemical shift values as these values are not universally adopted and will vary with temperature.
Response: Comment incorporated. A few relevant references were added.
Comment Summary #63: The commenters requested consistency with IUPAC for the chemical shift reference values.
Response: Comment partially incorporated. To better align with IUPAC for the chemical shift reference values, a few relevant references were added.

Spectrometer Calibration
Comment Summary #64: The commenter suggested not to discuss probe arcing too much in the text.
Response: Comment not incorporated. Probe arcing is one of the major issues in practical applications.

Sample Preparation
Comment Summary #65: The commenter suggested the addition of the sample rotor diameter information for a sample size range of 40–100 mg.
Response: Comment incorporated. The sample rotor diameter information was added as follows: "…*40-400 mg of material is typically required to fill a sample rotor* **at the size of 4 and/or 7 mm rotors**."
Comment Summary #66: The commenter mentioned that aside from sample packing for irregularly granular material, minor damage to the spinner turbine blades is the most common reason for rotors failing to spin or spinning erratically. The commenter recommended to pack samples in multiple layers, each comprising about 10%–15% of the rotor volume.
Response: Comment partially incorporated. Part of the commenter's suggestion highlights best practices of sample packing for irregularly granular material; therefore, the text was revised as follows: "If a rotor does not spin, the first step is to **examine the intactness of the cap and the rotor. One can then** compress…"

General Test Procedure
Comment Summary #67: The commenter requested two corrections in the second paragraph of this section. a. ^{13}C lower gyromagnetic ratio should be ¼ not 1/3 of ^{1}H. b. ^{31}P is 40% of ^{1}H not 1/3.
Response: Comment incorporated. Corrections were made per request.
Comment Summary #68: The commenter noted that ^{15}N solids is only practical for samples with ^{1}H T_1's <5 s. ^{1}H indirect detection is not practical in solids for spinning speeds <70 kHz.
Response: Comment acknowledged. In this section, the purpose is not to specifically address the feasibility of ^{1}H detection. The EC appreciated the comments on the T_1 dependance of ^{15}N but would not exclude the feasibility of ^{15}N experiments with long T_1.
Comment Summary #69: The commenter disagreed with the statement that larger rotors, up to 7 mm, provide significantly improved sensitivity at the expense of spinning sidebands and lower decoupling powers. The commenter pointed out that a 3.2 mm rotor has better RF performance for a given power amplifier.
Response: Comment not incorporated. Without quantitative analysis on literatures, the EC would not put such information in the text.
Comment Summary #70: The commenter requested clarifying if the lowest spinning speed is 6 kHz.
Response: Comment incorporated. The text was revised by changing 6 kHz to 1 kHz.
Comment Summary #71: The commenter requested including °C or °F after the approximate temperature change (30°).
Response: Comment not incorporated. Temperatures are expressed in centigrade (Celsius) degrees in the USP-NF text.
Comment Summary #72: The commenter suggested noting that the CSA of ^{19}F typically results in much larger spinning sidebands even with relatively fast spinning, and the peak widths are relatively broad even in crystalline materials, regardless of whether ^{1}H decoupling is used.

Response: Comment not incorporated. The ^{19}F peaks of crystalline and amorphous forms have distinct linewidths.
Comment Summary #73: The commenter suggested revising “For fields above 600MHz” to “For magnetic field strengths greater than 14.1 Tesla (i.e., 600 MHz frequency for 1H)” for a more scientific and accurate description.
Response: Comment incorporated. The text was revised as requested.
Comment Summary #74: The commenter suggested adding more explanation about why faster spinning has less benefit for ^{19}F and ^{31}P than ^{1}H. The lower abundance of these nuclei does not explain the observations in any given spectrum. This could be due to factors such as relative peak widths, the number of expected isotropic peaks, and/or the size and number of spinning sidebands. Additionally, the commenter requested defining “faster” more clearly. It is not clear whether “faster” means relative to typical spinning speeds for ^{19}F and ^{31}P (which are not specified) or relative to typical or fast MAS speed for ^{1}H, which could be >100 kHz.
Response: Comment partially incorporated. The following text was added for clarity: “…because the dipolar interactions between the nuclei are much weaker as they are more dilute in the sample compared to ^{1}H."
Comment Summary #75: The commenter stated that the formal relaxation time measurements are rarely used in qualitative or semi-quantitative analysis. Determining ^{1}H T_1 and $T_{1\rho}$ relaxation times is unnecessary for most ssNMR experiments. Instead, optimal magnetization recovery or cross polarization can be achieved by running a ^{1}H-^{13}C CP/MAS experiment with an arrayed pulse delay or contact time and observing when peaks reach their maximum intensities. This method is effective for pure materials with relatively low molecular weights (i.e., <1000), requiring no more than four acquisitions per spectrum if the spectrometer is properly optimized. The commenter noted that several iterations of these experiments could be typically completed in a fraction of the time needed for a single formal ^{1}H T1 or T1ρ relaxation experiment. However, aromatics and carbonyls may have different cross polarization times than aliphatic carbons, necessitating a compromise in contact time. Despite this, it is quick and easy to determine the best acquisition parameters, even for molecules with unusually long 1H T1 or inefficient cross polarization. Real-time data processing allows for immediate assessment of timing estimates, enabling adjustments as needed.
Response: Comment not incorporated. This is dependent on the laboratory—some laboratories will automatically measure these values, as they can be useful for comparing crystalline and amorphous samples (among other reasons). The EC agrees that this method is often a useful practice to estimate the relaxation times. It is certainly used often by practitioners, but there are potential challenges for impure materials/mixtures.
Comment Summary #76: The commenter suggested adding a picture to Figure 4.
Response: Comment incorporated. Additional examples of various rotor sizes have been included in Figure 4, providing users with a comprehensive overview of the available rotor options.
Comment Summary #77: The commenter suggested using “analyte” or “compound” throughout the chapter instead of “drug substance molecules.”
Response: Comment partially incorporated. The EC evaluated the whole chapter text and made applicable changes. In this section, “drug substance molecule” was replaced by “API.”
Comment Summary #78: The commenter stated that the ^{1}H lines are broad, and ^{1}H direct detection relaxation experiments are faster (typically 1 scan) than CP experiments.
Response: Comment not incorporated. ^{13}C detection is often utilized for molecule-specific relaxation measurement. One would often want to differentiate drug substance and excipients in solid dosages.

Post-Acquisition Data Processing
Comment Summary #79: The commenter suggested considering the usage of a Gaussian window to better match the line shape. Solids lines are typically Gaussian.
Response: Comment incorporated. The following sentence was added in the text: “*Solid-state NMR peaks of solid materials, such as crystalline and amorphous forms, typically exhibit a Gaussian line shape. Therefore, Gaussian window function is often utilized for data processing*.”

Spectral Quality Considerations
Comment Summary #80: The commenter suggested using a target minimum S/N of a reference sample as a parameter to ensure the instrument performance.
Response: Comment not incorporated. This topic is covered under the section "spectrometer calibration," where S/N specification is given.

Table 4
Comment Summary #81: The commenter suggested changing "Low concentration" to "Low signal to noise" because "Low concentration" is not applicable for ssNMR.
Response: Comment partially incorporated. The text was changed to "Low concentration (in mixtures)."
Comment Summary #82: The commenter stated that "salt" is typically a problem unless the sample is wet. Increasing power will risk arching and may be impossible in some cases.
Response: Comment partially incorporated. The text "if possible" was added after "increase power" for more flexibility.
Comment Summary #83: The commenter suggested changing the solution for Long T_1 to "Try longer pulse delay (i.e., recycle delay) for clarification.
Response: Comment partially incorporated. The text was changed to "Try longer pulse or recycle delay."
Comment Summary #84: The commenter suggested adding a note that CP parameters need to be optimized at the temperature of sample acquisition.
Response: Comment incorporated. Added a row for "weak or no signal" with the text "Check sample mobility to confirm it will cross polarize."
Comment Summary #85: The commenter requested clarification on "turn off the bearing and reopen" for "Distorted spectrum, spikes in FID."
Response: Comment partially incorporated. To better clarify a solution of distorted spectrum, the text was changed to "Probe arcing-determine if arcing stops by decreasing power and/or stop spinning."

Sample-Related Issues
Comment Summary #86: The commenter requested to change "$^1H\ T_{1\rho}H$" to "$^1H\ T_{1\rho}$ or $T_{1\rho}H$."
Response: Comment incorporated. $^1H\ T_{1\rho}$ was used in the text.
Comment Summary #87: The commenter requested the addition of some text related to sample heating due to ^1H decoupling as well as suspension or emulsion samples.
Response: Comment incorporated. The following text was added at the end of the third paragraph: "*This is particularly helpful for samples that readily flow upon magic-angle spinning, as the rotor can act as a centrifuge. In addition to sample heating due to magic-angle spinning, radio-frequency heating via ^1H decoupling for conductive samples is also a possibility and can be minimized by cooling the sample*."

Physical Form Characterization
Comment Summary #88: The commenter suggested adding an additional paragraph after the first paragraph in this section, describing how the crystal structure affects the appearance of the ssNMR spectrum. Specifically in relation to Z', the number of distinct molecular conformations in the unit cell.
Response: Comment incorporated. A paragraph was added as requested: "The *solid-state NMR spectrum of different crystalline forms can provide detailed information about molecular conformations and the properties of the crystal lattice. Typically, each conformation of the molecule in the crystal lattice will have its own peak for that nucleus. For example, a unit cell with two asymmetric molecules in the crystal lattice (e.g. Z' = 2) will usually have two peaks for each carbon. The location of the peaks in the spectrum are also related to the molecular conformation in the crystal lattice, which provides complimentary information to techniques such as X-ray powder diffraction.*"
Comment Summary #89: The commenter requested the addition of "be" in the following sentence for grammar: "…and the S/N ratio will substantially lower, compared to the crystalline form…".

Response: Comment incorporated.

Component Quantitation
Comment Summary #90: The commenter requested the correction of Equation 2 because there are two terms "exp(-T/T_1 ρ)."
Response: Comment incorporated. Equation corrected by deleting the second "exp(-T/T_1 ρ)."
Comment Summary #91: The commenter requested the addition of some text on the deconvolution of NMR because it is much more commonly used in ssNMR than liquid NMR.
Response: Comment incorporated. Text was added as follows: "For quantitative analysis,…***obtained by integration or spectral deconvolution***,…"
Comment Summary #92: The commenter suggested the addition of an additional paragraph explaining the acceptability of using deconvolution techniques, particularly for nuclei such as ^{19}F where there may only be 1–2 signals, and broad amorphous signals are likely to be overlapped. In such circumstances, accurate deconvolution is a critical technique to use for quantitative analysis.
Response: Comment partially incorporated. Quantitative ssNMR (qssNMR) requires further development in areas such as acceptance criteria, LOD, LOQ, and robustness, which are not addressed in the current chapter.
Comment Summary #93: The commenter noted that CP is generally not quantitative.
Response: Comment not incorporated. The EC stated that although caution needs to be exercised in obtaining quantitative data, it can be done. This section highlights the challenges of obtaining quantitative data.

Formulation and Drug Product Analysis
Comment Summary #94: The commenter recommended listing formulation types where ssNMR may be considered an appropriate tool for analysis.
Response: Comment incorporated. The text "in solid or semi-solid samples" was added at the end of the first sentence.
Comment Summary #95: The commenter recommended deleting the sentence, "One of the advantages of ssNMR is that…the only peaks in spectrum are due to the drug substance." The commenter mentioned that an excipient could be in any carbon-containing products; therefore, this statement may not always be true.
Response: Comment not incorporated. The sentence already has "do not typically overlap" to recognize that some excipients may overlap.

NMR Crystallography
Comment Summary #96: The commenter recommended focusing on the increasing role of computation in adding value to solid NMR spectra in order to make the text easier for the stakeholders to understand.
Response: Comment not incorporated. The EC agreed that the section on NMR crystallography is non-specific. However, the purpose of this section is to make non-experts aware of the topic, so that it can potentially be followed up later. The EC also decided that the topic is almost too specific for a USP chapter.

Implementation-Choosing a Nuclei to Study
Comment Summary #97: The commenter requested changing the section title to "Choosing a Nucleus to Study" or "Choosing Nuclei to Study."
Response: Comment incorporated. The section title was changed to "Choosing a Nucleus to Study."

^{19}F Solid-State NMR
Comment Summary #98: The commenter recommended noting that ^{19}F ssNMR usually requires a probe that can decouple ^{1}H to give the best resolution.
Response: Comment incorporated. The following text was added at the end of the section: "*The best resolution is usually obtained by using a probe that allows for 1H decoupling, as magic-angle spinning may not be sufficient to remove ^{1}H-^{19}F dipolar couplings.*"

Comment Summary #99: The commenter suggested adding a statement that the lack of peaks in ^{19}F ssNMR spectra may necessitate using deconvolution for quantitative applications, particularly if quantitating amorphous drug substance.
Response: Comment not incorporated. Deconvolution is mentioned in previous sections, and in theory, it is valid for all nuclei.
Comment Summary #100: The commenter requested removing the final part of the last sentence, "and only recently has it been common to incorporate ^{19}F into a drug product API."
Response: Comment partially incorporated. Replaced "recently" with "since the early 2000."

^{15}N Solid-State NMR
Comment Summary #101: The commenter noted that the relative CP enhancement is higher for ^{15}N (10:1) than for ^{13}C (4:1), and spinning speeds do not have to be as fast due to smaller CSA and dipolar coupling for ^{15}N; therefore, it is still practical to analyze it for many small molecules. The commenter recommended revising the last sentence as follows: "Therefore, despite its relatively low sensitivity,^{15}N ssNMR still offer useful analysis in drug products."
Response: Comment partially incorporated. The sentence was revised as recommended. The EC agreed on the CP enhancement differences between ^{1}H-^{13}C and ^{1}H-^{15}N. However, the intensity comparison of ^{13}C vs ^{15}N in the current text offers more practical insight.

^{31}P Solid-State NMR
Comment Summary #102: The commenter recommended the addition of a comment that the lack of peaks in ^{31}P NMR ssNMR spectra may necessitate using deconvolution for quantitative applications, particularly if quantitating amorphous drug substance.
Response: Comment incorporated. The following sentence was added at the end of the section: "*It is worth noting that crystalline and amorphous forms may often exhibit similar ^{31}P chemical shifts, which requires peak deconvolution or reference spectra to assist in quantitative analysis."*

Implementation Time-Domain Solid-State NMR Spectroscopy at Low Magnetic Field Strengths
Comment Summary #103: The commenter requested changing the section title since low-field NMR could be in contrast to high-field NMR, while time-domain NMR is in contrast to Fourier-transform NMR. Low-field NMR and time-domain NMR cannot be equated.
Response: Comment incorporated. The section title was changed to "Time-Domain Solid-State NMR Spectroscopy at Low Magnetic Field Strengths."
Comment Summary #104: The commenter stated that commercially available spectrometers with permanent magnets are now up to 125 MHz (^{1}H), and ssNMR has been done with benchtop spectrometers at 60 MHz.
Response: Comment incorporated. The operation range was changed to 2–60 MHz.
Comment Summary #105: The commenter recommended revising this section to focus on solid-state NMR application without mixing different methods. The last sentence in this section also seems to mix translational-diffusion-based PFG-NMR and rotational-diffusion-based T_1/T_2. While T_1/T_2 can be performed in both solid and solution NMR, PFG is performed only in solution NMR.
Response: Comment not incorporated. The EC stated that in the scenario of ssNMR, all examples in the text are sound applications.

Monograph

Monograph/Section(s): ACETYLCYSTEINE INJECTION/Multiple Sections
Expert Committee: Small Molecules 2
No. of Commenters: 1

Comment Summary #1: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.

Response: Comment not incorporated. A new USP general chapter, ⟨477⟩ User-Determined Reporting Thresholds, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee will consider incorporating this new approach in future revisions, as applicable.
Comment Summary #2: The commenter requested revising the acceptance criteria for L-cysteine, acetylcysteine-related compound D and the total degradation products in the test for *Organic impurities* to be consistent with what has been approved.
Response: Comment partially incorporated. The acceptance criteria for L-cysteine are increased from NMT 1.0% to NMT 1.5%, and the acceptance criteria for Acetylcysteine related compound D are increased from NMT 0.4% to NMT 0.5%. The acceptance criteria for the total degradation products remain unchanged as the acceptance criteria proposed represent the widest approved limits available to USP.
Comment Summary #3: The commenter requested revising the acceptance criteria for *pH* to be consistent with what has been approved.
Response: Comment incorporated. The acceptance criteria for the test for *pH* has increased from 6.5–7.5 to 6.0–7.5.

Monograph/Section(s): ALBENDAZOLE/Organic Impurities
Expert Committee: Small Molecules 3
No. of Commenters: 1

Comment Summary #1: The commenter recommended removing the “reporting threshold” in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, ⟨477⟩ User-Determined Reporting Thresholds, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee will consider incorporating this new approach in future revisions, as applicable.
Comment Summary #2: The commenter indicated that in the test for *Limit of Albendazole Related Compound D*, the acceptance criteria for Albendazole Related Compound D are different from what has been approved and recommended revising the acceptance criteria to be consistent with what has been approved.
Response: Comment partially incorporated. The Expert Committee determined that the test for *Limit of Albendazole Related Compound D* should be removed from the proposal to avoid compliance issues.
Comment Summary #3: The commenter recommended removing albendazole ethyl carbamate in the test for *Organic Impurities* because it is a common impurity and suggested that listing this impurity with a relative retention time in Table 1 may be acceptable.
Response: Comment partially incorporated. In the acceptance criteria of *Organic impurities* listed in Table 2, “if present” is added to the entry for albendazole ethyl carbamate.
Comment Summary #4: The commenter indicated that in the test for *Organic Impurities*, the limit for Total Impurities is different from what is approved and recommended revising the acceptance criteria to be consistent with what has been approved.
Response: Comment not incorporated. The proposed acceptance criteria for Total impurities are consistent with the sponsor’s approved specifications. If needed, a future revision can be considered upon receipt of approved specifications and supporting data.

Monograph/Section(s): ALBENDAZOLE TABLETS/Multiple sections
Expert Committee: Small Molecules 3
No. of Commenters: 2

Comment Summary #1: The commenter recommended removing the “reporting threshold” in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, ⟨477⟩ User-Determined Reporting Thresholds, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee will consider incorporating this new approach in future revisions, as applicable.

Comment Summary #2: The commenter indicated that the acceptance criteria for Albendazole Related Compound D are different from what has been approved and recommended revising the acceptance criteria to be consistent with what has been approved.
Response: Comment partially incorporated. The Expert Committee determined that the test for *Limit of Albendazole Related Compound D* should be removed from the proposal to avoid compliance issues.
Comment Summary #3: The commenter indicated that in the test for *Organic impurities*, their approved limit for total impurities is wider (NMT 1.3%) than the proposed limit of NMT 0.8% and requested that USP consider their wider approved limit.
Response: Comment incorporated. The acceptance criteria for the total degradation products are increased from NMT 0.8% to NMT 1.3%.
Comment Summary #4: The commenter indicated that the proposed limit for Albendazole Related Compound D (NMT 1.875 ppm) is different from the limit list in EP (NMT 0.2%). The commenter also indicated that Albendazole Related Compound D is not a part of their approved drug product specification and requested that USP consider omission of Albendazole Related Compound D from the drug product monograph.
Response: Comment incorporated. The Expert Committee determined the test for *Limit of Albendazole Related Compound D* should be removed from the proposal to avoid compliance issues.
Comment Summary #5: The commenter requested adding UV spectrum match to *Identification A* as an alternate method because diode array detector (DAD) is not commonly available.
Response: Comment not incorporated. USP does not typically include flexible identification tests. The Expert Committee determined that *Identification A* is suitable for its intended use. Identification tests based on UV-PDA can be found in many USP-NF drug product monographs.
Comment Summary #6: The commenter requested a provision for 0.27N Hydrochloric acid (i.e., 1%w/w Hydrochloric acid) for 400 mg tablet strength (USP tablet strength is 200mg only) of the Albendazole tablet.
Response: Comment not incorporated. The comment is out of scope for this proposal. An additional dissolution test can be considered upon receipt of supporting data for an FDA-approved product.
Comment Summary #7: The commenter stated that the *Assay* mobile phase buffer preparation (1.25 g/L of ammonium phosphate, monobasic in water) is different from the *Organic impurities* test (1.67 g/L of ammonium phosphate, monobasic in water) even though the procedures use the same chromatography except for flow rate and injection volume. The commenter proposed to align the buffer preparation concentration as 1.67 g/L of ammonium phosphate, which is monobasic in water.
Response: Comment not incorporated. The buffer preparations in the *Assay* and *Organic impurities* tests are consistent with the sponsor's methods and validation. The Expert Committee determined that the proposed *Assay* and *Organic impurities* tests are suitable for their intended use.

Monograph/Section(s): APIXABAN/Multiple Sections
Expert Committee: Small Molecules 2
No. of Commenters: 6

Comment Summary #1: The commenter observed that the proposed chemical name for apixaban in the section for chemical information, "1*H*-Pyrazolo[3,4-c]pyridine-3-carboxamide,4,5,6,7-tetrahydro-1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxo-1-piperidinyl) phenyl]-onobenzenesulfonate" ends with "benzenesulfonate." They indicated that the molecule does not have a sulfonate functional group and that the name should be corrected accordingly.
Response: Comment incorporated. The chemical name for apixaban is revised to remove "-onobenzenesulfonate."
Comment Summary #2: The commenter requested revising the acceptance criteria for apixaban acid and the total impurities in the test for *Organic impurities* to be consistent with what has been approved.

Response: Comment not incorporated. The proposed acceptance criteria represent the widest approved limits available to USP. If needed, a future revision can be considered upon receipt of approved specifications and supporting data.

Comment Summary #3: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.

Response: Comment not incorporated. A new USP general chapter, ⟨477⟩ User-Determined Reporting Thresholds, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee will consider incorporating this new approach in future revisions, as applicable.

Comment Summary #4: The commenter requested that analytical solutions containing apixaban in the *Assay* be stored at 5 °C because the storage conditions described in the *Organic impurities* test indicate that solutions are not stable at room temperature.

Response: Comment not incorporated. The Expert Committee determined that the proposed *Assay* is suitable for its intended use. The validation data indicates that analytical solutions containing apixaban in the *Assay* are stable at 25°.

Comment Summary #5: The commenter suggested revising the tailing factor to NMT 1.5 in the *Assay.*

Response: Comment not incorporated. The Expert Committee determined that the proposed *Assay* and system suitability requirements are consistent with validation data and are suitable for their intended use.

Comment Summary #6: The commenter suggested adding a resolution requirement to the *Assay* to ensure the specificity of the chromatographic system during the analysis.

Response: Comment not incorporated. The Expert Committee determined that the proposed system suitability requirements are adequate for the *Assay* test.

Comment Summary #7: The commenter stated that the values of relative response factor (RRF) for apixaban open ring acid, apixaban acid, apixaban related compound E, and apixaban ethyl ester described in the test for *Organic impurities* and the correction factors obtained by the commenter are different.

Response: Comment not incorporated. The Expert Committee determined the proposed RRF values are consistent with the validation data and are suitable for their intended use.

Comment Summary #8: The commenter requested that the reference standard for USP Apixaban Related Compound G RS be available in the USP store.

Response: Comment acknowledged. USP Apixaban Related Compound G RS is currently available.

Comment Summary #9: The commenter indicated that the relative response factor (RFF) of the apixaban open ring acid should be 1.1 by their study.

Response: Comment not incorporated. The Expert Committee determined the proposed RRF values of 0.74 for the apixaban open ring acid is consistent with the validation data and suitable for its intended use.

Comment Summary #10: The commenter recommended optimizing the test method for *Organic Impurities,* indicating that while the three batches' samples tested meet the requirements of the proposed monograph, their in-house impurity and chloro apixaban are not well separated with a resolution of 0.63.

Response: Comment not incorporated. The Expert Committee determined that the proposed *Organic Impurities* procedure is consistent with the validation data and suitable for its intended use. If needed, a future revision can be considered upon receipt of approved specifications and supporting data. In addition, use of alternate procedures is discussed in General Notices "6.30. Alternative and Harmonized Methods and Procedures."

Comment Summary #11: The commenter indicated that the proposed monograph was applied and that tested samples passed the USP specification for *Organic impurities*, Assay determination, Determination of APX Related Compound H, G, and F by HPLC. The commenter indicated that the results for *Organic impurities* and *Assay* determination of the proposed and commenter's methods were comparable, and the equivalence between USP's and the commenter's in-house methods was demonstrated.

Response: Comment acknowledged.

Comment Summary #12: The commenter indicated that the proposed *Organic impurities* procedure was found to be sensitive and specific for all of their detectable and specified impurities, as well as specific for additional process-related impurities APX desmethoxy and APX acid, which are specified for the commenter's product. The commenter recommended that these impurities be added as specified impurities with specifications of NMT 0.15%.
Response: Comment not incorporated. The commenter's product is not currently approved. The commenter's additional process-related impurities can be considered once they receive approval from the FDA.
Comment Summary #13: The commenter suggested adding a note on the test "Limit of Apixaban Related Compound H, Apixaban Related Compound G, and Apixaban Related Compound F" that impurities should be tested if relevant to the employed route of synthesis. Alternatively, the commenter indicated that the test could be replaced by a general note about user responsibility for product safety and ICH M7 compliance.
Response: Comment partially incorporated. The test is revised to indicate "if present" as follows: Limit of Apixaban Related Compound H, Apixaban Related Compound G, and Apixaban Related Compound F (if present).
Comment Summary #14: In the test for *Limit of Apixaban Related Compound H, Apixaban Related Compound G, and Apixaban Related Compound F,* the commenter recommended relaxing the System Suitability criteria for %RSD to NMT 10.0%, based on the result obtained during their evaluation.
Response: Comment not incorporated. The Expert Committee determined that the proposed requirement for %RSD is consistent with the validation data and suitable for its intended use.

Monograph/Section(s): APIXABAN TABLETS/Multiple Sections
Expert Committee: Small Molecules 2
No. of Commenters: 3

Comment Summary #1: The commenter stated that they have a tentatively approved ANDA for apixaban tablets of 2.5 mg and 5.0 mg strength and that their FDA-approved specifications for dissolution test are wider than USP's proposed specification. They requested that USP kindly consider their wider approved limit for the dissolution test in the monograph.
Response: Comment not incorporated. If needed, USP can consider incorporating an additional dissolution test once the commenter receives the final FDA approval.
Comment Summary #2: The commenter stated that they have a tentatively approved ANDA for apixaban tablets of 2.5 mg and 5.0 mg strength and that their FDA-approved specification for total impurities is wider than what is proposed. They requested that USP kindly consider their wider approved limit for total impurity specification in the monograph.
Response: Comment not incorporated. If needed, USP can consider incorporating the commenter's total impurity limit once the commenter receives the final FDA approval.
Comment Summary #2: The commenter observed that in the tests for *Assay* and *Organic impurities,* the sample solution does not pass through the 0.45-µm nylon filter paper during sample preparation. They indicated that the issue is resolved if the sample solution is centrifuged prior to filtering. The commenter requested to add a centrifugation step to the sample preparation instructions during further revision of monograph.
Response: Comment not incorporated. The Expert Committee determined that the proposed Sample solution preparation is consistent with the validation data and suitable for its intended use. The Expert Committee will consider a future revision if needed. Use of alternate procedures is discussed in General Notices "6.30. Alternative and Harmonized Methods and Procedures."
Comment Summary #2: The commenter indicated that the dissolution time of 15 min with Q = 80% may not be suitable and suggested 30 min based on the available literature recommendations and dissolution experiments in their laboratory.
Response: Comment not incorporated. If needed, a future revision to incorporate an additional dissolution test can be considered upon receipt of approved specifications and supporting data.

Monograph/Section(s): ASPIRIN AND DIPYRIDAMOLE EXTENDED-RELEASE CAPSULES/Organic Impurities
Expert Committee: Small Molecules 2
No. of Commenters: 3

Comment Summary #1: The commenter requested clarification as to whether the impurities listed in Tables 5 and 8 are for information only (i.e., whether specification limits are applicable for these impurities).
Response: Comment not incorporated. The frequently-asked-questions section of the USP.ORG website addresses the commenter's question under the Organic Impurities topic (see https://www.usp.org/frequently-asked-questions/organic-impurities).
Comment Summary #2: Commenters observed a discrepancy in the test for *Organic Impurities, Procedure 2: Dipyridamole*. In the acceptance criteria Table 9 for dipyridamole tartaric acid ester, the limit value should be 0.50% instead of 050%.
Response: Comment incorporated. The acceptance criteria for dipyridamole tartaric acid ester has been corrected to 0.50%.
Comment Summary #3: The commenter observed a discrepancy in the test for *Organic Impurities, Procedure 2: Dipyridamole*. Under sample preparation, the proposal states that a rotary shaker is used for intermittent shaking of the sample solution for 50 min. The commenter indicated that once a sample is on the rotary shaker, it will continuously shake until the set/defined timepoint, making intermittent shaking impossible. The commenter requested clarification as to whether there is a typo in the proposal or if such a rotary shaker is available as mentioned.
Response: Comment not incorporated. The Expert Committee determined that the proposed sample solution preparation is consistent with the validation data and suitable for its intended use.

Monograph/Section(s): BIMATOPROST/ Multiple sections
Expert Committee: Small Molecules 3
No. of Commenters: 5

Comment Summary #1: Commenters requested the addition of flexibility for <197D> (diffuse reflection) to *Identification A*.
Response: Comment incorporated.
Comment Summary #2: The commenter indicated that in the *Assay,* while it was confirmed that the system suitability test was met, impurities (5,6-trans and 15-beta) were not separated under the method's conditions. Therefore, the correct content of Bimatoprost API cannot be calculated under the conditions of the proposed Assay method.
Response: Comment not incorporated. The Expert Committee determined that the proposed *Assay* procedure is consistent with the validation data and suitable for its intended use. The use of alternate procedures is discussed in General Notices "6.30. Alternative and Harmonized Methods and Procedures."
Comment Summary #3: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, ⟨477⟩ User-Determined Reporting Thresholds, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee will consider incorporating this new approach in future revisions, as applicable.
Comment Summary #4: The commenter observed that for *Organic Impurities*, Procedure 1, Orilistat related compound C interferes with 15-keto bimatoprost (the same relative retention time)
Response: Comment not incorporated. *Organic Impurities*, Procedure 2 is intended to be used when Orlistat related compound C is a potential impurity. *Organic impurities* procedure 2 separates Orlistat related compound C and 15-Keto bimatoprost with RRTs of 1.17 and 1.25, respectively.

Comment Summary #5: The commenter indicated that in *Organic impurities*, Procedure 2, the *Sensitivity solution* was not detected with an injection volume of 10 μL. At 100 μL, the signal to noise was 6 and 11 on different instruments.
Response: Comment not incorporated. The Expert Committee determined that the proposed *Organic impurities* procedure is consistent with the validation data and suitable for its intended use and noted evaluation of the *Sensitivity Solution* indicated acceptable signal to noise results.
Comment Summary #6: Commenters observed that for *Organic impurities* Procedure 2, the retention time of bimatoprost is about 30 min, but the briefing indicates 42 min.
Response: Comment not incorporated. A review of the data indicates that the retention time of bimatoprost is about 30 min. However, the retention time is only listed for information in the PF briefing, so no change is required.
Comment Summary #7: The commenter requested changing the *Organic impurities*, Procedure 2, to their in-house procedure. The commenter is concerned that the concentration of the standard solution is too high and may result in insufficient separation.
Response: Comment not incorporated. The Expert Committee determined that the proposed *Organic impurities* procedure is suitable for its intended use. The proposed procedure is based on the sponsor's method, except to use the USP Reference Standard for the quantitation of Orlistat related compound C. Use of alternate procedures is discussed in General Notices "6.30. Alternative and Harmonized Methods and Procedures."
Comment Summary #8: The commenter proposed to widen limit for Residue on Ignition to NMT 0.2%.
Response: Comment incorporated. The acceptance criteria for Residue on Ignition are increased from NMT 0.20% to NMT 0.2%.
Comment Summary #9: The commenter proposed to widen the limits for Optical Rotation to (+33°)–(+39°°) and to change the solvent to 20 mg/mL methanol according to their approved application. They indicated that Bimatoprost is difficult to dissolve in acetonitrile, which is expensive and considered to have supply insecurities. All results met the specification using the commenter's and USP proposed methods; however, the results were lower for the USP method.
Response: Comment not incorporated. Changing the solvent from acetonitrile to methanol requires a revision proposal to be published for public comment. If needed, a future revision can be considered upon receipt of approved specifications and supporting data. Use of alternate procedures is discussed in General Notices "6.30. Alternative and Harmonized Methods and Procedures."
Comment Summary #10: Commenters requested to add water determination method 1a (volumetric titration) to the moisture section or change from the coulometric method to the volumetric method. They indicated that volumetric titration is a more common method for KF moisture testing and that the coulometric titration method requires weighing small amounts of sample that may result in errors because the sample may be affected by moisture absorption during sample preparation.
Response: Comment not incorporated. The Expert Committee determined that the proposed water determination procedure is suitable for its intended use. Use of alternate procedures is discussed in General Notices "6.30. Alternative and Harmonized Methods and Procedures."
Comment Summary #11: The commenter proposes removing the test for Escherichia coli since this test is specified for nonaqueous and aqueous preparations for oral use. Alternatively, the test should indicate that it applies only for this category.
Response: Comment incorporated. The test for Specified Microorganisms <62> is removed.

Monograph/Section(s): CHLORZOXAZONE/ Organic Impurities
Expert Committee: Small Molecules 4
No. of Commenters: 1

Comment Summary #1: The commenter indicated that under the Limit of Chlorzoxazone Related Compound A test, that Chlorzoxazone Related Compound A has been determined to be mutagenic in Ames assay,[1] and there is also a published carcinogenicity study[2]. Therefore, the commenter suggested the acceptance criterion for Chlorzoxazone Related Compound A is

not appropriate for a public standard and should be significantly tightened. The commenter recommended that this impurity be listed in the monograph without an acceptance criterion and with a footnote to refer to ICH M7 guidelines.

[1] NTP. 2-Amino-4-chlorophenol (95-85-2). Chemical Effects in Biological Systems (CEBS). Research Triangle Park, NC (USA): National Toxicology Program (NTP). https://manticore.niehs.nih.gov/cebssearch/test_article/95-85-2

[2] Yamazaki K et al, Oral Carcinogenicity and Toxicity of 2-Amino-4-chlorophenol in Rats. Journal of Occupational Health, 51: 249-260, 2009 at: https://onlinelibrary.wiley.com/doi/abs/10.1539/joh.L8178

Response: Comment not incorporated. The Expert Committee determined that the test for the *Limit of Chlorzoxazone Related Compound A* should not be included, reverting to the currently official test for *Chromatographic purity.* A new test may be proposed in the future once a more robust strategy regarding mutagenic and potentially mutagenic impurities is developed.

Comment Summary #2: The commenter indicated that under the *Organic Impurities* test, 3-Chloroaniline exhibits genotoxicity as per the National Toxicology Program report[3]. Therefore, the acceptance criterion for this impurity is not appropriate for a public standard and should be significantly tightened. The commenter recommended that this impurity be listed in the monograph without an acceptance criterion and with a footnote referring to the ICH M7 guidelines.

[3] NTP Technical Report on Comparative Toxicity Studies of o-, m-, and p-Chloroaniline. https://ntp.niehs.nih.gov/ntp/htdocs/st_rpts/tox043.pdf

Response: Comment not incorporated. The Expert Committee determined that the test for *Organic impurities* should not be included, reverting to the currently official test for *Chromatographic purity.* A new test may be proposed in the future once a more robust strategy regarding mutagenic and potentially mutagenic impurities is developed.

Comment Summary #3: The commenter recommended removing the “reporting threshold” in the test for *Organic impurities* as it will vary based on product-specific factors.

Response: Comment not incorporated. A new USP general chapter, ⟨477⟩ User-Determined Reporting Thresholds, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee will consider incorporating this new approach in future revisions, as applicable.

Expert Committee-Initiated Change: The retention time information for 3-chloroaniline and chlorzoxazone is moved from Table 2 within the proposed test for *Organic Impurities* to the system suitability section of the revised *Assay*.

Monograph/Section(s): CHLORZOXAZONE TABLETS/Impurities
Expert Committee: Small Molecules 4
No. of Commenters: 2

Comment Summary #1: The commenter indicated that under the Limit of Chlorzoxazone Related Compound A test, that Chlorzoxazone Related Compound A has been determined to be mutagenic in Ames assay,[1] and there is also a published carcinogenicity study[2]. Therefore, the commenter suggested the acceptance criterion for Chlorzoxazone Related Compound A is not appropriate for a public standard and should be significantly tightened. The commenter recommended that this impurity be listed in the monograph without an acceptance criterion and with a footnote to refer to the ICH M7 guidelines.

[1] NTP. 2-Amino-4-chlorophenol (95-85-2). Chemical Effects in Biological Systems (CEBS). Research Triangle Park, NC (USA): National Toxicology Program (NTP). https://manticore.niehs.nih.gov/cebssearch/test_article/95-85-2

[2] Yamazaki K et al, Oral Carcinogenicity and Toxicity of 2-Amino-4-chlorophenol in Rats. Journal of Occupational Health, 51: 249-260, 2009 at: https://onlinelibrary.wiley.com/doi/abs/10.1539/joh.L8178

Response: Comment not incorporated. The Expert Committee determined that the test for *Limit of Chlorzoxazone Related Compound A* should be removed. A new test may be proposed in the future once a more robust strategy regarding mutagenic and potentially mutagenic impurities is developed.

Comment Summary #2: The commenter indicated that under the *Organic Impurities* test, 3-Chloroaniline exhibits genotoxicity per National Toxicology Program report[3]. Therefore, the commenter suggested that the acceptance criterion for this impurity is not appropriate for a public standard and should be significantly tightened. The commenter recommended that this impurity be listed in the monograph without an acceptance criterion and with a footnote referring to the ICH M7 guidelines.

[3] NTP Technical Report on Comparative Toxicity Studies of o-, m-, and p-Chloroaniline. https://ntp.niehs.nih.gov/ntp/htdocs/st_rpts/tox043.pdf

Response: Comment partially incorporated. The Expert Committee determined that the test for *Organic impurities* should be removed. A new test may be proposed in the future once a more robust strategy regarding mutagenic and potentially mutagenic impurities is developed.

Comment Summary #3: The commenter indicated that in the test for *Organic impurities*, the acceptance criterion for the total degradation products are different from what has been approved and recommended revising the acceptance criteria to be consistent with what has been approved.

Response: Comment not incorporated. The Expert Committee determined that the test for *Organic impurities* should not be included. A new test may be proposed in the future once a more robust strategy regarding mutagenic and potentially mutagenic impurities is developed.

Comment Summary #4: The commenter recommended removing the “reporting threshold” in the test for *Organic impurities* as it will vary based on product-specific factors.

Response: Comment partially incorporated. The Expert Committee determined that the test for *Organic impurities* should be removed. A new test may be proposed in the future once a more robust strategy regarding mutagenic and potentially mutagenic impurities is developed.

Expert Committee Initiated Change: The retention time information for 3-chloroaniline and chlorzoxazone is moved from Table 2 within the proposed test for *Organic Impurities* to the system suitability section of the revised *Assay*.

Monograph/Section(s): DESONIDE CREAM/Multiple Sections
Expert Committee: Small Molecules 5
No. of Commenters: 1

Comment Summary #1: The commenter indicated that the impurity profile is inconsistent with the Desonide drug substance monograph, specifically for Desonide related compound A. The Desonide monograph lists Desonide glyoxal, whereas the Desonide Cream proposal lists Desonide related compound A. The commenter requested investigating and clarifying in the family monographs whether the impurities are seen at the same retention time to avoid confusion for the monograph users. The commenter indicated in additional communication that the proposed limits are different from what has been approved and recommended reaching out to approved manufacturers.

Response: Comment partially incorporated. The proposal is updated to clarify the impurity names as “Desonide glyoxal/Desonide related compound A” with a footnote indicating “Desonide related compound A is in equilibrium with desonide glyoxal in the presence of water and they are interconvertible.” The proposed acceptance criteria represent the widest approved limits available to USP. If needed, a future revision can be considered upon receipt of approved specifications and supporting data.

Comment Summary #2: The commenter indicated that the acceptance criteria for the total degradation products are different from what has been approved and recommended revising the acceptance criteria to be consistent with what has been approved.

Response: Comment incorporated. The acceptance criteria for the total degradation product are increased from NMT 4.0% to NMT 5.1%.
Comment Summary #3: The commenter recommended removing the “reporting threshold” in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, ⟨477⟩ User-Determined Reporting Thresholds supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee will consider incorporating this new approach in future revisions, as applicable.
Comment Summary #4: The commenter recommended adding a test for Burkholderia Cepacia Complex (BCC) per USP<60> under Specific Tests.
Response: Comment not incorporated. A future revision will be considered based on available approved specifications and supporting data.

Monograph/Section(s): DESONIDE LOTION/Multiple Sections
Expert Committee: Small Molecules 5
No. of Commenters: 1

Comment Summary #1: The commenter indicated that the impurity profile is inconsistent with the Desonide drug substance monograph, specifically for Desonide related compound A. The Desonide monograph lists Desonide glyoxal, whereas the Desonide Cream proposal lists Desonide related compound A. The commenter requested investigating and clarifying in the family monographs whether the impurities are seen at the same retention time to avoid confusion for the monograph users. The commenter indicated in additional communication that the proposed limits are different from what has been approved and recommended reaching out to approved manufacturers.
Response: Comment incorporated. The proposal is updated to clarify the impurity names as “Desonide glyoxal/Desonide related compound A” with a footnote indicating “Desonide related compound A is in equilibrium with desonide glyoxal in the presence of water and they are interconvertible.” The proposed acceptance criteria for Desonide glyoxal/Desonide related compound A are increased from NMT 2.0% to NMT 3.40%.
Comment Summary #2: The commenter indicated that the acceptance criteria for Total degradation products are different from what has been approved and recommended revising the acceptance criteria to be consistent with what has been approved.
Response: Comment incorporated. The acceptance criteria for the total degradation products are increased from NMT 3.0% to NMT 4.4%.
Comment Summary #3: The commenter recommended removing the “reporting threshold” in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, ⟨477⟩ User-Determined Reporting Thresholds, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee will consider incorporating this new approach in future revisions, as applicable.
Comment Summary #4: The commenter recommended adding a test for Burkholderia Cepacia Complex (BCC) per USP<60> under Specific Tests.
Response: Comment not incorporated. A future revision will be considered based on available approved specifications and supporting data.

Monograph/Section(s): DESONIDE OINTMENT/Multiple Sections
Expert Committee: Small Molecules 5
No. of Commenters: 1

Comment Summary #1: The commenter indicated that the impurity profile is inconsistent with the Desonide drug substance monograph, specifically for Desonide related compound A. The Desonide monograph lists Desonide glyoxal, whereas the Desonide Cream proposal lists Desonide related compound A. The commenter requested investigating and clarifying in the family monographs whether the impurities are seen at the same retention time to avoid confusion for the monograph users.

Response: Comment incorporated. The proposal is updated to clarify the impurity names as "Desonide glyoxal/Desonide related compound A" with a footnote indicating "Desonide related compound A is in equilibrium with desonide glyoxal in the presence of water and they are interconvertible."
Comment Summary #2: The commenter indicated that the acceptance criteria for Desonide related compound B, 16α-Hydroxyprednisolone, and total degradation products are different from what has been approved and recommended revising the acceptance criteria to be consistent with what has been approved.
Response: Comment not incorporated. The proposed acceptance criteria represent the widest approved limits available to USP. If needed, a future revision can be considered upon receipt of approved specifications and supporting data.
Comment Summary #3: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, ⟨477⟩ User-Determined Reporting Thresholds, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee will consider incorporating this new approach in future revisions, as applicable.

Monograph/Section(s):	DEXTRAN 40
Expert Committee:	Biologics Monographs 3 - Complex Biologics and Vaccines
No. of Commenters:	2

Molecular Weight Distribution and Weight and Number Average Molecular Weights

Comment Summary #1: The commenter inquired about how to calculate the weight average molecular weight of dextrose in the system suitability evaluation.
Response: Comment not incorporated. This is a query regarding the calculation of the weight average molecular weight of dextrose, not a public comment on the revision.
Expert Committee-initiated Change #1: The Expert Committee decided to delete the defined column temperature and flow rate from the Chromatographic System to maintain the flexibility of the method. The column temperature was changed back to "constant temperature."

Monograph/Section(s):	DEXTRAN 70
Expert Committee:	Biologics Monographs 3 - Complex Biologics and Vaccines
No. of Commenters:	2

Molecular Weight Distribution and Weight and Number Average Molecular Weights
Comment Summary #1: The commenter indicated that controlling the temperature at 40 degrees is difficult in most quality control laboratory settings because it would require a very large heating compartment for three columns or one heating compartment for each column. The commenter also suggested specifying the temperature of the RI detector.
Response: Comment partially incorporated. The defined column temperature was deleted from the Chromatographic System, and the column temperature was changed back to "constant temperature." The RI detector temperature will not be specified to maintain the flexibility of the method.
Expert Committee-initiated Change #1: The Expert Committee decided to delete the defined flow rate from the Chromatographic System to maintain the flexibility of this method.

Monograph/Section(s):	DIBASIC CALCIUM PHOSPHATE DIHYDRATE (12000)/Multiple Sections
Expert Committee:	Simple Excipients
No. of Commenters:	3

Comment Summary #1: The commenter indicated the current procedure for *Identification A* did not give a positive result and suggested changing the procedure by dissolving the sample in water first and adding only a small amount of hydrochloric acid. The detail of the suggested change is: Dissolve sample in 10 ml of water, add 1 mL of HCL, and sufficiently dissolve sample. Then, add 2 mL of Ammonia TS dropwise with shaking, and then add 1 mL of ammonium oxalate TS.A white precipitate is formed.
Response: Comment not incorporated. This comment is out of scope for the proposed revision. *Identification A* is based on validation data and is currently harmonized with other pharmacopeias. This change requires additional supporting data and would result in the procedure no longer being harmonized. The Expert Committee will consider a future revision to this test upon receipt of supporting information.
Comment Summary #2: The commenter requested clarification on whether a requirement for the square of the correlation coefficient, r^2, for the calibration curve should be added to the Limit of Fluoride test.
Response: Comment not incorporated. This comment is out of scope for the proposed revision. A requirement for the square of the correlation coefficient for the calibration curve used in the titration method is not typically included. The Expert Committee will consider future revisions to this monograph upon receipt of supporting information.
Comment Summary #3: The commenter suggested including a requirement for microbial analysis referencing total aerobic microbial count and total combined yeasts and molds count in Additional Requirements, which can be found in <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests. The commenter stated that dibasic calcium phosphate is highly susceptible to fungal growth and can cause contamination in or carry contamination into finished products.
Response: Comment not incorporated. This comment is out of scope for the proposed revision. This additional requirement requires supporting data and would result in the monograph no longer being harmonized. The Expert Committee will consider a future revision to this test upon receipt of supporting information.

Monograph/Section(s): GLIPIZIDE EXTENDED-RELEASE TABLETS/Multiple Sections
Expert Committee: Small Molecules 3
No. of Commenters: 3

Comment Summary #1: The commenter noted that their approved method of analysis for *Assay* is different from the method proposed in PF. They indicated that their evaluation found the proposed method to not be suitable for extraction of their formulation and requested that their registered method be incorporated as an alternate assay in the USP monograph.
Response: Comment not incorporated. The Expert Committee determined that the proposed *Assay* procedure is consistent with the validation data and suitable for its intended use. Additionally, USP does not include multiple *Assay* procedures in USP/NF. Use of alternate procedures is discussed in General Notices “6.30. Alternative and Harmonized Methods and Procedures.” A flexible sample preparation can be considered in a future revision with supporting data.
Comment Summary #2: The commenters noted that their approved methods of analysis for dissolution are entirely different from the method proposed in PF and that their registered specification is different from the USP forum proposed specification.
Response: Comments not incorporated. This comment is out of scope for the proposed revision. USP will work with the commenters to consider whether their dissolution procedure in the monograph may be implemented via Revision Bulletin.
Comment Summary #3: The commenter noted that their approved method of analysis for *Organic Impurities* is entirely different from the method proposed in PF. They indicated that the extraction time given in the proposed PF method is not suitable for extraction of their formulation; hence, the USP forum method is not appropriate for them. The commenter requests that USP include their method as an additional method for *Organic Impurities*. They also indicated that their registered and approved specification only has related compound A,

where USP has additionally proposed limits for any unspecified degradation product and total degradation products.
Response: Comment not incorporated. The Expert Committee determined that the proposed *Organic Impurities* procedure is consistent with the validation data and suitable for its intended use. The submitted *Organic Impurities* procedure is specific to the commenter's specified impurity Related compound A, which does not justify a flexible approach to add a second *Organic Impurities* procedure. Use of alternate procedures is discussed in General Notices "6.30. Alternative and Harmonized Methods and Procedures." A flexible sample preparation can be considered in a future revision with supporting data.
Comment Summary #4: The commenter observed that in the test for *Organic impurities* there is a typo in the sensitivity solution preparation. They indicate that the solution should be prepared from Stock solution A rather than Stock solution B, as proposed.
Response: Comment incorporated. The text has been revised to indicate that the sensitivity solution is prepared from Standard stock solution A.
Comment Summary #5: The commenter indicated that for Dissolution Test 3, the Factor for Acid and Buffer Standard Solutions should be 714, not 740.
Response: Comment not incorporated. The standard solution preparations in the PF proposal are consistent with the information submitted by the sponsor. The value 740 is based on the volume of the buffer stage medium of the dissolution.

Monograph/Section(s): HYDROCODONE BITARTRATE AND ACETAMINOPHEN ORAL SOLUTION/Impurities
Expert Committee: Small Molecules 2
No. of Commenters: 1

Comment Summary #1: The commenter noted that the acceptance criteria for any unspecified degradation product and total degradation products are different from what has been approved and recommends revising the acceptance criteria to be consistent with what has been approved.
Response: Comment not incorporated. The proposed acceptance criteria represent the widest approved limits available to USP. If needed, a future revision can be considered upon receipt of approved specifications and supporting data.
Comment Summary #2: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, ⟨477⟩ User-Determined Reporting Thresholds, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee will consider incorporating this new approach in future revisions, as applicable.

Monograph/Section(s): HYDROXYAMPHETAMINE HYDROBROMIDE/Impurities
Expert Committee: Small Molecules 3
No. of Commenters: 1

Comment Summary #1: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, ⟨477⟩ User-Determined Reporting Thresholds, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee will consider incorporating this new approach in future revisions, as applicable.
Comment Summary #2: The commenter indicated that in the test for *Organic Impurities*, the limit for "Total impurities" is different from what is approved. The commenter recommended revising the acceptance criteria to be consistent with what has been approved.
Response: Comment not incorporated. The Expert Committee determined that the proposed *Organic impurities* test, which replaces the TLC test for *Ordinary Impurities* <466>, is suitable for its intended use. If needed, a future revision can be considered upon receipt of approved specifications and supporting data.

Monograph/Section(s): MOMETASONE FUROATE CREAM/Specific Tests
Expert Committee: Small Molecules 2
No. of Commenters: 1

Comment Summary #1: The commenter indicated that, based on the recommendations in USP <1111>, a suitable microbial enumeration test requirement would be Total Aerobic Microbial Count NMT 10^2 cfg/g, Total Combined Yeast and Mold Count NMT 10^1 cfg/g, and an absence of Staphylococcus aureus and Pseudomonas aeruginosa in 1 g.
Response: Comment not incorporated. The reference to Microbial Enumeration Tests ⟨61⟩ was removed because no related acceptance criteria were listed. A future revision can be considered upon receipt of approved specifications and supporting data.

Monograph/Section(s): PERPHENAZINE/Multiple Sections
Expert Committee: Small Molecules 4
No. of Commenters: 3

Comment Summary #1: The commenter recommended removing the “reporting threshold” in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, ⟨477⟩ User-Determined Reporting Thresholds, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee will consider incorporating this new approach in future revisions, as applicable.
Comment Summary #2: The commenter indicated support of the proposed tests for *Assay* and *Organic impurities.* The proposed tests are considered feasible, and the relative retention times of all observed impurities were aligned with the PF proposal. In addition, the proposed system suitability criteria could be met.
Response: Comment acknowledged.
Comment Summary #3: The commenter indicated that from their comparison of the current *Organic impurities* procedure to the PF 48(5) proposal, it can be concluded/observed that while the proposed chromatographic conditions have increased the performance as impurities eluting close to the main peak are better resolved, by adopting the proposed method, late eluting impurities detected by the current method at about 35 min are not detected with the proposed method. Therefore, the proposed *Organic Impurities* test cannot be considered suitable for the intended purposes compared to the method currently official procedure as it is not possible to detect/quantify impurities potentially present in the drug substance.
Response: Comment not incorporated. The Expert Committee determined that the proposed *Organic Impurities* procedure is consistent with the validation data and suitable for its intended use. There are no late eluting impurities specified in the proposal. While the run time in the proposed procedure stipulates NLT 3.2 times the retention time of perphenazine, the run time can be extended as needed. Use of alternate procedures is discussed in General Notices “6.30. Alternative and Harmonized Methods and Procedures.”

Monograph/Section(s): TECHNETIUM TC 99M MEBROFENIN INJECTION/Specific Tests
Expert Committee: Small Molecules 4
No. of Commenters: 1

Comment Summary #1: The commenter requested the addition of "colorless" to the acceptance criteria for *Appearance* in the Specific Tests section.
Response: Comment not incorporated. If needed, the acceptance criteria for colorless can be added to the test for *Appearance* in a future revision upon receipt of approved specifications and supporting data.

Monograph/Section(s): TRIPTORELIN PAMOATE
Expert Committee: Biologics Monographs 1 - Peptides

No. of Commenters: 1

Comment Summary #1: The commenter asked for the reason of the proposed acceptance criteria in the microbial enumeration test and the justification of deviating from USP <1111> recommendation.
Response: Comment not incorporated. The proposed limit was approved by the regulatory agency.