Commentary

USPNF 2026 ISSUE 1

July 25, 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
<41> BALANCES
<202> IDENTIFICATION OF FIXED OILS BY THIN-LAYER CHROMATOGRAPHY
<317> ICP OES TESTING FOR SODIUM HYDROXIDE AND POTASSIUM HYDROXIDE
<785> OSMOLALITY AND OSMOLARITY
<1040> QUALITY CONSIDERATIONS OF PLASMID DNA AS A STARTING MATERIAL FOR CELL AND GENE THERAPIES INCLUDING RNA PRODUCTS
<1085> GUIDELINES ON ENDOTOXINS TEST
<1251> WEIGHING ON AN ANALYTICAL BALANCE
<1785> - OSMOLALITY AND OSMOLARITY - PRACTICAL CONSIDERATIONS

Monographs
ANHYDROUS LACTOSE
DICLOFENAC SODIUM COMPOUNDED TOPICAL FOAM
DIETHYLENE GLYCOL MONOETHYL ETHER
FONDAPARINUX SODIUM INJECTION
LACTOSE MONOHYDRATE
POMEGRANATE FRUIT DRY EXTRACT
PROPYL GALLATE

No comments were received for the following proposals:

Monographs
BITTER ORANGE FRUIT FLAVONOIDS DRY EXTRACT
BROMELAIN
CONSTRUCT HUMAN FIBROBLASTS IN POLYGLACTIN SCAFFOLD
MESO-ZEAXANTHIN
PAPAIN

General Chapter

General Chapter/Section(s):	<41> BALANCES
Expert Committee:	General Chapters - Measurement and Data Quality
No. of Commenters:	12

[This section was updated on October 3, 2025 to correct section headings]

General Comment
Comment Summary #1:
The commenter requested to keep the formulas in GC<41> and GC<1251> to be consistent.
Response: Comment acknowledged. Although no specific formula was mentioned, the editorial staff checked the chapters to ensure consistent formula usage.

Repeatability
Comment Summary #2:
The commenter requested to add text for clarity as "…Weighing of the test sample (during routine usage) shall not be less than the derived Minimum Weight value during calibration."

Response: Comment not incorporated. The minimum weight is the smallest weight that can be measured on the balance while meeting the repeatability requirement, as stated in GC<41>.

General Comment

Comment Summary #3: The commenter recommended clarifying the intent of the 10-point repeatability for performance check as the practice would put burden on the analytical lab.

Response: Comment not incorporated. Performance checks (accuracy and repeatability) are required in the current official GC <41>. There is no requirement for a daily repeatability test. The frequency of all performance checks is risk-based. The intent of the repeatability test is to limit the random error of the balance; the random error is dominant when weighing small samples and used to calculate the minimum weight. As the minimum weight (and the random error) is not constant over time, monitoring the random error of the balance through the repeatability performance check is an important activity to comply with the requirements of GC <41>.

General Comment

Comment Summary #4: The commenter stated that GC<41> applicability should not be defined by the location of the balance. Analytical balances used in manufacturing spaces but generating analytical data or data that would be used in analytical procedures should be in the scope of GC<41>. Industrial bench balance used to weigh and dispense material not generating analytical data or used in analytical procedures would be out of scope of GC<41>.

Response: Comment not incorporated. Balances used in manufacturing in-process control (IPC) are out of the scope of GC<41>. However, the IPC balance should still follow the measurement best practice. This topic is discussed in GC<1251>: “This chapter should not be considered all-inclusive, and other sources of information (e.g., the US National Institute of Science and Technology and balance manufacturers) may be useful and applicable when analysts perform a weighing operation or implement a weighing procedure.”

Repeatability

Comment Summary #5: The commenter requested clarification on why using a test weight of NMT 5% of the balance capacity if the standard deviation is nearly a constant.

Response: Comment not incorporated. The standard deviation is nearly constant across the lower measurement range. Using a 5% test load effectively tests the balance in the lower measurement range; however, it is not necessary to use a test load as small as the material weighed on the balance during normal usage. Moving to higher test loads—such as 5%—suggests that in a higher region, the standard deviation might not be considered nearly constant.

Accuracy

Comment Summary #6: The commenter stated that the following two statements are contradictory. 1. For accuracy test weight selection is 5% to 100%. 2. Balance should be calibrated for usage range. The commenter gave an example of a balance with maximum capacity of 220 g. If the usage range is 10 mg to 200 g then the analyst needs to calibrate from 10 mg to 200 g. However 5% of 200 g is 10 g so that the analyst can’t select weight less than 10 g for accuracy test.

Response: Comment not incorporated. Calibration is an activity described by international standards, e.g., EURAMET cg-18 and ASTS E898. Calibration is a comprehensive activity comprising the repeatability test, eccentricity test, and error-of-indication test. Additionally, it also requires determining the measurement uncertainty of the device. The first error-of-indication test point is usually zero, and typically, a minimum of four subsequent error-of-indication test points covering the measurement range of the balance are applied. In the example given by the commenter, an analytical balance with a measurement range of 220 g is

considered; therefore, 0, 50 g, 100 g, 150 g, and 220 g would be commensurate with the procedures suggested by the calibration guidelines. By fixing zero as the calibration point, any measurement on the balance is bracketed between this point and the subsequent test points. The accuracy test involves a different activity and is not calibration; it focuses on the assessment of the systematic error of the balance. Since the systematic error is very small and usually hidden within the repeatability of the balance, a meaningful accuracy test is performed using a test load of at least 5% of the capacity of the balance.

Repeatability

Comment Summary #7: The commenter requested to revise the text to state that the test weight should ideally be approximately 5% of the maximum capacity of the balance to allow slight variations as necessary.

Response: Comment not incorporated. The test weight requirement is not revised in the PF50(5) draft version. USP suggests being specific with regard to limit values to avoid ambiguities.

Comment Summary #8: The commenter requested to clarify the formula in Repeatability section: Result=$2s/m_{snw}$. The result formula multiplied 100 is not mentioned for result in %.

Response: Comment not incorporated. The factor of 100 must not be used because the acceptance criterion is indicated in percentage, which is 1/100 already.

Comment Summary #9: The commenter requested to add the following for editorial clarification: “If the standard deviation obtained is less than 0.41d, replace this standard deviation with 0.41d. In this case, the minimum weight is described by the following equation: m_{min} = 2000*0.41d = 820d.”

Response: Comment partially incorporated.

General Comment

Comment Summary #10: The commenter requested to rethink the approach of monitoring daily sensitivity test.

Response: Comment not incorporated. There is no requirement for a daily sensitivity test. The user decides the frequency in the framework of the applicable quality management system on a risk-based approach. The accuracy test must be carried out between 5% and 100% of the balance capacity, thereby avoiding the use of very small weights and the related handling issues. Additionally, in the repeatability test, the test load could be up to a few percent of the balance capacity, and test loads below 100 mg are not allowed to prevent handling errors with very small weights.

General Comment

Comment Summary #11: The commenter requested more examples should be given instead of formulas as well as causes of anomalies. The comments stated that more training on the topic would be beneficial.

Response: Comment not incorporated. The EC recommends basic training on balance for analysts in the lab.

General Comment

Comment Summary #12: The commenter requested adding “multiple 100” or "× 100" to three "Result" equations in GC <41> to express the final results as the unit of percentage, %.

Response: Comment not incorporated. Percentage, %, is 1/100, and the factor 100 is included by means of the % symbol. Therefore, adding "× 100" and expressing the unit as a percentage is unnecessary and redundant.

Introduction

Comment Summary #13: The commenter requested to revise the “bases” to “basis” in the 2nd paragraph of the Introduction section.
Response: Comment incorporated.

Repeatability
Comment Summary #14:
The commenter proposed to revise the definition of m_{snw} as “the smallest net weight (grams, e.g.) to be used on the balance or user-defined requirement”.
Response: Comment not incorporated. The proposed change does not add further clarification of the text.

General Comment
Comment Summary #15:
The commenter recommended to add blank space between numeric value and “%” to comply with ISO 80000 requirements.
Response: Comment not incorporated. The format is consistent with the USP style guide.

General Comment
Comment Summary #16: The commenter requested to add the requirement that ASTM 0 and 2 weighs to be used regarding applicable standards.
Response: Comment not incorporated. The requirements for the weight selection are clearly stated, i.e., the weight MPE (alternatively, the calibration uncertainty) shall not exceed 0.05%. Using this information, it is possible to select weights of an appropriate weight class that fulfill this requirement. For example, using a 100-g weight, the MPE of the weight will not exceed 50 mg (which is 0.05% of 100 g). An ASTM 0 weight with a nominal mass of 100 g has an MPE of 0.13 mg, which is orders of magnitudes smaller than the requirement and is not needed for the given example. An ASTM weight of class 6 would suffice from a metrological point of view. The weight selection criterion is not revised in PF 50 (5), and it is the current official text in GC<41>.

Repeatability
Comment Summary #17: The commenter suggested to revise the proposal to allow for slight deviations in test weight. The commenter proposed to change the term “shall” to “should” in the statement “Test weights below 100mg shall not be used” to “Test weights below 100mg **should** not be used.”
Response: Comment partially incorporated. For clarity, the sentence "Test weights below 100 mg shall not be used” was revised to read: "**Test weights with a nominal mass below 100 mg shall not be used.**" The conventional mass may not be considered for the selection of the test load. Not using test loads below 100 mg aligns with Ph. Eur. and JP.

Calibration
Comment Summary #18: The commenter requested to clarify that “leveling and adjusting” are stated as examples.
Response: Comment not Incorporated. In the chapter, it is stated that less significant operations include leveling and adjustment with internal weights. This syntax suggests that these two activities are examples.

Accuracy
Comment Summary #19: The commenter requested clarification and an example for the formula: Result=|m− I|/m.
Response: Comment not incorporated. The accuracy test is performed using one test load and is done independently from the repeatability test; therefore, “I” does not refer to the average of 10 measurements. Moreover, the accuracy test is performed with a test load between 5% and

100% of the capacity of the balance, while the repeatability test is done using a test. GC <1251> provides some more explanation (under Performance Qualification section) regarding this. This requirement is unchanged from the current official GC <41>.

Accuracy
Comment Summary #20: The commenter requested to change the “Result=|m− I|/m” to “Result=|I-m|/m” to align with the international metrological practices as well as the formulas in Ph. Eur. And JP.
Response: Comment incorporated.

Calibration
Comment Summary #21: The commenter stated that conformity decision rule used to determine pass or fail should be documented in calibration certificate since the measurement uncertainty is to be included in the calibration. While calibration uncertainty informs conformity decisions, it does not significantly enhance the value of the calibration certificate on its own. Moreover, a simple rule based on specific risk should not be applied unless it is aligned with a global risk framework. The commenter proposed the following recommendations for flexibility and clarity. 1. Change the term 'shall' to 'should' to provide organizations the opportunity to evaluate their specific circumstances over time and determine the most appropriate conformity decision rule to follow. 2. Include additional clarification about potential conformity decision rules in the documentation. Based on these recommendations, the revised sentence should read: “Calibration results should include measurement uncertainty, and the conformity decision rule used to make pass or fail decisions.”
Response: Comment not incorporated. Estimating uncertainty serves different purposes: 1. Ensuring traceability of measurement results (as mentioned in GC <41>). 2. Allowing the user to estimate the uncertainty of the whole analysis (as mentioned in NMR chapters, e.g., GC<761>). 3. Including the uncertainty, if decided by the user or stipulated by regulations, into decision rules for conformity assessment. While USP is fostering 1. and 2., uncertainty is currently not included in decision rules, i.e., simple decision rules are applied (e.g., in GC <41>). The uncertainty of the weights is not included in the conformity decision of the accuracy requirement. By requiring that the uncertainty of the weights shall not exceed 1/3 of the acceptance criterion, the residual risk of false-acceptance and false-rejection results are acceptable and, therefore, the rationale for applying a simple decision rule. This is in line with the conformity decisions of legal metrology (e.g., OIML R76 or HB 44), where simple decision rules are applied. Many users are not familiar with the concept of decision rules in metrology; therefore, we do not want to explicitly discuss that topic in GC <41> and GC<1251>. In the future, it would be better to discuss that in stimuli articles or general chapters that apply for the whole compendium. For GC <41>, it is evident that simple decision rules are applied for the conformity assessment. The EC decided to maintain the "shall" for the calibration requirement, emphasizing, as already stated in GC <41>, the traceability of measurement results, which allows uncertainty estimation of the whole analysis process.

General Comment
Comment Summary #22: The commenter requested to add text referring to GC<1251> for further explanation on some terminologies.
Response: Comment not incorporated. GC<41> has the following statement: "For example, glossary and a discussion of the theoretical bases of these requirements, see Weighing on an Analytical Balance <1251>, which is a helpful—but not a mandatory—resource."

Repeatability
Comment Summary #23: The commenter requested clarity on the definition of “low range”.

Response: Comment not incorporated. The current official text of GC<41> has the definition. The text uses the expression "measurement range," which is an accepted term for the "region" in which an instrument may be used. The explanation with regards to the lower measurement range is only used to illustrate that there is no requirement to use a repeatability test load that reflects the material mass that is routinely weighed on the balance.

General Comment

Comment Summary #24: The commenter mentioned that a five-place balance typically has a readability (d) of 0.00001g. The previous version of this proposal has a statement " For example, for a 4-palce analytical balance, d is 0.0001."

Response: Comment not incorporated. A five-place balance was used as an example as it reflects more pharmacopeial applications.

Repeatability

Comment Summary #25: The commenter found that "ranges" and "intervals" are confusing in the chapter text. For example, it is stated that multi-interval balances repeatability should be performed in the finest "range" even if the balance does not have multiple ranges. The commenter requested clarity on the difference between large scale interval and small-scale interval. In addition, it is unclear about the maximum capacity. Specifically, the commenter requested clarity on whether it applies to all balance types or only for the single range balances in the statement "the maximum capacity refers to the capacity of the coarsest weighing range...".

Response: Comment not incorporated. The definitions of multi-interval and multiple range balances are listed in the glossary in GC<1251>. The definitions do not use the word "interval," but "partial weighing range" with respect to multi-interval balances. The numerical value of a large-scale interval is larger than the numerical value of a small-scale interval. The sentence "Specifically, the maximum capacity..." refers to multi-interval and multiple range balances as this text is part of the paragraph that specifically addresses multiple range and multi-interval balances.

Repeatability

Comment Summary #26: The commenter suggested revising the sentence "The same rules for the test weight apply to single range balances" to " The same rules for the test weight apply as for single range balances."

Response: Comment incorporated. Use "apply as for single range balances" to clearly convey that the same rules are shared, not uniquely assigned.

Repeatability

Comment Summary #27: The commenter requested clarification on whether Standard Deviation based on Population (SDVP) or Standard Deviation based on Sample (SDVS) is required.

Response: Comment not incorporated. Based on the test (10 repeated measurements), it is evident that the standard deviation refers to the sample and not the population. This requirement has been noted in the chapter since many years and seems to be evident to the user.

General Chapter/Section(s):	<202> IDENTIFICATION OF FIXED OILS BY THIN-LAYER CHROMATOGRAPHY
Expert Committee:	Excipients Test Methods
No. of Commenters:	2

Comment Summary #1: The commenter requested to clarify "Apply the Sample as narrow bands of 8-mm length at a distance of 8 mm from the lower edge of the plate. The distance between bands, center-to-center, is NLT 11 mm." to indicate whether each of the three system suitability solutions (as previously mentioned in the section of System suitability solution) will all be applied. The commenter also requested to change the wording to "apply narrow bands of 8-mm width" if each band is applied in parallel to the front (lower) edge of the TLC plate.
Response: Comment incorporated. The sentences have been modified to improve the clarity as follows:
In the Identification section, Method III, System Suitability: Changed "Apply the Sample as narrow bands of 8-mm length" to "Apply the Sample as a narrow band of 8-mm width."
"The distance between bands, center-to-center, is NLT 11 mm" has been moved to the analysis section after "Follow the procedure mentioned in System suitability."
Comment Summary #2: The commenter recommended providing information on methodology application instructions in the chapter since there are three methods in the chapter.
Response: Comment incorporated. To avoid confusion, the sentence "If no method is specified in the monograph, then either of the methods can be applied" has been added at the end of the introduction.

General Chapter/Section(s):	<317> ICP OES TESTING FOR SODIUM HYDROXIDE AND POTASSIUM HYDROXIDE
Expert Committee:	Excipients Test Methods
No. of Commenters:	2

Comment Summary #1: The commenter requested more flexibility in the choice of diluent and calibration for the ICP-OES procedure.
Response: Comment not incorporated. The established procedure was developed and validated using hydrochloric acid as the diluent. We currently lack data supporting the use of alternative diluents. Stakeholders are welcome to adapt the procedure using a different diluent. However, any modifications would require validation to ensure the accuracy and reliability of the results. Stakeholders were advised to refer to the General Notice 6.30. Alternative and Harmonized Methods and Procedures and the General Chapter <1225> for guidance on alternative procedures and method validation.
Comment Summary #2: The commenter suggested revising the description of the diluent in the Methods section to specify the use of trace metal grade hydrochloric acid and deionized water as "1% Trace metal grade hydrochloric acid in de-ionized water".
Response: Comment partially incorporated. Based on USP Reagent definition and <1730>, the diluent in the method has been changed as "1% Hydrochloric acid, Utlratrace in deionized water at least 18 MΩ." A reagent citation has been added to the hydrochloric acid, Ultratrace.

General Chapter/Section(s):	<785> OSMOLALITY AND OSMOLARITY
Expert Committee:	General Chapters - Physical Analysis 2020
No. of Commenters:	4

Comment Summary #1: Under Test solutions, the commenter suggested deleting the last sentence starting with "It should be noted...."
Response: Comment incorporated.
Comment Summary #2: The commenter requested further clarification that the instrument be calibrated to a range that complies with the test solution range or wider.
Response: Comment not incorporated. The text explains the procedure for neat and diluted samples.

Comment Summary #3: Under method 1: Freezing-Point Osmometry, Troubleshooting, the commenter requested further explanation on how to remove or avoid the entrained air.
Response: Comment partially incorporated. The text was revised to include that the interference by bubbles must be reported.
Comment Summary #4: Under Method 1: Freezing-Point Osmometry, the commenter suggested aligning the procedure for daily calibration check with the European Pharm.
Response: Comment not incorporated. The EC, on advice from the Expert Panel, finds that the procedure in the chapter is appropriate as it is written. This chapter is not under harmonization.
Comment Summary #5: The commenter suggested following the instrument manufacturer recommendations for troubleshooting in the cases where the equipment fails calibration.
Response: Comment incorporated.
Comment Summary #6: Under Test Solutions, the commenter requested the inclusion of an explanation for high concentration.
Response: Comment partially incorporated. Text edited to state, “when the concentration interferes.”
Comment Summary #7: The commenter suggested adding an example of bracketing for solution with 1000 - 3000 mOsmol/kg samples.
Response: Comment not incorporated. It will be added to the FAQ page at www.usp.org.

General Chapters/Sections:	<1040> QUALITY CONSIDERATIONS OF PLASMID DNA AS A STARTING MATERIAL FOR CELL AND GENE THERAPIES INCLUDING RNA PRODUCTS
Expert Committee:	Biologics Monographs 5-Advanced Therapies Expert Committee
No. of Commenters:	23

General Comments
Comment Summary #1: The commenter requested that the General Chapter be updated to include non-E. coli strains that are being developed or cell-free production processes.
Response: Comment partially incorporated. In the General Chapter, the use of E. coli was replaced with microbial host to include the use of other cell types. The chapter remains focused on the plasmid DNA process, not other polynucleotide processes; therefore, cell-free production processes are not included.
Comment Summary #2: The commenter requested that the General Chapter be revised to describe the possibility of using other bacteria as potential cell substrates.
Response: Comment partially incorporated. In the General Chapter, the use of E. coli was replaced with microbial host to include the use of other cell types.
Comment Summary #3: The commenter suggested that the General Chapter focus on the fit for purpose quality of the polynucleotide product generated rather than specifying specific sources such as E. coli.
Response: Comment partially incorporated. In the General Chapter, the use of E. coli was and replaced with microbial host to include the use of other cell types. However, the chapter remains focused on the plasmid DNA process, not other polynucleotide processes.
Comment Summary #4: The commenter suggested that there should be a statement in the General Chapter that it is possible to use alternative technologies, methods and/or procedures if they provide advantages in terms of performance (e.g. accuracy, sensitivity, precision, selectivity, robustness), handling (e.g. sample preparation), throughput (e.g. run time or suitability for automation) or in other circumstances.
Response: Comment partially incorporated. Two sentences were added to the end of the Introduction to clarify that the General Chapter is not all inclusive and that other approaches or strategies can be used. Language was changed in Section 3. *Manufacturing Considerations* and

Table 2 to expand the use of other microbes and the use of methods other than what is included in the table.

Comment Summary #5: The commenter suggested to broaden the requirements by including a general remark that exceptions can occur for small plasmid batch sizes for e.g. individualized products or rare disease products.

Response: Comment not incorporated. The content of this General Chapter should not be considered as a requirement but that it is intended to discuss the recommended practices. Where specific issues, such as small batch size, are considered, discussions with regulators on reasonable approaches for establishing necessary quality assurance should be included.

Comment Summary #6: The commenter suggested to remove text that is already in other guidance documents and just cite the guidance document.

Response: Comment not incorporated. The General Chapter cross references relevant USP and regulatory guidance documents. In some instances, explanatory text and content are included to provide context for plasmids used as starting material, which would not be present in the referenced documents.

Comment Summary #7: The commenter requested clarification of a phased approach (as per clinical trial development phase) and the expectations for analytical testing and acceptance criteria of a plasmid used as starting material.

Response: Comment not incorporated. The General Chapter already discusses a phased approach to analytical testing under the section on “Quality control assays” and notes in the Conclusion section, and the user is expected to justify the use of a phase-appropriate control strategy to comply with the company’s quality management system and regulatory guidelines.

Comment Summary #8: The commenter suggested to have a summary table with the considerations for plasmid and MCB quality options across Phase I to Phase III.

Response: Comment not incorporated. User should review appropriate FDA guidances, and the quality options depend on the intended end use.

Comment Summary #9: The commenter requested clarification from guidance perspective, including specifically why starting material was the chosen terminology and how that relates to the other terms used previously and in other guidances. As the most recent guidance this should at least contextualize prior guidances if the plan is to outright change the language.

Response: Comment not incorporated. Table 1 of this General Chapter addresses this issue in some, but not all, instances. A starting material could also be an ancillary material. See footnote “b” in Table 1. However, given the scope of this chapter, “starting material” is more representative than using other terms mentioned by the commenter.

Comment Summary #10: The commenter requested that a distinction be made between those quality attributes which should be analyzed and understood as part of the characterization of the plasmid DNA, and those which would be considered to be necessary for (phase dependent) release testing (for example, on circular pDNA would the identity of homologous regions including LTRs, ITRs and poly(A) tails be considered as part of the characterization).

Response: Comment not incorporated. Section 5.1states: “See Table 3 and Table 4 for an overview of quality attributes, test methods, and example acceptance criteria” and “The User should select a subset of these tests or other procedures not listed as appropriate for the intended application.”

Comment Summary #11: The commenter suggested to include section on expectations for manufacturing controls and expectations.

Response: Comment not incorporated. Manufacturing controls are covered in section 4.1 Phase Appropriate Quality Systems and Facilities and section 5.6 General Acceptance Criteria and Manufacturing Considerations.

Comment Summary #12: The commenter requested that the chapter include suggested specifications or guidance on specification setting. The commenter also requested that the General Chapter discuss how to establish "end-use" or "end-user" specific values.

Response: Comment not incorporated. The General Chapter includes general information on specification setting, such as in “Table 3. Plasmid DNA Quality Attributes.” End-user-specific values depend on the specific uses of the plasmid and is beyond the scope of this chapter.
Comment Summary #13: The commenter requested that for plasmids used as a starting material in a commercial product, the General Chapter recommends that either GMP plasmids are used or that the plasmid has been independently verified to meet appropriate GMP standards for intended use.
Response: Comment partially incorporated. One of the reasons for writing this chapter was the inconsistent use of statements of GMP compliance by the plasmid manufacturer. Irrespective of the “claim” of GMP by a vendor, it is the entity using the plasmid as a starting material that is obligated to determine if the “quality” of the plasmid is acceptable.
Comment Summary #14: The commenter suggested that the structure of the document should be aligned with other general chapters (e.g., <1046> and <1047>), for example the chapter could include a Glossary.
Response: Comment not incorporated. The chapter structure is consistent with <1046> and <1047>. The current USP style for general chapters does not include a Glossary at the end of the chapter.
Comment Summary #15: The commenter suggested to add tables listing activities and processes and the applicability to development and manufacturing phase.
Response: Comment not incorporated. The General Chapter contains Tables 2, 3, and 4 that list the testing and quality attributes relevant to plasmid manufacturing.
Comment Summary #16: The commenter suggested to align to EMA terminology “GMP Principles” for the intermediate level of GMP being described here for early phase plasmids (EMA/246400/2021).
Response: Comment not incorporated. Reference to EMA/246400/2021 is made in section 4 Quality Management. There is already a general alignment between this chapter and the EMA document; both indicate that the relevant quality requirements for using the plasmid in a CGT (ATMP) should be determined by the product manufacturer.
Comment Summary #17: The commenter requested that clear guidance on requirements around storage of retention or reserve samples required be added to the chapter because the guidance would be beneficial. Annex 13/19 can be interpreted in different ways.
Response: Comment not incorporated. There are too many variables with regard to appropriate number and volume of retention or reserve samples to make a general recommendation in this General Chapter.
Comment Summary #18: The commenter suggested that it would be useful to expand scope to include plasmid use for vaccines as there is significant overlap to what is outlined in this General Chapter. It would be important to call out differences with vaccines also.
Response: Comment not incorporated. Vaccines are out of scope because several guidances for plasmid used in vaccines are available.
Comment Summary #19: The commenter requested to introduce the concept about how the usage of the plasmid dictates the GMP controls implemented that was a big focus of EMA document (EMA/246400/2021).
Response: Comment not incorporated. The concept that the usage of the plasmid dictates the level of controls is extensively discussed in section 4.1 “Phase Appropriate Quality Systems and Facilities” and is reiterated in several places in the chapter.
Comment Summary #20: The commenter requested clarification around phase appropriate method readiness as validated methods should be applied for release and stability testing for late stage or pivotal material.
Response: Comment partially incorporated. Phase appropriate methods are already covered in section 4.1 Phase Appropriate Quality Systems and Facilities under “Quality Control Assays,” and the pivotal material was added to Figure 2.

Introduction

Comment Summary #21: The commenter suggested the following change: "…including linearization and subsequent ~~translation~~ transcription for mRNA drug production" because DNA is transcribed to mRNA, not translated.

Response: Comment partially incorporated. The word "translation" was corrected to "transcription." The sentence was also edited to include in vitro transcription (IVT) in response to another commenter's comment. The revised sentence is, "Plasmids are double-stranded, circular DNA biological molecules that can be used as starting materials during the manufacture of cell and gene therapy (CGT) products, including linearization and subsequent ~~translation~~**in vitro transcription (IVT)** for mRNA drug production."

Comment Summary #22: The commenter recommended to change the text to, "**including mRNA drug product"** then move the information on linearization and subsequent translation of mRNA to chapter 2, Scope.

Response: Comment not incorporated. The information about the linearization and subsequent translation of mRNA was not moved to the Scope section because it needed to remain in the sentence as mRNA is in scope of the chapter and should be described in this introductory sentence.

Comment Summary #23: The commenter suggested to revise the first sentence of the chapter to further the definition of plasmid to include or exclude some of the newer plasmid systems such as Nanoplasmids, minicircle DNA, and doggybone (db)DNA.

Response: Comment not incorporated. Plasmid DNA covers nanoplasmids and minicircle DNA; these are proprietary plasmids; therefore, there is no need to mention the proprietary plasmids in this General Chapter. Doggybone DNA is not considered a plasmid; therefore, it does not need to be mentioned in the introductory sentence.

Comment Summary #24: The commenter suggested to make the following edits in bold to introduce IVT earlier in the chapter. "Plasmids are double-stranded, circular DNA biological molecules that can be used as starting materials during the manufacture of cell and gene therapy (CGT) products, including linearization and **subsequent in vitro transcription (IVT) for mRNA drug production."**

Response: Comment incorporated.

Comment Summary #25: The commenter has suggested the following edits to the first sentence in the Introduction to be aligned with <1047>. "Plasmids are double-stranded, circular DNA biological molecules **that exist in bacteria as extrachromosomal, self-replicating molecules** that can be used as starting materials during the manufacture of cell and gene therapy (CGT) products, including linearization and subsequent translation for mRNA drug production."

Response: Comment not incorporated. The suggested revision precludes consideration of newer, non-bacterial sources of plasmids that were not in use at the time <1047> was written.

Comment Summary #26: The commenter suggested to change the second introductory sentence to "Plasmid DNA is used for a number of biotechnology applications, including X, Y and Z which are the subject of this guidance."

Response: Comment not incorporated. The current wording in the chapter is clear, and the specific applications are cell and gene therapy products. The chapter does not cover all biotechnology applications; therefore, adding that phrase could cause confusion.

Comment Summary #27: The commenter suggested to add a fourth bullet point, "Plasmid supplier qualification process including a site audit".

Response: Comment not incorporated. The chapter states that the plasmid supplier qualification should be a part of the quality risk management system. The supplier qualification process and site audit are already captured in the second bullet, "Quality and Risk Assessment."

Comment Summary #28: The commenter suggested to edit this sentence as follows,

"Because these materials are used ~~to introduce a recombinant nucleic acid sequence into a CGT product~~ **as starting materials for the manufacture of Cell and Gene Therapies**"
Response: Comment incorporated.
Comment Summary #29: The commenter suggested the following change, "to establish whether the plasmid supplier meets the quality systems requirements of a CGT manufacturer **for a starting material**."
Response: Comment incorporated. The following edits were made to the sentence, "Therefore, it is **the responsibility of** ~~incumbent upon~~the end user, who is using the plasmid to manufacture a CGT product, to conduct a quality audit of the supplier of the plasmid to document the level of compliance with quality standards and in conjunction with other objective evidence, such as test results, to establish whether the plasmid supplier **and plasmid** meets the **quality system requirements for a starting material.**" ~~a CGT manufacturer.~~
Comment Summary #30: The commenter suggested the following edits, "Therefore, it is incumbent upon the end user, who is using the plasmid to manufacture a CGT product, to conduct a quality ~~audit~~ **evaluation** of the supplier of the plasmid to document the level of compliance with quality standards and in conjunction with other objective evidence…"
Response: Comment not incorporated. Audit is the correct term that is used by Quality Assurance. Introducing a new term such as evaluation does not provide clarity.
Comment Summary #31: The commenter suggested the following edits, "Therefore, it is **the responsibility of** ~~incumbent upon~~ the end user."
Response: Comment incorporated.

Scope
Comment Summary #32: The commenter requested that an introductory sentence be added to the Scope section that discusses plasmids from biological origin verses synthetic plasmids, and a statement about whether or not synthetic plasmids are in scope.
Response: Comment incorporated. The following text was added to the scope section, "Given the diverse uses of plasmid DNA in CGT and mRNA-based drug product development and manufacturing, the scope of this chapter is to discuss plasmids of biological origin that are used as starting materials. A 'synthetic plasmid' is a plasmid that has been artificially created in a laboratory by scientists, essentially a piece of DNA designed and constructed de novo with specific genetic components, rather than being isolated from a biological source. Synthetic plasmids are considered as out of scope for this chapter."
Comment Summary #33: The commenter requested to add a phrase about plasmid use for mRNA-based drug products to the first sentence of the Scope section.
Response: Comment incorporated. The first sentence of the scope section was updated to include plasmids for mRNA-based drug products.
Comment Summary #34: The commenter suggested requirements for genetic stability should be considered, as appropriate, prior to commercial application.
Response: Comment partially incorporated. The following sentence was added, "Plasmids of biologic origin contain both genetic components that are necessary for maintenance (origin, selective marker, etc.) and those that are not constitutively expressed in the host but are vital for construction of the chosen active pharmaceutical ingredient."
Comment Summary #35: The commenter suggested to add the text in bold to clarify that the linearized plasmid has been accepted as a starting material for mRNA products.
"Given the diverse uses of plasmid DNA in CGT drug development, the scope of this chapter is to discuss plasmids that are used as starting materials. **For mRNA products, the linearized plasmid is used as starting material**."
Response: Comment partially incorporated. The following text was added to the scope to clarify that linearized plasmid is also considered a starting material, "In general, plasmids including linearized forms used as a starting material for the manufacture of cell, gene and mRNA therapies are in scope."

Comment Summary #36: The commenter suggested to add to Table 1: “plasmid starting materials used to generate genetically modified cells” because in the text of the Scope section, “plasmids used as starting materials to genetically modify cells” are in scope but are not mentioned out in Table 1. A distinction should be made between the GOI plasmids mentioned in Table 1 for ex vivo cell therapy manufacturing which are considered as Drug Substance.
Response: Comment partially incorporated. The text in the scope section and in Table 1 was edited and aligned. Genetically modified cells were removed from the phrase, “plasmid starting materials to generate genetically modified cells” in the scope section. The scope was broadened to include cell and gene therapies.
Comment Summary #37: The commenter suggested the following edits in bold, “In general, plasmids used as a starting material for the manufacture of gene therapies, including genetically modified cells, are in scope. **These plasmids are templates from which the active substance is manufactured and are not intended to be present in the final product (non-constitutive).**
Response: Comment partially incorporated. The sentence was added but was edited to remove “non-constitutive” because this was already incorporated in another edited sentence in the paragraph.
Comment Summary #38: The commenter suggested the following edits, “In general, plasmids used as a starting material for the manufacture of **mRNA vaccines** and gene therapies, including genetically modified cells, are in scope.”
Response: Comment partially incorporated. The sentence was modified to “In general, plasmids **including linearized forms** used as starting material for the manufacture of **cell,** gene, and **mRNA therapies** are in scope. The general chapter provides guidance for starting materials for cell, gene, and mRNA therapies.”
Comment Summary #39: The commenter suggested to rephrase to include "cell therapy" to align with other parts in document “In general, plasmids used as starting material for the manufacture of cell and gene therapies, including genetically modified cells, are in scope.”
Response: Comment partially incorporated. The sentenced was modified to “In general, plasmids including linearized forms used as a starting material for the manufacture of **cell,** gene and mRNA therapies are in scope.” Cell therapies were included in the scope. The other edits to the sentence have been made in response to previous comments.
Comment Summary #40: The commenter recommended to add information on the linearization and subsequent translation for mRNA drug production.
Response: Comment partially incorporated. The sentence was modified to “In general, plasmids **including linearized forms** used as a starting material for the manufacture of cell, gene and **mRNA therapies** are in scope” to include linearization and mRNA therapies.
Comment Summary #41: The commenter recommended to add “It is out of scope for the plasmids used for rBV constructions as those plasmids are not starting materials for AAV production in Sf9/rBV systems.”
Response: Comment not incorporated. The use of plasmids to prepare the bacmid in E. coli is out of scope as they are not starting materials, and this specific case is not mentioned in the chapter because very specific use cases are not included in the scope section.
Comment Summary #42: The commenter requested alignment on use of words of manufacturing and development together and separate and it should imply both.
Response: Comment partially incorporated. The sentence, “In the general chapter, the definition of Supplier is the manufacturer of the plasmid, and the User is the drug substance or drug product developer and **may be the manufacturer**” was edited to clarify that the developer may be the manufacturer.
Comment Summary #43: The commenter requested justification for the reference to USP <1043> *Ancillary Materials for Cell, Gene and Tissue-Engineered Products* reference.

Response: Comment not incorporated. The reference to <1043> *Ancillary Materials for Cell, Gene and Tissue-Engineered Products* was added because the helper plasmid may be considered a starting material or an ancillary material.

Comment Summary #44: The commenter suggested to consider expanding the scope of the general chapter to include enzymatic and non-standard plasmid DNA as well as ex vivo DS; as an emerging space would be valuable to have guidance and support.

Response: Comment not incorporated. Synthetic DNA will not be included in the scope because at this time, the use of synthetic DNA has not matured enough to write a general guidance.

Comment Summary #45: The commenter suggested to add a "Product type" column for each of the plasmid descriptions in Table 1.

Response: Comment not incorporated. The product type is a downstream use of the plasmid, which is too specific for this general chapter. There are several uses of the plasmid, and it would be too difficult to capture all of them.

Comment Summary #46: The commenter suggested to make helper plasmid plural (helper plasmids) in Table 1 because often multiple plasmids are used. The commenter suggested that rows 1 and 3 of Table 1 could be combined.

Response: Comment not incorporated. The helper plasmid was not changed to plural because it is understood that more than one helper plasmid may be used. Rows 1 and 3 in Table 1 cannot be combined because the helper plasmids are the only plasmids that could also be considered as ancillary material.

Comment Summary #47: The commenter recommended that it be clearly stated in Table 1 that reprogramming plasmids or gene editing plasmids (cas9) are considered in this case as "research grade" reagents and not in scope of the USP based on Table 1 column for "Scope".

Response: Comment partially incorporated. A line was added to Table 1, which states, "Plasmid vectors used for generic cloning activities including production cell line design" to describe what is not in the scope relating to cloning and cell line design.

Comment Summary #48: The commenter suggested that it may be confusing to reference USP <1043> and to have the footnote that states that helper plasmids can be considered an ancillary material because the ISO ancillary material document doesn't differentiate between helper, GOI and packaging plasmids.

Response: Comment not incorporated. The ISO ancillary material document included plasmids as within the scope by mistake. There has been a request for revision of the ISO standard submitted.

Comment Summary #49: The commenter requested that USP provide a definition on the difference between a helper and packaging plasmid and clarification on why the helper and packaging plasmid are separated in Table 1.

Response: Comment not incorporated. According to the FDA, a starting material is a substance that is incorporated into a drug substance as a significant structural fragment and has a defined chemical structure and properties. Helper plasmids can be considered as ancillary material in some cases, as noted in the footnote in Table 1.

Comment Summary #50: The commenter requested to provide more precision on the difference between helper plasmid and packaging plasmid, and noted that there may not be any difference.

Response: Comment partially incorporated. To provide clarity, the term "packaging plasmid" was removed from Table 1 because it includes helper plasmids, Rep, and Cap. The text was modified to separate Rep and Cap from the helper plasmid in the table.

Comment Summary #51: The commenter requested clarification if the "packaging plasmid used for generating viral vectors meant capsid and requested that an example be provided.

Response: Comment partially incorporated. To provide clarity, "packaging plasmid" was removed from the table because it includes helper plasmids, Rep, and Cap. The text was modified to separate Rep and Cap from the helper plasmid in the table.

Comment Summary #52: The Commenter suggested to clarify in a footnote or in the text, whether the “GOI plasmid used as the GOI in viral vectors” also applies to plasmids used to generate stable producer cells. The commenter noted that plasmids used in the generation of stable producer cells for VSN manufacturing should not be in scope, as the genetic modification of the packaging cell line occurs early in development prior to laying down eukaryotic MCB and WCB, which are considered themselves as starting material for viral vector production.
Response: Comment partially incorporated. The text and examples are provided in Table 1 to clarify that these examples are out of scope.
Comment Summary #53: The commenter suggested the following edit, “**Linearized** plasmids used as templates for mRNA products”. The commenter also requested to differentiate between circular and linearized plasmids, and which may be in scope or out of scope of the chapter.
Response: Comment partially incorporated. Table 1 was revised to include “linearized” plasmids as a starting material. A differentiation between circular and linearized plasmids was not made because information on circular plasmid is provided in the chapter to support the cell and gene therapy use.
Comment Summary #54: The commenter suggested using plasmid used for generating viral vectors (e.g. Helper, Packaging, GOI) instead of separating out Helper, packaging and GOI plasmids in Table 1.
Response: Comment not incorporated. The types of plasmids are separated in the table for clarity and because the helper plasmid can be considered as ancillary material in some cases.
Comment Summary #55: The commenter requested that a reference for GOI plasmids used directly in ex-vivo cell therapy manufacturing be added to the chapter for guidance since these plasmids are out of scope.
Response: Comment incorporated. A reference to USP <1047> Gene Therapy Products was added to Table 1.
Comment Summary #56: The commenter indicated that “GOI plasmid used directly in ex vivo cell therapy manufacturing” is still considered starting material.
Response: Comment not incorporated. The GOI plasmid that is used directly in ex vivo cell therapy is considered a drug substance and not a starting material.
Comment Summary #57: The commenter recommended to include a CMC reference for GOI plasmids that are directly injected into humans or animals.
Response: Comment incorporated. The reference, “Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)” was added to Table 1.
Comment Summary #58: The commenter suggested to add a footnote to Table 1 to clarify that DNA manufacturing through an in vitro process includes DNA enzymatically amplified from an initial plasmid.
Response: Comment partially incorporated. A footnote was added to clarify that these materials were created through a cell-free process. “In vitro” was changed to “cell-free.”
Comment Summary #59: The commenter suggested that a guidance reference be added to the DNA manufactured through an in vitro process in Table 1.
Response: Comment not incorporated. A guidance document does not exist for plasmids made by recombinant enzymes, and this synthetic DNA falls outside the scope of the chapter.
Comment Summary #60: The commenter suggested to change the GOI plasmid used directly in ex vivo cell therapy manufacturing to in scope because the plasmid is considered a starting material.
Response: Comment not incorporated. The FDA guidance, Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) classifies the plasmid as a drug substance in this case. However, Table 1 does allow for discussion with the regulatory agency, which may give the plasmid a different designation.
Comment Summary #61: The commenter requested to include plasmids used as templates to manufacture DNA through an in vitro process be included in the scope of the chapter.

Response: Comment not incorporated. The general guidance in this chapter can be applied to different cases, but this specific example falls outside the scope of the chapter.
Comment Summary #62: The commenter suggested to delete the reference to “CMC, chemistry, manufacturing and control; INDs, investigational new drug applications” from footnote d in Table 1.
Response: Comment not incorporated. Footnote *d* is not a reference to a CMC guidance but is used for defining abbreviations used in Table 1.
Comment Summary #63: The commenter requested clarification if footnote b in Table 1 is applicable to helper plasmids or for all materials being labelled as starting materials in this chapter.
Response: Comment not incorporated. Footnote *b* can only be applied in some cases to helper plasmids and not the other plasmids covered in the chapter.
Comment Summary #64: The commenter suggested to remove footnote e from Table 1, as it is not clear where De Novo synthesis as a starting material for pDNA production fits in. The description says enzymatic and chemical. The footnote only mentions enzymatic. Excluding chemical could put De Novo synthesized constructs out of scope, when they could be in scope.
Response: Comment partially incorporated. The phrase “enzymatic processes” was removed from footnote *e* to clarify that enzymatic or synthetic processes could be used to produce synthetic DNA, which is out of scope.
Comment Summary #65: The commenter suggested to revise and in some cases remove the footnotes for Table 1, for example provide alternative guidance for plasmid used directly in ex vivo cell therapy manufacturing. The commenter recommended to remove footnote e because it had additional information for material that is out of scope of the chapter.
Response: Comment not incorporated. The footnotes were added for further clarification even for examples that are out of scope. No guidance is provided for synthetic DNA because there is no existing guidance to cite.
Comment Summary #66: The commenter suggested to edit in Table 1 “for mRNA” to “for RNA” to account for other enzymatic RNA processes that utilize plasmid templates such as circular RNA.
Response: Comment incorporated. The “mRNA” was changed to RNA in the table.

Manufacturing Considerations
Comment Summary #67: The commenter suggested removing section 3, and only keeping select, specific sentences and cross reference appropriate guidance for more details e.g., ICH Q5a-d.
Response: Comment not incorporated. ICH Q5D is referenced in section 3.1. The reference was changed to include all of ICH Q5. Important details not contained in the ICH reference are included in the chapter.
Comment Summary #68: The commenter suggested to rewrite section 3 utilizing process flow diagrams and figures to illustrate. The commenter also suggested to incorporate de novo synthesis as an option prior to clone and include clone screening (and what to screen for, e.g. ITR/LTR integrity) prior to clone selection and remove bullet point number 5 as it is out of place and inconsistent with even other raw-materials related.
Response: Comment partially incorporated. Bullet point number 3 was revised for clarification. Text was added to clarify that clones are screened and selected: “A clone is **screened and** selected for production purposes and is expanded into a master cell bank (MCB). If required, a working cell bank (WCB) is prepared, often later in development.” Bullet point number 5 was moved to the first bullet point for better flow of the process.
Comment Summary #69: The commenter suggested that bullet number 5 should be the first bullet as it is a prerequisite for manufacturing. The commenter suggested to change the figure and text of the manufacturing flow 1-5, so they are completely aligned. The commenter also suggested to move the reference to Figure 1 to the first sentence of the section.

Response: Comment incorporated. Bullet number 5 was moved to number 1 to align the flow with the manufacturing process. A reference to Figure 1 was added to the first sentence of the section.
Comment Summary #70: The commenter suggested to remove language that specified E. coli for the bacterial substrate. For bullet point 1., the commenter suggested this edit:" 1.The initial plasmid is created using standard molecular biology techniques and transformed into a suitable cell substrate. ~~(typically E.coli).~~
Response: Comment partially incorporated. "E. coli" was replaced with "microbial" throughout the chapter to allow for more flexibility in the cell substrates used. E. coli was mentioned as an example; "typically E. coli" was replaced with "for example E.coli."
Comment Summary #71: The commenter suggested to change substrate to cellular host or cell host as substrate in the context of cells are often used to reference the growth media or matrix on/in which cells are grown as this will help with clarity.
Response: Comment incorporated. Substrate was changed to cellular host.
Comment Summary #72: The commenter suggested to add the phrase, "is typically, but not always" to say that an MCB is not always created.
Response: Comment incorporated.
Comment Summary #73: The commenter suggested the following change, " A clone is selected for production purposes and, **unless only small plasmid DNA batch sizes are needed, e.g., for an individualized product,** is expanded […].
Response: Comment partially incorporated. This sentence was added to the end of bullet #3: "An MCB may not be needed if only small plasmid DNA batch sizes need to be manufactured, e.g., for an individualized product."
Comment Summary #74: The commenter suggested to change the language to, "A clone is **screened and** selected for production purposes…" in bullet point number 3.
Response: Comment incorporated.
Comment Summary #75: The commenter suggested to add a statement that the steps in bullet points 2 and 3 should be GMP compliant.
Response: Comment not incorporated. Discussion regarding GMP compliance is discussed in a later section (4.1) and does not need to be discussed in this section.
Comment Summary #76: The commenter suggested that production can also be initiated by repeated sampling from a single vial as this is frequently the case at plasmid manufacturers.
Response: Comment not incorporated. Multiple sampling from a single vial is not a common practice.
Comment Summary #77: The commenter suggested the following edits to make bullet point 4 less E. coli specific: "4. The **bacterial** ~~E. coli~~ containing plasmid is lysed and the plasmid is purified."
Response: Comment partially incorporated. The wording was changed to "microbial host" to make the statement not E.coli specific .
Comment Summary #78: The commenter suggested to change "E.coli" to host bacteria to allow for flexibility for different bacterial organisms to be used.
Response: Comment partially incorporated. The wording was changed to "microbial" to focus more on E.coli.
Comment Summary #79: The commenter suggested adding more detail and clarification by changing the text of bullet 4 to " The E. Coli is isolated by harvest and plasmid is extracted by lysis. Subsequently the plasmid is purified for removal of cellular components and impurities."
Response: Comment partially incorporated. The wording was changed to "microbial host containing plasmid is lysed and the plasmid is purified (column or filtration techniques)," which is a more concise way of describing the process.
Comment Summary #80: The commenter suggested to change "may include" to "can be" since not every sequence to be delivered is synthesized (and only a relatively recent option) in this

statement, “The resulting construct contains a bacterial origin of replication, which results in plasmid reproduction and persistence, a selectable marker to ensure growth of bacteria that have been transformed, and the gene of interest, which ~~may include~~ **can be** multiple sequences of promoters, regulatory elements, and therapeutic genes.
Response: Comment partially incorporated. The text was changed to, “The construct also **typically** contains a selectable marker to ensure growth of the microbes that have been transformed, and the gene of interest, which may include multiple sequences of promoters, regulatory elements, coding sequence (CDS) and/or open reading frame (ORF) for therapeutic genes.” Adding the word “typically” allows for flexibility in whether or not the elements are included.
Comment Summary #81: The commenter requested to revise this statement about using multiple vials of the cell bank until the production volume is achieved “Production is initiated with one or more vials of the cell bank until the production volume is achieved.”
Response: Comment partially incorporated. The language in the bullet point was revised to clarify that one or more vials should be from the same cell bank. It is acceptable to use more than one vial to obtain the required seed volume.
Comment Summary #82: The commenter suggested to add one sentence on the general purification techniques used for plasmids after bullet point #4.
Response: Comment incorporated. Examples of purification techniques were added to bullet point 4.
Comment Summary #83: The commenter suggested to remove the reference to Figure 1 and to revise this statement, “Identity testing for critical process raw materials… should be considered prior to use in plasmid manufacture and is essential **when material suppliers do not have appropriate quality systems to ensure data integrity and traceability (e.g. ISO, GMP) and ensure notification of changes** ~~(Figure 1)~~.
Response: Comment partially incorporated. The reference to Figure 1 was moved to the beginning of the section to clarify that Figure 1 describes the process for plasmid manufacturing. The text was incorporated but as an example of best practice. The following sentence was added: “As a best practice, identity testing should be done for material suppliers that do not have appropriate quality systems.”
Comment Summary #84: The commenter suggested to make Figure 1 a more thorough overview of upstream processes, downstream processes, fill and release.
Response: Comment not incorporated. Figure 1 is meant to be high level. The text in the chapter describes plasmid clone screening and sequencing.
Comment Summary #85: The commenter requested clarification of what is meant by “critical process raw materials” as the point seems to be out of context here. The commenter suggested removing the critical process raw materials and discuss it elsewhere.
Response: Comment not incorporated. The critical raw materials, including media and buffers, should be tested before use in plasmid production and should be included in the section that discusses plasmid production.
Comment Summary #86: The commenter suggested adding to assess risk of adventitious agents (including BSE/TSE) and cross-contamination with other plasmids and microbial cells to the Manufacturing Section.
Response: Comment partially incorporated. The text about adventitious agent was added. Cross-contamination is discussed in section 3.2 Facility Design.
Comment Summary #87: The commenter requested to remove and not introduce new definitions such as critical process materials. Also, the commenter questioned the identification requirements for buffers. The commenter suggested the following edits, “Identity testing for ~~critical process~~ raw materials critical for the process ie media ~~(media, buffers, etc.)~~ should be considered prior to use in plasmid manufacture.”

Response: Comment partially incorporated. “Critical process raw materials” was revised to “raw materials critical for the process.” The examples of buffer and media were retained as examples because these may be raw materials that are critical to the process.
Comment Summary #88: The commenter suggested to change the text in bullet #5 to “Plasmid formulation, storage and QC testing.” The commenter also suggested removing the current text in bullet 5 because the identity testing of raw materials should not be step 5.
Response: Comment partially incorporated. In response to previous comments, the text in bullet point 5, which refers to identity testing of raw materials, was moved to bullet point 1. The bullets in Section 3 were meant to be considerations and not necessarily a sequence of events. The numbers were changed to bullets to clarify that it is not a sequence of events.
Comment Summary #89: The commenter suggested editing the bullet point 5 to “5. Identity testing for critical process raw materials (media, buffers, etc.) ~~should~~ **can** be considered prior to use in plasmid manufacture (Figure1).”
Response: Comment not incorporated. The sentence says “should be,” which is a best practice and not a requirement; therefore, it does not need to be changed to “can be.”
Comment Summary #90: The commenter suggested editing the text in bullet point #5 to, "Phase appropriate consideration of raw materials control for plasmid manufacturing".
Response: Comment not incorporated. The text was not changed because the chapter describes best practices. While testing the identity of critical raw materials is recommended as a best practice, it is not mandatory.
Comment Summary #91: The commenter suggested the following edits, “Animal derived components in the manufacturing process need to be described and ~~explained~~ **the risk of adventitious agent transmission should be assessed**.”
Response: Comment incorporated.
Comment Summary #92: The commenter suggested to add this sentence to the end of the first paragraph after the bulleted list, “In addition, identity testing for critical process raw materials (media, buffers, etc.) should be considered prior to use in plasmid manufacture (Figure 1).”
Response: Comment not incorporated. Instead of moving the text from bullet point 5 into the section text, bullet point 5 was moved to bullet point 1 to reflect the flow of the manufacturing process.
Comment Summary #93: The commenter suggested to add, "coding sequence (CDS) and/or open reading frame (ORF)" into the sentence " The construct also typically contains a selectable marker to ensure growth of the microbes that have been transformed, and the gene of interest, which may include multiple sequences of promoters, regulatory elements, **coding sequence (CDS) and/or open reading frame (ORF)** for therapeutic genes.".
Response: Comment incorporated.
Comment Summary #94: The commenter requested clarification on what is meant by “well documented”.
Response: Comment not incorporated. The chapter already includes a sentence that defines the acceptable documentation supporting this best practice.
Comment Summary #95: The commenter suggested to add guidance to help harmonize identity testing of raw materials at receipt for plasmid manufacturers. The commenter suggested to add "5. Identity testing for critical process raw materials… should be considered prior to use in plasmid manufacture and is essential when material suppliers do not have appropriate quality systems to ensure data integrity and traceability (e.g., ISO, GMP) and ensure notification of changes"
Response: Comment not incorporated. Identity testing and management of raw materials is the responsibility of the quality management system of the user.
Comment Summary #96: The commenter recommended “Risk based acceptance testing for critical process raw materials….”
Response: Comment not incorporated. A raw material is determined as critical through a process. There is no need to identify that as “risk based.”

Comment Summary #97: The commenter recommended adding another figure to describe the process for constructing the initial plasmid DNA for transformation.
Response: Comment not incorporated. There are many ways to construct a plasmid. Adding a figure would bias the reader to one particular way of constructing a plasmid.
Comment Summary #98: The commenter recommended "The resulting construct contains a bacterial origin of replication. This results in plasmid reproduction and persistence, a selectable marker to ensure growth of bacteria that have been transformed, and the gene of interest. T**his gene of interest** may include multiple sequences of promoters, regulatory elements, and therapeutic genes."
Response: Comment partially incorporated. The sentence was revised and divided into two sentences to reduce the length of the sentence. The suggested edits were not included because of the way the sentences were divided.
Comment Summary #99: The commenter recommended to edit as follows: "The design, synthesis, and cloning of this initial plasmid DNA should be well documented and controlled; and **environmental/reagents/other carryover concerns of cloning that may influence the process should be considered**. Any errors in sequence or cross-contamination with other DNA could be propagated in subsequent manufacturing operations and **these records of deviations should be included in the documentation**."
Response: Comment partially incorporated. The text was edited to, "The design, synthesis, and cloning of this initial plasmid DNA should be well documented and controlled, as any error in **the** sequence or cross-contamination with other DNA could be propagated in subsequent manufacturing operations. **For example, carryover concerns of cloning that may influence the process should be considered**." A statement about carryover concerns of cloning that may influence the process was added. A statement about deviations was not included because this is covered in subsequent sentences in the section.
Comment Summary #100: The commenter recommended the following edits, "The use of animal derived components in the manufacturing process needs to be described ~~and explained~~ **as well as qualified, controlled and justified**."
Response: Comment partially incorporated. The phrase "the use of" was added to the beginning of the sentence. The sentence had already been revised to "The use of Animal derived components in the manufacturing process need to be described and ~~explained~~ the risk of adventitious agent transmission should be assessed" from a previous comment.
Comment Summary #101: The commenter suggested the following edits for clarification, "Animal derived components in the manufacturing process (steps 2-5) need to be described and explained."
Response: Comment not incorporated. The manufacturing process is sufficient, and there is no need to explain the animal-derived components in the steps described in the chapter.
Comment Summary #102: The commenter suggested to change to "typically" and then, "and/or". The edited sentence would be, "The initial plasmid DNA for transformation is **typically** produced through a combination of gene synthesis **and/or** cloning.
Response: Comment incorporated.
Comment Summary #103: The commenter requested to consider inclusion or exclusion of "cloning scars", if present (if synthesized, to omit).
Response: Comment not incorporated. Cloning scar is not a well-known term and not normally a part of a quality analysis.
Comment Summary #104: The commenter suggested adding the phrase "if synthesized" to this sentence, "Documentation may include "records of derivation; reports related to design, synthesis, and cloning; describing how the plasmid was made; and the nucleic acid sequence as verified by scientifically justified sequencing techniques."
Response: Comment not incorporated. The sentence states "may include"; therefore, other qualifiers are not needed.

Comment Summary #105: The commenter recommended moving Figure 1 directly before the 5-point descriptive text and changing the caption to “General description of process for producing plasmid DNA”.
Response: Comment partially incorporated. The figure will not be moved because it supports the flow of the section. The title of the figure was revised as the commenter suggested.
Comment Summary #106: The commenter suggested to revise Figure 1 to add a footnote that other manufacturing steps or methods may be possible, “typical” to be considerate of manufacturer dependent process improvements such as PCR amplification, and linearization of plasmid DNA vs. microbial expansion.
Response: Comment partially incorporated. The title of the figure has been revised to “General description of process for producing plasmid DNA.”
The current text provides a general description; the details suggested by the commenter are too process-specific and were therefore not included.
Comment Summary #107: The commenter requested clarification if formulation of the DNA in Figure 1 refers to the solution of plasmid in water or suitable buffer or if this refers to the formulation of the DNA plasmid as a drug product for final vector manufacturing.
Response: Comment not incorporated. The term “formulated” is meant to be general and should describe whatever is needed for the next steps of the manufacturing process. Drug substance is outside the scope of the chapter; therefore, it does not mean plasmid formulation for the drug substance.
Comment Summary #108: The commenter suggested to clarify that a clone should be screened first before using the clone to create a bank in Figure 1. The commenter indicated that Figure 1 omits critical steps (e.g. plasmid clone screening, sequencing and selection). The commenter suggested adding these details to Figure 1.
Response: Comment not incorporated. Figure 1 was intended to be a high-level overview and not overly detailed with all possibilities of testing.
Comment Summary #109: The commenter suggested to clarify the language around the gene of interest (GOI) because it should be the genetic product to be expressed, and not the other elements.
Response: Comment not incorporated. The gene of interest can include more than the therapeutic gene. For example, Helper genes, Rep/Cap can be the gene of interest in a plasmid used as a starting material for cell and gene therapy.
Comment Summary #110: The commenter requested clarity on whether risk assessment is sufficient in the case that animal-derived components cannot be excluded.
Response: Comment partially incorporated. To provide clarity, this sentence was revised as follows, “**The use of** animal derived components in the manufacturing process need to be described and explained **the risk of adventitious agent transmission should be assessed**.”
Comment Summary #111: The commenter suggested to describe controls that need to be applied if animal derived components are present, including the appropriate risk assessment package and adventitious agents safety evaluation to be filed in 3.2.A.2.
Response: Comment partially incorporated. The text has been revised to include risk assessment, and a reference to a new FDA guidance on the risk assessment was added.

3.1 Master Cell Bank
Comment Summary #112: The commenter recommended including language such as, “considerations will include manufacturability and potential for product quality performance" and then any callouts should be more descriptive of current considerations e.g. ITR retention, plasmid topology, etc.
Response: Comment partially incorporated. The first sentence of section 3.1 was re-worded to “considerations will include manufacturability and whether the strain of microbe can deliver suitable plasmid quality.”

Comment Summary #113: The commenter suggested to remove: "Competent cells are usually purchased by the Supplier and should include quality control testing for strain identity, lytic phage contamination, and viability at a minimum". The commenter suggested to edit the third sentence of section 3.1 to “MCB sourcing and generation should be managed within suppliers’ quality and materials management system and consistent with end users quality management requirements".
Response: Comment partially incorporated. The sentence "Competent cells are usually purchased by the Supplier and should include quality control testing for strain identity, lytic phage contamination, and viability at a minimum" was deleted. The third sentence in Section 3.1 was changed to “Cell bank sourcing and generation should be managed within suppliers’ quality and materials management system and consistent with end users quality management requirements."
Comment Summary #114: The commenter requested clarity on the (clinical phase dependent) requirements for establishment of an MCB (e.g. is an MCB required for plasmid manufacturing for clinical phase 1? Phase 2?), and the extent of testing thereof.
Response: Comment incorporated. Text was added to specify the phase-dependent requirements for the MCB.
Comment Summary #115: The commenter suggested to reword the second paragraph in 3.1, “Once the microbes ~~bacteria~~ have been transformed with the initial plasmid DNA **and a clone is selected, a cell bank** ~~an MCB~~ of the transformed microbes ~~bacteria~~ will be established.” The commenter suggested to add in section 3.1 “**MCB and WCB are generally prepared under full cGMP conditions because they may be used for commercial supply.**
When producing plasmid for phase I/II clinical trial supply, a pre-MCB can be acceptable if manufactured under the principles of GMP based on Quality Risk principles and tested for strain identity, phage contamination, and viability at minimum.”
Response: Comment partially incorporated. The sentence was re-worded to “Once the microbes ~~bacteria~~ have been transformed with the initial plasmid DNA and **a clone is selected, a cell bank** of the transformed microbes ~~bacteria~~ will be established.” The sentence was added as suggested, except the addition of the testing requirements. “MCB and WCB are generally prepared under full cGMP conditions because they may be used for Pivotal and commercial manufacturing. When producing plasmid for phase I/II clinical trial supply, a non-GMP cell bank can be acceptable if manufactured under the principles of GMP based on Quality Risk principles.”
Comment Summary #116: The commenter recommended the following edits, “Once the bacteria have been transformed with the initial plasmid DNA, an MCB of the transformed bacteria ~~will~~ **may** be established. **A MCB is recommended, but not required. Establishing a MCB depends on the need for establishing a bank.** The MCB can be is used in subsequent manufacturing events, resulting in more controlled and consistent production.
Response: Comment partially incorporated. The text has already been edited from a previous comment to allow for a non-GMP compliant cell bank. The sentence “Establishing an MCB depends on the need for establishing a bank” was not incorporated because subsequent sentences address this point.
Comment Summary #117: The commenter recommended the following edits, “Table 2 lists a panel of **release** tests that can be performed on the MCB to ensure quality and safety. Although not all assays are required, the testing panel should include tests for identity, ~~stability,~~ and purity (6) at a minimum. The commenter recommends removing “stability”.
Response: Comment not incorporated. The word “release” was not added as some tests can involve characterization. The stability will not be removed as it is commonplace to test for cell bank stability for an MCB.
Comment Summary #118: The commenter requested to clarify that 2 identity tests are required: one for the bacterial strain identity and one for plasmid identity. The commenter recommended differentiating Research grade and GMP plasmid in this place in the chapter.

Response: Comment partially incorporated. The sentence was modified to “should include tests for microbial strain identity, plasmid identity, stability and purity.” Text was added to clarify when a cell bank can be manufactured under non-GMP compliant conditions. Text was added to specify when cell banks should be made under GMP conditions and when it would be acceptable not to make the cell bank under GMP conditions.

Comment Summary #119: The commenter requested clarification on how many of the identity tests are needed. For example, if the identity test are unique identity tests that query different aspects of identity and thus should be selected in combination (separate rows). It would be helpful to industry to provide a reasonable panel and a comment on how much orthogonality is required, or more critically, what is required as a baseline if one is proposed**.**

Response: Comment not incorporated. The number and types of identity tests used is specific to the use of the plasmid and cannot be specified in this general chapter.

Comment Summary #120: The commenter suggested to add the following sentence, “**Stability of the MCB should also be demonstrated**.” The commenter recommended to include a table for WCB release testing or add a footnote in Table 2 for the tests that would be repeated from the MCB in the WCB.

Response: Comment not incorporated. Stability is included as a test that should be conducted; therefore, it does not need to be restated. The recommendation to add release testing for WCB is too complex for this general chapter as there are different scenarios that can occur when generating a WCB (e.g., if MCB testing is not performed, complete testing of each WCB is expected).

Comment Summary #121: The commenter recommended the following edits, “The choice of ~~bacterial~~ ***microbial*** strain will vary based on the nature of the plasmid DNA; considerations will include plasmid stability, copy number, and doubling time.”

Response: Comment incorporated. “Bacterial” has been changed to “microbial” throughout the general chapter.

Comment Summary #122: The commenter recommended the following edits: “Competent **microbial** cells are usually purchased by the Supplier and should include quality control testing for strain identity, lytic phage contamination, and viability at a minimum.”

Response: Comment partially incorporated. The sentence was deleted because the step on purchasing competent cells is too detailed and not needed in the chapter.

Comment Summary #123: The commenter suggested making the following edits to this sentence, “The provider of competent cells should be managed within the Supplier’s quality management system **or qualified in-house at the manufacturer using a risk based approach**.”

Response: Comment partially incorporated. The sentence was modified to, “Cell bank sourcing and generation should be managed within the Supplier’s quality management system and consistent with end users' quality management requirements.”

Comment Summary #124: The commenter suggested to add the word usually because an MCB may not be established in all applications. The text will be revised as follows, “Once the bacteria have been transformed with the initial plasmid DNA, an MCB of the transformed bacteria will **usually** be established.”

Response: Comment partially incorporated. The sentence was modified to “an MCB of the transformed microbe **may** be established.”

Comment Summary #125: The commenter requested definition and further explanation of how to differentiate from cell bacteria to master cell bacteria (e.g. additional detail regarding clonal selection etc.).

Response: Comment not incorporated. It is not within the scope of this General Chapter to discuss general molecular biology principles.

Comment Summary #126: The commenter suggested that it should be clarified that identity refers to both microbial (bacterial) identity and identity of the sequence.

Response: Comment incorporated. The sentence was modified to “should include tests for bacterial strain identity, plasmid identity, stability and purity.”
Comment Summary #127: The commenter suggested mentioning additional examples of when an MCB would not be created. The commenter also stated that it would be very helpful to assess the regulatory requirement of establishing a MCB. e.g., clarification if a MCB for a PCR based mRNA manufacturing process would be required if the IVT template is linearized and expanded by PCR requiring only small amounts of plasmid, compared to the current process of enzymatic linearization of large plasmid quantities.
Response: Comment not incorporated. The scope of the general chapter is the use of a plasmid as a starting material. In the example provided by the commenter on PCR-based mRNA manufacturing, the plasmid is not a starting material; therefore, it will not be included in the chapter.
Comment Summary #128: The commenter requested to add business risk as not creating a cell bank would be a business risk.
Response: Comment not incorporated. The chapter focuses on quality and not business-associated decisions.
Comment Summary #129: The commenter requested to remove the recommendation for microbial MCB stability as it is not a necessary practice and will add unnecessary cost to sponsors throughout industry. E. coli cell lines are well accepted as stable, and this is not common practice.
Response: Comment not incorporated. Master cell bank (MCB) stability is a common practice and, therefore, will not be removed from the chapter.
Comment Summary #130: The commenter recommended clarifying the testing of WCB as it seems inconsistent with the recommendation for the MCB testing.
Response: Comment partially incorporated. The statement about the WCB testing being less extensive than the MCB testing was removed from the chapter.
Comment Summary #131: The commenter suggested to clarify that the requirement of the stability test program that includes viability and plasmid retention are required for commercial supply only.
Response: Comment not incorporated. Stability testing is listed as optional in the general chapter, therefore, there is no need to specify that it is for commercial supply only.
Comment Summary #132: The commenter recommended the following edits, “To ensure that the MCB is suitable for manufacturing of the plasmid DNA throughout the product life cycle, the MCB ~~may~~ **should** be put on a stability testing program **that will include viability**.”
Response: Comment not incorporated. There may be situations where users will take on the business risk of not carrying a stability program until commercialization.
Comment Summary #133: The commenter suggested that the stability testing program should provide the type of program which deemed acceptable with examples such as genetic stability, sterility, how many cycles or how long the bacteria should be utilized.
Response: Comment not incorporated. Adding descriptions of the stability testing programs is too detailed and prescriptive for this General Chapter.
Comment Summary #134: The commenter suggested to remove plasmid retention as a requirement for MCB stability testing.
Response: Comment partially incorporated. The sentence was modified to “MCB stability testing may include viability and plasmid retention” to allow flexibility in the plasmid retention testing.
Comment Summary #135: The commenter requested clarification on the statement, “limited use of WCB”. The commenter recommended that the text be edited to state that the MCB will be used to refer to both MCB and WCB bacterial cell banks in the remainder of the chapter.
Response: Comment partially incorporated. The statement about the limited use of the WCB was removed from the text. The sentence was modified to, “MCB will be used to refer to both MCB and WCB microbial cell banks in the remainder of the chapter.”

Comment Summary #136: The commenter suggested the following edits, “Table 2 lists a panel of tests that ~~can~~ **should** be performed **as applicable** on the MCB to ensure quality and safety based upon an established framework of risk assessment to determine the appropriate tests.”
Response: Comment not incorporated. The EC finds the current wording of this statement to be sufficient.
Comment Summary #137: The commenter recommended to group all identity tests together in Table 2.
Response: Comment incorporated. Table 2 was edited to group the identity tests together.
Comment Summary #138: The commenter suggested to organize Table 2 with quality attributes grouped together.
Response: Comment incorporated.
Comment Summary #139: The commenter recommended to include purity tests in Table 2 because these tests are needed to demonstrate the cell bank is free of bacterial or fungal contaminants in addition to bacterial phages.
Response: Comment not incorporated. A microbial strain identity test that will detect contaminants is included in Table 2.
Comment Summary #140: The commenter suggested to include 16S ribosomal DNA and phage typing to identify the E.coli host strain and exclude other strains.
Response: Comment not incorporated. This general chapter has been written to include microbial strains, not specifically E. coli; therefore, assays specific to E. coli are not highlighted.
Comment Summary #141: The commenter recommended to group the methods in Table 2 as identity test for the microbial host and identity tests for the plasmid.
Response: Comment incorporated.
Comment Summary #142: The commenter suggested that all Identity attributes and all purity attributes should be grouped together in Table 2. The commenter also suggested that within a group the table should indicate hierarchy from most general to more specific testing.
Response: Comment partially incorporated. The tests were grouped together in the Table, but a hierarchy was not assigned because that may lead to subjective interpretation of priority.
Comment Summary #143: The commenter suggested to put “identity” in a more specific order where quality attributes are identified (microbial vs sequence identity or plasmid identity), like sequence identity.
Response: Comment incorporated.
Comment Summary #144: The commenter suggested to include “expression of the desired product” as an identity test to be in alignment with ICH Q5D 2.3.1.2.
Response: Comment not incorporated. The table includes a test for plasmid identity, which is the “expressed product.”
Comment Summary #145: The commenter suggested to make the following additions to the table: streak plate method for purity, nucleic acid fingerprinting and profiling for cell bank host identity and DNA sequencing for plasmid identity.
Response: Comment not incorporated. Table 2 is a non-exhaustive list of tests that allows for other tests that are not listed in the table to be included.
Comment Summary #146: The commenter suggested adding more specificity for the sequencing test method.
Response: Comment not incorporated. Adding specificity for the sequencing test method would limit flexibility in the type of sequencing that could be used and would be too specific for this general chapter.
Comment Summary #147: The commenter suggested that the test with selective media is only a purity test, and the desired host bacterial is identified as acceptance criteria.
Response: Comment not incorporated. Selective media is an identification test that uses the selective marker to identify the presence of the plasmid with the selectable marker in the organism.

Comment Summary #148: The commenter requested clarification if the “selective media” test method is meant to be for confirmation of antibiotic resistance.
Response: Comment partially incorporated. The text in the table was revised to “identifies organism containing the plasmid with the selectable marker” to clarify that the presence of the plasmid in the organism is identified.
Comment Summary #149: The commenter suggested to clarify that selective media does not only identify E.coli, but E. coli of interest (that contains the plasmid).
Response: Comment partially incorporated. The text in the table was revised to “identifies organism containing the plasmid with the selectable marker” to clarify that the presence of the plasmid in the organism is identified.
Comment Summary #150: The commenter suggested to remove MCB stability testing recommendation from the chapter, or if the MCB testing is included, have clarity and consistency of language.
Response: Comment not incorporated. The text in the chapter clearly describes that the MCB stability testing and the testing is optional.
Comment Summary #151: The commenter suggested to provide an alternative example that does not involve Ethidium Bromide Agarose Gel Electrophoresis.
Response: Comment partially incorporated. Ethidium Bromide and Agarose Gel Electrophoresis were replaced with sizing chromatography to allow for other options of testing.
Comment Summary #152: The commenter suggested to remove the abbreviation of Ethidium Bromide.
Response: Comment incorporated. Ethidium Bromide was removed from Table 2.
Comment Summary #153: The commenter suggested that there are other, safer and more sensitive dyes for DNA, so remove Ethidium Bromide to include the other dyes and change to DNA Agarose Gel Electrophoresis.
Response: Comment incorporated. Ethidium Bromide and Agarose Gel Electrophoresis were replaced with sizing chromatography to allow for other options of testing.
Comment Summary #154: The commenter recommended to remove the Identity testing by Ethidium Bromide-stained Agarose Gel Electrophoresis for size verses supercoiled marker because the plasmid DNA topology is tested for purified plasmid.
Response: Comment partially incorporated. Ethidium Bromide and Agarose Gel Electrophoresis were replaced with sizing chromatography.
Comment Summary #155: The commenter suggested the following edits to Table 2, “Exemplary Test Method” in the header of the second column; revise the header of the third column to “Example Acceptance Criteria”. The commenter also suggested to add a footnote, stating that “alternative methods and /or acceptance criteria could be established”
Response: Comment incorporated. Table 2, column 2 header has been changed to “Exemplary Test Method” and Table 2, column 3 heading was changed to “Example Acceptance Criteria”. A footnote was added to indicate that alternative methods and /or acceptance criteria could be established.
Comment Summary #156: The commenter suggested to add a footnote to the Ethidium Bromide that alternative staining methods may be used.
Response: Comment partially incorporated. Ethidium Bromide and Agarose Gel Electrophoresis were replaced with sizing chromatography.
Comment Summary #157: The commenter suggested to change the proposed acceptance criteria for Ethidium Bromide-stained Agarose Gel Electrophoresis to “Matches user-supplied material or (physical or in silico) reference pattern”
Response: Comment incorporated. The example acceptance criteria for the size exclusion chromatography (see previous comments for edits) were edited as suggested by the commenter.
Comment Summary #158: The commenter suggested changing “Identifies organism as E. coli” to “Identifies organism as gram negative rods”

Response: Comment not incorporated. The text and table were edited to replace “E. coli” with “microbial”; therefore, gram-negative rods is not suitable and has not been added to the table.
Comment Summary #159: The commenter suggested to delete E. coli because the selective media does not identify E. coli in the selective media Identity test.
Response: Comment incorporated. “E. coli” was deleted and replaced with “microbial.” The acceptance criteria were edited to state that the identity test with selective media identifies the organism containing the plasmid with the selective marker.
Comment Summary #160: The commenter suggested to delete the Identity test that was tested by Ethidium Bromide-stained Agarose Gel Electrophoresis and delete the acceptance criteria that the DNA co-migrates with reference DNA or size verses a supercoiled marker.
Response: Comment partially incorporated. Ethidium Bromide Agarose Gel Electrophoresis has been replaced with sizing chromatography.
Comment Summary #161: The commenter suggested to add text that the restriction digest could be designed to exclude contamination with other plasmids in the facility.
Response: Comment not incorporated. A restriction digest is not a good method to detect contamination because of the limit of detection.
Comment Summary #162: The commenter suggested to remove the footnote from the Identity test (Restriction digest plus Ethidium Bromide-stained Agarose Gel Electrophoresis) because Identity cannot change over time and therefore cannot be a stability indicating test.
Response: Comment not incorporated. Restriction digest can detect degradation and therefore could be stability-indicating.
Comment Summary #163: The commenter suggested to specify the type of DNA sequencing.
Response: Comment not incorporated. The type of sequencing should not be specified to allow flexibility in the type of sequencing used (e.g., Sanger or NGS).
Comment Summary #164: The commenter suggested to replace “sequence identity” with “plasmid sequence identity” in Table 2 as this will distinguish plasmid DNAs from bacterial chromosomal DNA.
Response: Comment incorporated. Plasmid was added to sequence the identity in Table 2.
Comment Summary #165: The commenter suggested revising the acceptance criteria for Sequence identity to “Identical to user-supplied sequence or (physical or in silico) reference material”.
Response: Comment incorporated.
Comment Summary #166: The commenter suggested that the Viability test should be called Viable Cell Count if the results are being reported as cfu/mL. The commenter suggested that the method name should be included rather than repeating the quality attribute as the method such as microbial enumeration.
Response: Comment incorporated. The quality attribute was renamed to viable cell count, and the exemplary test method was changed to microbial enumeration.
Comment Summary #167: The commenter suggested that a method be described in the test method column rather than repeating the quality attribute. The commenter suggested to change the test method to “growth in the presence of specific selectable markers”.
Response: Comment incorporated.
Comment Summary #168: The commenter suggested to provide a suggested method such qPCR or ddPCR for plasmid copy number rather than restate the quality attribute.
Response: Comment incorporated. PCR (which would include qPCR and ddPCR) was added to the example test method column.
Comment Summary #169: The commenter recommended removing the quality attribute “End of Production cells” from the Sequence Identity of Table 2.
Response: Comment not incorporated. The end of production cells can be tested as a stability indicating test. A footnote was added to clarify that the testing would be stability-indicating.
Comment Summary #170: The commenter requested more clarity be provided regarding EOP and the tests needed.

Response: Comment incorporated. Footnote *C* was added to clarify that EOP testing could be used as a stability indicating test.
Comment Summary #171: The commenter suggested to add the detail that "**The entire cassette (gene of Interest of plasmid and flanking regions) on purified circular plasmid is** identical to the user-supplied sequence of reference Material. The commenter also suggested to add that the "entire backbone is also sequenced as characterization".
Response: Comment not incorporated. Additional details about sequencing specific parts of the plasmid and to what extent is outside the scope of this general chapter.
Comment Summary #172: The commenter suggested to rename quality attribute to "Absence of bacteriophage" because the definition of quality attributes does not comply with the definition of purity. The commenter requested to define between elemental impurities and other impurities.
Response: Comment incorporated. The purity attribute has been revised to "absence of bacteriophage."
Comment Summary #173: The commenter suggested to consider specifying sequencing for identity is done on "the entire cassette (gene of Interest of plasmid and flanking regions) of purified circular plasmid" for greater clarity.
Response: Comment not incorporated. Specifying which regions need to be sequenced is too prescriptive for this general chapter.
Comment Summary #174: The commenter recommended to add Topology as new row in Table 2 (MCB test panel).
Response: Comment not incorporated. Table 2 refers to MCB testing and not plasmid release testing. Topology testing would be included in the plasmid release testing and not the MCB testing.
Comment Summary #175: The commenter suggested to consider adding Topology as Quality attribute on MCB, if deemed necessary based on process.
Response: Comment not incorporated. Table 2 refers to MCB testing and not plasmid release testing. Topology testing would be included in the plasmid release testing and not the MCB testing.
Comment Summary #176: The commenter suggested adding a footnote to the End of Production (EOP) Table 2 that will allow EOP testing on WCB prior to BLA filing, which is common practice for other biologic cell substrate starting materials.
Response: Comment not incorporated. Adding this text would describe a very specific situation and requirements of the regulatory authority, which are out of the scope of the chapter.
Comment Summary #177: The commenter requested more guidance on which analysis may be appropriate for EOP of a pre-starting material bank. The commenter requested clarity on whether the focus is only on genetic stability or if the focus is also on endogenous phage/virus sequences.
Response: Comment not incorporated. The commenter requested the addition of details that are too specific for the scope of the chapter.
Comment Summary #178: The commenter suggested to add a footnote: "EOP bank should be prepared from the bank used for routine production (MCB or WCB) and tested for genetic stability (e.g., gene copy number) prior to filing for marketing authorization."
Response: Comment not incorporated. The commenter requested the addition of details that are too specific for the scope of the chapter.
Comment Summary #179: The commenter suggested to add "MCB and WCB are generally prepared under full cGMP conditions because they may be used for commercial supply. When producing plasmid for phase I/II clinical trial supply, a pre-MCB can be acceptable if manufactured under the principles of GMP based on Quality Risk principles and tested for strain identity, phage contamination, and viability at minimum."
Response: Comment partially incorporated. The following text was added to the chapter, "MCB and WCB are generally prepared under full cGMP conditions because they may be used for Pivotal and commercial manufacturing. When producing plasmid for phase I/II clinical trial

supply, a cell bank which was not made under GMP conditions may be acceptable if manufactured under the principles of GMP based on Quality Risk principles."
Comment Summary #180: The commenter requested clarification if the quality attribute of identity sequencing in Table 2 is targeted for the bacterial cells or the plasmid contained in the bacterial cells.
Response: Comment incorporated. The quality attribute description was updated to "plasmid sequence identity" for clarification.
Comment Summary #181: The commenter suggested to remove the mention of E. coli in Table 2. E. coli can be replaced with the cell substrate used or something similar.
Response: Comment partially incorporated. "E. Coli" was replaced with "microbial strain."
Comment Summary #182: The commenter suggested adding a true viability test where the result would be percent viable.
Response: Comment not incorporated. There are many viability assays, and the user must determine which is most appropriate. Being prescriptive about the type of viability assay is out of the scope of the general chapter.
Comment Summary #183: The commenter suggested removing footnote d and just state, "establish per experience/vendor recommendation".
Response: Comment partially incorporated. The acceptance criteria were updated to "establish per experience/vendor recommendation." Footnote *d* was not removed because it is used in more instances in Table 2.
Comment Summary #184: The commenter requested to name the viability test method expected to be used.
Response: Comment not incorporated. There are many viability assays, and the user must determine which is most appropriate. Being prescriptive about the type of viability assay is out of the scope of the general chapter.
Comment Summary #185: The commenter requested to name the plasmid retention test method to be used.
Response: Comment not incorporated. The intent of the general chapter is to allow flexibility to use the most appropriate plasmid retention test and not be too prescriptive.
Comment Summary #186: The commenter suggested to replace the quality attribute "plasmid retention" with "purity" and replace the test method "plasmid retention" with "DNA homogeneity" in Table 2.
Response: Comment partially incorporated. Plasmid Retention and DNA homogeneity are not synonymous. DNA homogeneity is a population where the individuals have the same genetic constitution. Plasmid retention measures the percentage of colonies that retain the plasmid DNA. The plasmid retention method has been updated to "growth in the presence of specific selectable markers."
Comment Summary #187: The commenter requested clarification and explanation of methods verses readouts. For example, copy number is a readout, and the commenter would like clarification of the suggested method to measure the readout.
Response: Comment incorporated. The plasmid copy number was replaced with the method to measure the copy number, Polymerase Chain Reaction (PCR).
Comment Summary #188: The commenter requested to provide a method or a preferred method for analyzing the plasmid copy number.
Response: Comment incorporated. The plasmid copy number was replaced with the method to measure the copy number, Polymerase Chain Reaction (PCR).
Comment Summary #189: The commenter requested more clarity on the definition of plasmid retention and the quality attribute it would analyze. The commenter recognizes it is a potential critical quality attribute that may be considered for testing. The commenter suggested to keep the plasmid retention test as optional but remove the plasmid retention acceptance criteria for the MCB as it has not been demonstrated that results of less than 80% will result in the manufacture of plasmid that is not of sufficient quality for downstream processing.

Response: Comment partially incorporated. The plasmid copy number was replaced with the method to measure the copy number, Polymerase Chain Reaction (PCR). The acceptance criteria were updated to remove the greater than 80% requirement and was replaced with, "establish per experience/vendor recommendation."

Comment Summary #190: The commenter requested to remove plasmid copy number from the recommended testing panel because plasmid copy numbers should not be required for the characterization of a non-stable transformed microbial cell bank for starting material production as these tests are not effective indicators for quality and stability. The commenter also noted that, a distinction should be made between cell bank requirements for plasmids used as starting materials versus for human use.

Response: Comment not incorporated. Table 2 is a list of suggested tests, and it is up to the user to determine which tests to perform on the MCB to ensure quality and safety. There are examples where testing the plasmid copy number has been requested by regulatory authorities. In Table 2, a distinction does not need to be made for the plasmid requirements and plasmids for human use because the human use is out of the scope of the chapter.

Comment Summary #191: The commenter recommended to remove Plasmid Copy Number from Table 2 because the value obtained is subjective and is unlikely to be a critical quality attribute.

Response: Comment not incorporated. Table 2 lists examples of tests that can be performed on the MCB and are not mandatory.

Comment Summary #192: The commenter suggested to add USP chapters as references <1104>, <621>, <736>, <853>, <857>, <1053>, <1056>, <1065>, <1103>, <1104>, <1125>, <1126>, <1127>, <1129>, <1132> and <1736>).

Response: Comment partially incorporated. The above 1000 chapters are too general to be useful references. USP <621> *Chromatography* was added to Table 2 as a reference for chromatographic separation. Chapters <736>, <853>, and <857> are not relevant.

Comment Summary #193: The commenter requested to remove plasmid retention from the recommended testing panel. Plasmid retention should not be required for the characterization of a non-stable transformed microbial cell bank for starting material production as these tests are not effective indicators for quality and stability. The commenter also requested that a distinction should be made between cell bank requirements for plasmids used as starting materials versus for human use.

Response: Comment not incorporated. Table 2 lists examples of the tests that can be performed on the MCB and are not mandatory. The plasmids for human use are out of the scope of this chapter.

Comment Summary #194: The commenter recommended removal of Plasmid Copy Number in recommended tests. The commenter requested clarification of what is meant by EOP cells, what testing is expected and against what outcomes of concern (e.g. genetic instability of inserted pDNA).

Response: Comment not incorporated. Table 2 is an example list of assays that can be performed on the MCB to ensure quality and safety. EOP testing can be done to check for contamination and verify the identity of the microbial cells.

Comment Summary #195: The commenter requested to remove the End of Production cell testing or allow for more flexibility on at scale evaluation.

Response: Comment not incorporated. Table 2 is an example list of assays that can be performed on the MCB to ensure quality and safety. EOP testing should be included in this list as the EOP can be tested to detect contamination and to verify the identity of the microbial cells.

Comment Summary #196: The commenter suggested to remove the end of production cells from table 2 and include a paragraph after the table. This statement would be included in the paragraph, "End of Production Cells (EOP) should have appropriate release tests repeated as determined by the specific test panel."

Response: Comment not incorporated. Table 2 is an example list of assays that can be performed on the MCB to ensure quality and safety. EOP testing should be included in this list as the EOP can be tested to detect contamination and to verify the identity of the microbial cells.
Comment Summary #197: The commenter suggested the following edits, "[…] may preclude the creation and release of a MCB prior to using the cells for manufacture **or only one DNA batch, e.g. for an individualized product, is needed and a MCB not feasible; […]".**
Response: Comment incorporated.
Comment Summary #198: The commenter suggested to consider adding plasmid DNA topology as a quality attribute for bacterial MCB (Master Cell Bank) for information purpose only.
Response: Comment not incorporated. Plasmid topology is not a quality attribute for an MCB.
Comment Summary #199: The commenter suggested to consider adding recommendations for stability testing of MCB and/or WCB as there is limited information from current guidance.
Response: Comment not incorporated. The stability-indicating assays are included in the footnote in Table 2.
Comment Summary #200: The commenter suggested the following revision for clarification: "... therefore, the quality of the cell bank is assessed by testing the final **plasmid** material."
Response: Comment incorporated.
Comment Summary #201: The commenter requested clarification on under what conditions is an MCB not created? The commenter indicated that business or financial should not be part of consideration for making an MCB. The commenter requested examples of when an MCB would not be created (e.g., personalized medicine).
Response: Comment partially incorporated. The text was updated to include examples of when an MCB is not created or released before using the cells for manufacturing.
Comment Summary #202: The commenter recommended to either clarify this paragraph or remove it since it is already stated that an MCB may not be created. ""Establishing an MCB may not be needed for all plasmid products. In some cases, the speed at which plasmid DNA must be produced, from the time the sequence is established, may preclude the creation and release of a MCB prior to using the cells for manufacture; therefore, the quality of the cell bank is assessed by testing the final material. A small bacterial cell bank can be maintained so that it can be examined as part of an investigation of a failed production run."
Response: Comment partially incorporated. For clarification, the text has been updated to include examples of when an MCB is not created or released before using the cells for manufacturing.
Comment Summary #203: The commenter suggested that MCB should be in compliance with GMP for Phase I because the MCB and plasmid will eventually require GMP plasmid control later in the development process.
Response: Comment not incorporated. The chapter is not intended to be this prescriptive. It is up to the user to define their filing strategy.
Comment Summary #204: The commenter recommended providing additional clarification around the sentence; "Establishing an MCB may not be needed for all plasmids: in some cases, the speed at which plasmid DNA must be produced, from time of sequence established, may preclude the creation of MCB prior to use of the cells in manufacturing…"
Response: Comment partially incorporated. For clarification, the text was updated to include examples of when an MCB is not created or released before using the cells for manufacturing.

3.2 Facility Design and Cross Contamination Controls
Comment Summary #205: The commenter requested clarification when discussing facility design if this is manufacturing production facility design or other aspects of the process.
Response: Comment not incorporated. This chapter states that plasmid DNA was used as starting material for cell and gene therapies. The facility would be the plasmid production facility.

Comment Summary #206: The commenter suggested to add text to the section to elaborate on Phage contamination control and supply planning suggestions.
Response: Comment partially incorporated. A statement regarding potential phage contamination was included; however, further details were not included as they were beyond the level of detail of this general chapter.
Comment Summary #207: The commenter suggested to divide the section into two sections: Facility Design and Equipment requirements.
Response: Comment not incorporated. The equipment is part of the facility design; therefore, there is no need to have a separate section.
Comment Summary #208: The commenter requested consistent terminology.
for intermediate GMP status for Phase I/II (non-pivotal) plasmid, with a preference for "GMP principles" because it is already in use.
Response: Comment not incorporated. The term "GMP principles" was introduced in Section 4.1, but in this section, GMP compliance is more appropriate.
Comment Summary #209: The commenter suggested the following edits, "In general, for facility design, consideration should be provided to…. and cleaning. ~~as well as to prevent contamination and cross-contamination by foreign DNA.~~ (moved down to new sentence) … For clinical or commercial use, the level of GMP compliance should be phase appropriate. **Not all GMP requirements intended for drug substances and drug products are required for plasmid starting materials; however, it is mandatory that the principles of GMP are complied with according to Quality risk principles. The relevant aspects of GMP for starting materials must be established based on the potential for impact to the final product considering the risk of contamination and cross-contamination with foreign DNA."**
Response: Comment partially incorporated. The suggested text was added to the chapter, but the statement about preventing contamination and cross contamination was not removed. This statement was expanded on by adding phage contamination and antibiotic contamination in response to other comments received. This statement included important information that should be included in the chapter.
Comment Summary #210: The commenter suggested the following edits: "[…], the level of GMP **/quality** requirements should be phase appropriate."
Response: Comment partially incorporated. Quality management was added to the text instead of quality requirements.
Comment Summary #211: commenter requested clarification on what level of written / documented evidence should be available to meet authorities' expectations on application of GMP principles.
Response: Comment not incorporated. Describing regulatory requirements that the user should define in the strategy to respond to regulatory feedback is not included in the purpose and scope of the general chapter.
Comment Summary #212: The commenter noted that cleaning verification is generally accepted if validation not yet completed. The commenter suggested to change the text to "validated or verified" cleaning procedures".
Response: Comment partially incorporated. The sentence was edited to "validated or under validation." Verified was not used because it is not well defined.
Comment Summary #213: The commenter suggested the changes to the title of Section 3.2 to "3.2 Facility Design **and Cross Contamination Controls".** The commenter suggested to revise other text as follows: "Processing one specific plasmid at a time, **room segregation, and equipment segregation** should be evaluated, as well as validated **or verified** cleaning procedures to address change over between products and/or unique plasmids." And "Cleaning and change over procedures should be established and validated **or verified** for any equipment that is used between production of different plasmids."

Response: Comment partially incorporated. The section title was edited as suggested. The wording was edited to “validated or under validation.” Verified was not used because it is not well defined. The wording of this sentence “Cleaning and change over procedures should be established and validated” was not changed because “established and validated” includes processes under validation.
Comment Summary #214: The commenter suggested moving the text, “materials that are introduced should be released” to Section 4.1
Response: Comment not incorporated. The raw material control strategy is part of facility management, which is why it is referenced in Section 3.2.
Comment Summary #215: The commenter suggested to add a recommendation to risk assess potential cross-contaminants and impact to user facility to determine if any additional testing is required (i.e. beta lactams in a multi-product facility).
Response: Comment incorporated. Potential cross contamination by antibiotics was added to the section.
Comment Summary #216: The commenter suggested to delete, “(Unidirectional flow is preferred)”
Response: Comment not incorporated. Unidirectional flow is described as preferred, which provides flexibility.
Comment Summary #217: The commenter recommended the following edits: “Commissioning, qualification, and certification of heating, ventilation, and air conditioning (HVAC) systems and high efficiency particulate air (HEPA) filtration should be designed **and documented** to meet appropriate clean room classifications, with routine environmental monitoring of the facility and production process. **Biosafety levels and other activities in the cleanroom need to be considered to determine what is appropriate.”**
Response: Comment partially incorporated. The phrase “and documented” was added. Biosafety levels are mentioned in Section 4.1; therefore, it does not need to be discussed in this section.
Comment Summary #218: The commenter recommended the following text, “There are several areas of focus in facility design to reduce the risk of cross-contamination when operating in a multiproduct facility. Equipment selection and the utilization of single use equipment should be implemented whenever possible to reduce the risk of cross-contamination. It is recommended that a risk assessment be conducted as facilities are designed to identify areas of potential risk and how design and engineering controls should be implemented. “**A multiproduct facility should be justified and detailed how separation will be accomplished to avoid cross-contamination.** Processing one specific plasmid at a time, room segregation, and equipment segregation as well as validated cleaning procedures will address change over between products and/or unique plasmids. **Segregation should be designed or considered at every point to prevent cross-contamination.”**
Response: Comment not incorporated. The text would be redundant with other statements in the chapter about preventing cross-contamination. Justification in a multiproduct facility would occur during an audit but does not need to be included in the chapter as it is outside the scope of the general chapter.
Comment Summary #219: The commenter suggested that the second paragraph should start with facility—to the room---then materials (logical thought process) “Cleaning and change over procedures should be established and validated for **all single use versus shared equipment** that is used between production of different plasmids as well as cleaning of the facilities. The shared equipment should not be in direct contact with the final product to minimize the risk of cross-contamination.
For classified cleanrooms, cleaning efficacy studies should demonstrate that cleaning agents are effective on all materials used in that area of the facility and representative isolates identified through environmental monitoring.”

Response: Comment not incorporated. The Expert Committee found the text is clear as written, and the suggested edits would not add clarification.
Comment Summary #220: The commenter suggested the following edits, “Equipment selection and utilization of single use equipment ~~should~~ **could** be implemented whenever possible.”
Response: Comment not incorporated. The use of single-use equipment is recommended when possible, and “should” specifies this recommendation.
Comment Summary #221: The commenter suggested the following edits, “Cleaning and changeover procedures should be established and validated for any equipment that is used between production of different plasmids. ~~as well as cleaning of the facilities”~~ ...
Response: Comment incorporated.
Comment Summary #222: The commenter suggested to remove this sentence “Materials that are introduced should be released and tested per the procedures for raw material testing and if animal sources are necessary, efforts should be made to minimize the risk of transmission of transmissible spongiform encephalopathies (TSEs), including not obtaining materials from specific geographic regions.” from this section because TSE should not be in this area - it should be moved to the next section of “Quality Management.
Response: Comment partially incorporated. The statement was removed from section 3.2 Facility Design and was moved to the Quality Management section.
Comment Summary #223: The commenter recommended moving this statement to section 4.1 Phase Appropriate Quality Systems and Facilities. ”... Raw material and Supplier management-research material may have only the basic raw material and Supplier management, whereas clinical and CGMP-compliant material would have ~~full~~ **phase appropriate** raw material and Supplier management oversight by quality assurance...**Materials that are introduced should be released and tested per the procedures for raw material testing and if animal sources are necessary, efforts should be made to minimize the risk of transmission of transmissible spongiform encephalopathies {TSEs), including not obtaining materials from specific geographic regions.”**
Response: Comment partially incorporated. The statement was moved to the Quality Management section but not in the specific place recommended.

4. Quality Management
Comment Summary #224: The commenter recommended to provide guidance on the media in which plasmids are stored (e.g., in glycerol). Include that storage conditions should be qualified for the intended solution.
Response: Comment partially incorporated. A statement that storage conditions should be studied was added to the Stability section. Providing options for media used to store plasmids is too detailed for this general chapter.
Comment Summary #225: The commenter suggested the text “Risk management is a systematic process for the assessment, control, communication, and review of risk. Quality oversight of risk management should be established and continually maintained to ensure company procedures match regulatory commitments and industry guidance.” to be the first sentence of this section – setting the tone for the remainder of this part. After the statement, cite ICH Q10: Guidance for Industry- Pharmaceutical Quality System in describing the specific quality system and management responsibilities for the lifecycle of the product. This section should then build out what should be then developed by the plasmid supplier.
Response: Comment incorporated. Text was moved to the beginning of the section as suggested by the commenter. A reference to ICH Q10 was added.
Comment Summary #226: The commenter recommended the following edits, “Plasmid **and MCB** Supplier qualifications should be part of the User’s quality risk management system like any other Supplier.”

Response: Comment partially incorporated. The sentence was re-worded for clarification with the incorporation of MCB, as suggested by the commenter.
Comment Summary #227: The commenter recommended to remove most of the text and add citations to guidance. The commenter recommends retaining Figure 2.
Response: Comment not incorporated. The chapter is intended to provide guidance on plasmids as a starting material and provide additional information that is not found in the guidances.
Comment Summary #228: The commenter suggested the following edits: "The Supplier should be ~~audited~~ **evaluated** and have ~~their~~ **its** quality systems routinely reviewed in accordance with ICH Q9 (quality risk management)." The edit is suggested because qualification methods other than audits should be valid and are used in industry.
Response: Comment incorporated.
Comment Summary #229: The commenter noted that the Supplier Audit process needs to have a clear connection process to quality risk management process. The commenter suggested that audits be addressed further down in this paragraph. The commenter also suggested that this section have a general introduction to the type of quality management that is expected.
Response: Comment partially incorporated. Text was moved to introduce quality risk management first in the section. The word "audit" was removed and replaced with evaluation as suggested by another commenter.
Comment Summary #230: The commenter requested clarification on which the appropriate quality control and documentation to ensure that a material is not considered research grade when using non-GMP plasmid in Phase I.
Response: Comment not incorporated. Appropriate documentation is part of the regulatory process and quality management system of the user and thus cannot be defined in this chapter.
Comment Summary #231: The commenter suggested that other EU documents be cited, unless they are not because USP is U.S. based.
Response: Comment not incorporated. Appropriate documentation has already been cited in the chapter.
Comment Summary #232: The commenter suggested highlighting that non-GMP plasmid can be made from a cGMP MCB for Phase I studies with appropriate risk-based CMC controls.
Response: Comment incorporated. This sentence was added to Section 3.1 *Master Cell Bank*, "When producing plasmid for phase I/II clinical trial supply, a non-GMP cell bank can be acceptable if manufactured under the principles of GMP based on Quality Risk principles."

4.1 Phase Appropriate Quality Systems and Facilities
Comment Summary #233: The commenter suggested to add a table summary to section 4.1 to make it easier to read.
Response: Comment partially incorporated. The bulleted section of 4.1 was further bulleted with sub-bullets to increase readability.
Comment Summary #234: The commenter recommended to remove the non-GMP plasmid from Figure 2. The commenter also recommended to remove the "least conservative" and "most conservative" models because GMP sourcing should be considered a baseline requirement, and non-GMP sourcing would only be acceptable if the sponsor can justify the use of the material with appropriate risk management and regulatory strategy. The commenter requested clarification of phase-appropriate documentation packages.
Response: Comment partially incorporated. The use of GMP from the beginning of the development process is not required. Figure was revised to remove the term, "non-GMP plasmid" and to remove the "least conservative and most conservative" terms. Figure 2 was also edited to define the use of GMP plasmid for a pivotal trial. The appropriate documentation packages would be specific to the company filing for regulatory approval and the regulatory agency; therefore, this information was not added to the chapter.

Comment Summary #235: The commenter recommended the following edits, “Users may seek **cell** materials that are ~~made~~ ~~with~~ **manufactured under** the appropriate quality system ~~and facility~~ in a facility to enable consistent production and ensure that patient safety and product efficacy are never compromised throughout development.” **This will provide assurance that patient safety and product efficacy are never compromised throughout development.”**
Response: Comment not incorporated. The EC found that the proposed changes did not enhance the understanding of this section of the General Chapter.
Comment Summary #236: The commenter recommended to edit the text, “Taken together, these references and principles provide users with guidelines and principles ~~for manufacturing safe and efficacious material.~~ **on what is required for pre-clinical, clinical and commercial phases for manufacturing safe and efficacious material.”**
Response: Comment not incorporated. The suggested edits were not included because the edits would introduce regulatory language for the clinical phase requirements into the chapter. The purpose of the chapter is to describe general considerations for the manufacture and release of plasmids for use as starting materials and not a regulatory guidance.
Comment Summary #237: The commenter suggested to reference PDA book “Phase Appropriate GMP for Biological Processes”.
Response: Comment not incorporated. Reference to the PDA book is already included in the chapter.
Comment Summary #238: The commenter noted that a statement from FDA in a CMC Town Hall meeting that was held on September 22, 2022 said, “US regulations do not require that the level of detail for these materials is the same for drug substance" - only to qualify banks.” The commenter requested an example be provided that would show why the most-conservative approach in Figure 2 would be taken.
Response: Comment partially incorporated. The decision to use the most conservative path is a sponsor decision. Figure 2 was edited to remove the most conservative option because the purpose of this section is to discuss phase appropriateness.
Comment Summary #239: The commenter suggested to update Fig 2 to algin to text, whether the GMP (Research grade) plasmid should extend into Phase 1 or not. The commenter suggested to align terminology for intermediate level GMP for Phase I/II plasmid between figure and text (e.g. GMP Principles (phase appropriate)).
Response: Comment incorporated. The figure text was changed to provide additional clarity on plasmids that will enter phase I. These edits aligned the text in the figure with the text in the chapter.
Comment Summary #240: The commenter requested more information about the minimum requirements for “non-GMP” plasmid material quality systems and requested clarification if all of the GMP pieces are required to get the products to Phase III.
Response: Comment partially incorporated. Figure 2 was revised to clarify when GMP would be required (i.e., pivotal). The text in the chapter describes the minimal requirements for the use of a “non-GMP” plasmid material.
Comment Summary #241: The commenter suggested to include details of when to start working from a bacterial MCB in the least conservative and most conservative scenarios within Figure 2.
Response: Comment not incorporated. The level of detail of when to start working from a Master Cell Bank is not included in Figure 2.
Comment Summary #242: The commenter suggested to replace text within this paragraph to be directional in terms of what are the minimal expectations for each phase of development.
Response: Comment not incorporated. The minimal expectations for each phase are determined by a user risk assessment process. Providing more details about the user’s risk assessment process is out of the scope of this General Chapter.
Comment Summary #243: The commenter recommended to align to EMA terminology “GMP Principles” for this intermediate level of GMP allowed for early phase plasmids,

EMA/246400/2021. The commenter noted that a BSC hood use is good practice and useful guidance for clinical plasmid manufacturers who are potentially working in non-classified area. The following edits are suggested, “Plasmids intended for use in early phase I/II clinical trials are typically produced in a biosafety level 1 laboratory that is commissioned and operated under GMP ~~conditions~~ **principles using risk-based and phase-appropriate controls**. **Open culturing step should be protected within a unidirectional flow hood in all phases.”**
Response: Comment partially incorporated. The GMP principles were added to the sentence to better align with the EMA terminology; however, the use of the unidirectional flow hood was not added because it was too specific.
Comment Summary #244: The commenter recommended to focus on Pivotal trials as the milestone to achieve full cGMP because clinical programs may vary. The commenter indicated that Plasmid DNA starting material used in pivotal ~~Phase III~~ clinical trials should be made in a full cGMP facility.
Response: Comment incorporated. Figure 2 was edited to include Pivotal instead of Phase III clinical trials, and that Plasmid DNA starting materials used in pivotal trials should be made in a full CGMP facility.
Comment Summary #245: The commenter suggested to revise Figure 2 for "least conservative" pathway. If phased-separation is desired, suggest "pre-pivotal" and "pivotal" to distinguish registrational trials. The commenter requested clarification of bullet point 1 that indicates that clinical and CGMP compliant material would have full raw material and supplier management by QA.
Response: Comment partially incorporated. Figure 2 was revised to have Late Stage and Pivotal, Early Stage and Pre-clinical. The bullet point entitled, Raw material and Supplier management was edited for clarification: “*Raw material and Supplier management*—research or preclinical material may have only ~~the~~ basic raw material and Supplier management, whereas clinical phase appropriate and commercial phase ~~and CGMP-compliant~~ material would have full raw material and Supplier management oversight ~~by quality assurance~~ managed within the plasmid Supplier’s quality management system (QMS).”
Comment Summary #246: The commenter suggested to use the language, “non-GMP plasmid used for pre-clinical studies” and “principles of GMP are adopted as relevant. **Response:** Comment not incorporated. The text defines research grade as plasmids not intended for clinical use; therefore, it is clear how this term agrees with the revised Figure 2.
Comment Summary #247: The commenter requested clarification within this chapter whether plasmid manufactured under non-GMP with GMP principles applied can be used at Phase 3 and commercial phases.
Response: Comment partially incorporated. Figure 2 has been revised, and non-GMP plasmid can be used in clinical (which could contain Phase 3) but not in the late stage or pivotal phase 3. It should be noted that the requirements for pivotal need to match commercial.
Comment Summary #248: The commenter recommended that USP <1040> be aligned with the EMA guidance to support a more harmonized global approach to the management of the quality considerations for plasmid DNA as a starting material. A risk-based approach that incorporates end-user requirements, should determine whether there is a need to implement “full” cGMP, and the associated requirements related to cleanroom classifications, separate material airlocks (MALs) and personal air locks (PALs), aseptic processing, validated cleaning and changeover procedures, environmental monitoring etc. for late stage clinical (phase III trials) and commercial use.
Response: Comment not incorporated. This General Chapter provides guidance on best practices. It is recommended to use plasmids that are CGMP-compliant for pivotal and commercial manufacturing. The chapter describes best practices, but decisions would need to be managed under the company’s quality management system.
Comment Summary #249: The commenter recognizes these controls as a value adding parts of microbial control and cross-contamination mitigation strategies but does not believe they

need to be mandated to generate plasmid of suitable quality as it is well accepted low bioburden levels can be addressed as part of onward drug substance manufacturing operations.
Response: Comment not incorporated. Best practices for pivotal use the same plasmid manufacturing process that would be used for commercial purpose, which is why it is suggested in the General Chapter to use CGMP. The user's quality management system and risk assessment would have to be leveraged if pursuing phase-appropriate plasmid use in pivotal (other considerations include comparability).
Comment Summary #250: The commenter requested to consider if chapter <1116> would also apply.
Response: Comment not incorporated. USP chapter <1116> Microbiological Control and Monitoring of Aseptic Processing Environments has already been referenced in section 4.1.
Comment Summary #251: The commenter requested clarification if veterinary medicinal products are part of the scope of this document.
Response: Comment not incorporated. Veterinary medicinal products are a part of the scope of this chapter; therefore, no change to the text was needed.
Comment Summary #252: The commenter recommended to add a reference after this text, "It should be noted that veterinary medicinal products regulated by FDA do not go through a phased clinical trial process. The plasmids used in clinical studies to support target animal safety and effectiveness should follow the recommendations for plasmids used in phase III clinical trials of human drug products".
Response: Comment not incorporated. The Center for Veterinary Medicine of FDA does not have a guidance that could be referenced here.
Comment Summary #253: The commenter recommended to use consistent terminology with EMA to describe GMP principles.
Response: Comment not incorporated. Because terms used to describe different levels of GMP compliance are not applied consistently among plasmid manufacturers, it is not possible to harmonize or define the terms at this time. This general chapter has attempted to capture the terms used so that users can apply the terms to their application.
Comment Summary #254: The commenter suggested moving these sentences "Equipment used in the space is qualified for GMP use per facility procedures and regulatory expectations. Temperature-controlled units and process equipment are monitored, usually by a validated control system for control and recording any discrepancies related to manufacturing operations. Unit operations are typically defined and segregated appropriately to avoid any potential cross-contamination between products and processes. Segregation of unit operations is generally by upstream and downstream manufacturing operations." to Section 3.2 because they are more relevant to facility design. The commenter suggested defining which phase the equipment applies to for example applying GMP to equipment.
Response: Comment partially incorporated. The first two sentences are related to GMP manufacturing and should remain in section 4.2; however, a reference to section 3.2 was added. The last sentence was revised to specify plasmid manufacturing under the GMP conditions: "**For plasmid manufactured under GMP**, s~~S~~egregation of unit operations is generally by upstream and downstream manufacturing operations."
Comment Summary #255: The commenter suggested that it would be beneficial to create a matrix (table) based on topic and phase and what is required for each.
Response: Comment not incorporated. The lower part of Figure 2 illustrates this information, and it does not need to be additionally included in a table.
Comment Summary #256: The commenter recommended the following edits, "Plasmid DNA ~~starting material~~ used in phase III clinical trials ~~should be made~~ are made in full cGMP facilities and support transition to commercial manufacturing."
Response: Comment partially incorporated. The sentence was revised to "Plasmid DNA starting material used in **pivotal clinical trials** should be manufactured under ~~made in a~~ full CGMP facility, and certain clinical trial host countries require CGMP." The wording "starting

material" was retained in the sentence because that is the scope of the chapter. "Should be" was kept in the sentence because it still allows the option for people to use phase-appropriate plasmids with QMS risk assessment. Phase III was changed to pivotal to align with revisions that have been made to Figure 2.

Comment Summary #257: The commenter suggested to add guidance that phase appropriate GMPs should be based on potential impact to final product; aligns with EMA/246400/2021. The commenter suggested to consider adding the following text, "**Not all GMP requirements intended for drug substances and drug products are required for plasmid starting materials; however, it is mandatory that the principles of GMP are complied with according to Quality risk principles. The relevant GMP principles for starting materials must be established based on the potential for impact to the final product considering the risk of contamination and cross contamination with foreign DNA."** The commenter suggested to align terminology for intermediate level GMP for Phase I/II plasmid (e.g., GMP Principles).

Response: Comment not incorporated. Reference to the EMA guidance is already included in this section of the General Chapter. The suggested text was not included because GMP standards need to be followed independent of product stage (DS/DP). For this statement, the GMP references can be used (which are provided in the General Chapter). Since this is above 1000 chapter, the intention was to not be too prescriptive, and the terminology is aligned in the figure and the text.

Comment Summary #258: The commenter suggested to add a statement that the pivotal trial is the milestone to achieve full cGMP for plasmid starting material and update Figure 2 to reflect this point.

Response: Comment incorporated. Figure 2 and the text in the General Chapter were updated to state that only the GMP plasmids will be used in pivotal clinical trials.

Comment Summary #259: The commenter suggested to add, "Open culturing step should be protected within a unidirectional flow hood in all phases."

Response: Comment not incorporated. The use of phase-appropriate documentation and processes would include the use of a unidirectional flow hood to prevent contamination. The use of unidirectional flow hood is common practice and does not need to be called out in this General Chapter.

Comment Summary #260: The commenter suggested to delete "with late phase cGMP would use validated assays" and instead define the expectation for qualification, demonstrating adequate method performance including parameters such as accuracy, linearity, precision, as appropriate.

Response: Comment partially incorporated. Methods used in pivotal should be validated. The sentence was revised to provide additional clarity: "Clinical and CGMP-compliant materials would use qualified assays, with **pivotal** late phase CGMP **release** using validated assays."

Comment Summary #261: The commenter suggested for the entire section 4.1 to align starting material requirements mentioned in this chapter with the EMA ATMP GMP guide, i.e. GMP principles to apply for starting materials/ risk-based approach based on type of material).

Response: Comment not incorporated. This General Chapter is aligned with the EMA ATMP GMP guide.

Comment Summary #262: The commenter requested to revise this statement, "The EMA guidance document (10) provides additional information on phase appropriate manufacturing of starting materials." The commenter suggested this revision, "The EMA guidance document (10) provides additional information on ~~phase appropriate manufacturing of starting materials~~ **principles of GMP to be applied for biological starting materials used in ATMP manufacturing."**

Response: Comment not incorporated. Section 4.1 of this General Chapter is focused on phase-appropriate manufacturing of starting materials. The suggested edits would not provide clarification and could cause confusion.

Comment Summary #263: The commenter suggested to align wording or refer to EMA ATMP GMP guide for application of GMP principles for starting material manufacturing throughout development and commercialization.
Response: Comment partially incorporated. Additional clarity was added to the pivotal requirements for GMP production. Phase-appropriate, documented plasmid production covers the topics suggested by the commenter. The EMA ATMP GMP guide is mainly focused on cellular starting materials with minimal information on plasmid starting materials. Adding this reference would not add value to the chapter. The GMP guidance, EU GMP Guide Volume IV, is guidance for drug substance, and the drug product is out of the scope of the chapter.
Comment Summary #264: The commenter suggested the following edits to this sentence, " ~~However,~~ ~~p~~Plasmids used in commercial manufacturing, must follow CGMP."
Response: Comment partially incorporated. The sentence that the commenter suggested to edit was deleted, and the sentence before that was revised for clarification: "Plasmid DNA starting material used in ~~phase III~~ **pivotal** clinical trials **and in commercial manufacturing** ~~should~~ **must** be manufactured ~~in a~~ under full CGMPs."
Comment Summary #265: The commenter suggested to revise Phase 3 to 2 (or language as "pivotal" or "registrational") and modify lifecycle recommendations to remove flexibility of GMP source post-registrational trial. Also align PPQ with appropriate CGT phase (2, pivotal, registrational, whichever is the preferred language).
Response: Comment partially incorporated. The sentence was revised to use pivotal instead of Phase III, and the sentence, "However, plasmids used in commercial manufacturing, must follow CGMP" was deleted.
Comment Summary #266: The commenter suggested to add clarification around the considerations for plasmids used at different phases before the seven bullet points by reference the EMA Q&A on GMP principles for starting materials used to transfer genetic material for ATMPs.
Response: Comment partially incorporated. A reference to the EMA guidance was added to the text before the bullet points. This statement is present in the chapter: "In all cases, users are ultimately responsible for ensuring that the plasmids meet the necessary functional, quality, and documentation requirements expected by regulatory authorities." This sentence points out that the list is not exhaustive and that the user needs to determine the CGMP requirements to match their plasmid requirements.
Comment Summary #267: The commenter requested elaboration on the use of non-GMP materials that must be defended and rationalized.
Response: Comment not incorporated. The chapter is focused on guidance for use of plasmids, and the guidance includes the use of CGMPs in a phase-appropriate manner.
Comment Summary #268: The commenter suggests to align wording or refer to EMA ATMP GMP guide for application of GMP principles for starting material manufacturing throughout development and commercialization.
Response: Comment partially incorporated. The phrase "separation in time" was added to align with the EMA guidance.
Comment Summary #269: The commenter suggested to align wording or refer to EMA ATMP GMP guide for application of GMP principles for starting material manufacturing throughout development and commercialization because a distinction should not be made based on clinical trial stage but on what aspects of GMP are important to follow in order to manufacture starting material with expected quality.
Response: Comment partially incorporated. The wording has been changed to align with the EMA ATMP GMP guidance. The chapter provides flexibility for the use of CGMP throughout the process, if justified by the user. The premise of the chapter is that there should be a distinction based on clinical trial stage.
Comment Summary #270: The commenter requested clarification on this text. "Users are reminded that the FDA is the only source of policy on US pharmaceutical CGMPs. CGMP

requirements are found in statutes and regulations, and FDA's current thinking on these requirements is explained in guidance documents, including Questions and Answers on Current Good Manufacturing Practices-General Provisions (16)." Clarify if CGMP requirements as required to Drug Substance and Drug product apply entirely to starting materials or if a risk-based approach could be followed.
Response: Comment partially incorporated. A risk-based approach should be followed. The text was added to the chapter to clarify that risk assessment should be considered.
Comment Summary #271: The commenter recommended the following edits: "In all cases, ~~Users~~ **Drug Product manufacturers** are ultimately responsible for ensuring that the plasmids meet the necessary functional, quality, and documentation requirements expected by regulatory authorities."
Response: Comment not incorporated. The chapter defined users, and the term has been used throughout the chapter.
Comment Summary #272: The commenter suggested that "compliant" should be added to the "Some considerations for plasmids" section. Everything written above should be here and aligned with what has already been indicated. The commenter recommended to create a matrix table where each field appears with adjusted requirements per clinical phase: Raw material and Supplier management, segregated manufacturing, product contact materials, facilities, quality control assays, final fill and quality management oversight.
Response: Comment not incorporated. This is an informational general chapter numbered above 1000. The chapter provides suggestions and recommendations for each of the items in a phase-appropriate manner. It is up to the user to determine how to implement the considerations in specific phases of clinical trials.
Comment Summary #273: The commenter suggested edits to clarify the text. "In clinical or CGMP-compliant manufacturing, although columns and fermenters may be reused after thorough and validated cleaning. **Alternatively,** single-use technologies **can** ~~would~~ be used ~~widely~~ for clinical and CGMP-compliant plasmid DNA manufacturing."
Response: Comment incorporated.
Comment Summary #274: The commenter suggested to delete the sentence "Some considerations for plasmids used at different phases (for brevity, we will refer to them as non-CGMP-compliant, and CGMP-compliant) may include the following:" because it is not consistent with the bullet points below where research, clinical and CGMP material are mentioned. It is better to keep the distinction between the 3 "grades" instead of 2.
Response: Comment partially incorporated. Edits were made to the bulleted list and the sentence before the list to align on terminology for the grades.
Comment Summary #275: The commenter suggested that resins should be single use unless otherwise justified. Resins have large surface area and therefore it is hard to validate the cleaning process.
Response: Comment not incorporated. Resin single use is common and allowed in this chapter. However, re-use of resins can be done if the cleaning process is validated. The validation of the cleaning process is the justification.
Comment Summary #276: The commenter indicated that "Product contact materials - In clinical or CGMP-compliant manufacturing, although columns and fermenters may be reused after thorough and validated cleaning." is not a complete sentence and suggested combining with the previous sentence.
Response: Comment partially incorporated. The sentence was edited to ensure that it is a complete sentence. The second sentence was edited from a previous comment which improved the flow of the two sentences.
Comment Summary #277: The commenter suggested to move the sentence "Product contact materials - Single-use technologies would be used widely for clinical and CGMP-compliant plasmid DNA manufacturing." to the beginning of this Product contact materials section.
Response: Comment not incorporated. Edits based on other comments have been

incorporated in the sentence, which make the sentence a better fit in the current place in the paragraph.

Comment Summary #278: The commenter recommended to add a minimum requirement list to mitigate risk for raw materials used in clinical plasmid manufacture. The commenter suggested to add the text in bold. Raw material and supplier management - … clinical and CGMP-compliant **plasmid starting material** would have full raw materials and Supplier management oversight by quality assurance. **Clinical plasmid should be produced with the following controls on raw material, at minimum: COA, Statement of origin, and TSE/BSE, risk statement for animal derived material, Compendial grade, if available, Change notification from RM suppliers**

Response: Comment partially incorporated. Edits made from a previous comment included quality management system oversight to manage clinical phase appropriate and CGMP, which addresses the comment from this commenter.

Comment Summary #279: The commenter suggested the following edits: "Product contact materials - … In clinical or CGMP-compliant manufacturing, ~~although~~ columns and fermenters may be reused after thorough and validated **or verified** cleaning."

Response: Comment partially incorporated. The word "although" was removed from the sentence. The sentence was edited to state "validated or under validation." "Verified" was not used because it is not well defined.

Comment Summary #280: The commenter suggested the following edits, "Environmental monitoring should be performed and cleaning procedures for the equipment ~~and facility~~ should be validated. Pressure differentials and the use of airlocks should be employed with flows defined per classification of the space. ~~(unidirectional versus bidirectional flows)~~" ….

Response: Comment not incorporated. Making the suggested edits does not add clarification to the chapter. There are several references in this section of the chapter for equipment and facility monitoring.

Comment Summary #281: The commenter suggested to update the language to "validated or verified cleaning procedures.

Response: Comment partially incorporated. The text was edited as follows: "validated (or under validation) cleaning." Verified was not used because it is not well defined.

Comment Summary #282: The commenter requested clarification on this text from the General Chapter, "air handling and access control for research material may be limited. Significantly more control for clinical and CGMP-compliant plasmid manufacturing, air handling, personnel, and material flow, would be as expected and required by regulations." The commenter requested clarification on which regulations are applicable.

Response: Comment partially incorporated. References to the appropriate regulations were added, and the text was revised as follows: "*Facilities*—air handling and access control for research material may be limited. Significantly more control for clinical **phase appropriate** and CGMP-compliant plasmid manufacturing, air handling, personnel, and material flow, would be as expected and required by regulations **(12,13,14,15, 16).**"

Comment Summary #283: The commenter recommended to edit by switching the two sentences: "Significantly more control for clinical and CGMP-compliant plasmid manufacturing, air handling, personnel, and material flow, would be as expected and required by regulations. Air handling and access control for research material may be limited."

Response: Comment not incorporated. The chapter has been consistently starting with research grade and moving to more controlled environment, which follows the development process. Changing the order of the sentences would not match the flow of the rest of the chapter and would not provide additional clarity.

Comment Summary #284: The commenter requested to define and/or contrast qualified and validated assays. The commenter also requested to include reference to appropriate guidance documents or chapters addressing assay qualification and validation.

Response: Comment partially incorporated. Section reference was corrected to reference Section 5.1 Quality Attributes instead of section 4.1. Section 5.1 has many references to quality control assays. Qualified and validated assays are defined in Section 5.1 which is now referenced.
Comment Summary #285: T The commenter suggested to remove reference to Section 4.1 of this General Chapter (or refer to alternative reference such as ICH Q6 & Q9).
Response: Comment partially incorporated. The incorrect chapter reference of section 4.1 was corrected to cite section 5.1 of the chapter.
Comment Summary #286: The commenter suggested to change bioburden to sterility in this sentence "Clinical and CGMP-compliant plasmids will be filled in a controlled environment and tested at a minimum for ~~bioburden~~ **sterility**."
Response: Comment partially incorporated. The text indicates that the minimum testing to be performed is bioburden because certain clinical cell and gene therapy products do not require sterility-tested plasmid. A reference to section 5.1.13 was added for more information on bioburden and sterility testing.
Comment Summary #287: The commenter suggested the following revision to the statement, "Clinical and CGMP-compliant material will be overseen by the quality management ~~and regulatory~~ groups." The revision is proposed because oversight is done by quality groups only.
Response: Comment incorporated.
Comment Summary #288: The commenter suggested to move quality management oversight to the beginning of the section as a high-level statement.
Response: Comment partially incorporated. The quality management oversight bullet point was moved to the first bullet point in the list of some considerations of plasmids used at different phases.
Comment Summary #289: The commenter suggested the following edits because there could be different interpretations of what a "not 100% thorough batch record review" means.: "The actual activities for quality oversight of clinical material may be phase appropriate and ~~slightly limited, for example, less than 100% thorough batch record review."~~ concepts should be subject to a risk-based approach."
Response: Comment partially incorporated. The text was edited as follows: "The actual activities for quality oversight of clinical material may be phase appropriate. ~~and slightly limited, for example, less than 100% thorough~~ **As an example, batch record review concepts should be subject to a risk-based approach**."
Comment Summary #290: The commenter suggested to move this text, "Various aspects of the User's drug product development program will impact the quality of materials used at different phases, including proximity of the raw material to the patient and route of administration. Users should thoroughly analyze their drug product program requirements to determine the best options to choose." to the beginning of section 4.1 as a guide and clarify how it fits into the general concept of the section which is propagating a phase appropriate approach instead of a risk-based approach applicable for all phases.
Response: Comment not incorporated. This paragraph was deleted in response to another comment received.
Comment Summary #291: The commenter requested clarification on if there is a separate regulation for veterinary products that should be referenced or if veterinary products are included in the scope of the General Chapter as veterinary products are not listed in Table 1 as within the scope of the General Chapter.
Response: Comment not incorporated. CVM from FDA does not have regulatory guidance for plasmids used in veterinary medicines. The plasmids used for veterinary medicinal products are in scope of this chapter but do not need to be specifically called out in Table 1 because a different type of products are included in Table 1.
Comment Summary #292: The commenter suggested that more attributes should be listed according to the FDA guidance provided by the commenter.

Response: Comment not incorporated. The bullet point before the fill-finish bullet (Quality Control Assays) cites section 5.1, which lists multiple quality attributes that can be tested.
Comment Summary #293: The commenter recommended giving a different example than less than 100% thorough batch record review. Data integrity and good documentation practices are just as important in clinical as in commercial production, where batch record review should always be performed.
Response: Comment partially incorporated. ”Less than 100% batch record review” was removed and batch record review was used as an example, acknowledging that there are many other examples that could be used that are risk-based.
Comment Summary #294: The commenter suggested editing the sentence “In clinical or CGMP-compliant manufacturing, although columns and fermenters may be reused after thorough and validated cleaning” to make it a complete sentence.
Response: Comment incorporated. The sentence was revised to ensure it is a complete sentence.
Comment Summary #295: The commenter suggested to correct the typo and change “pote” to “potential”.
Response: Comment incorporated. The typo was corrected.
Comment Summary #296: The commenter suggested to delete this paragraph “Various aspects of the User’s drug product development program will impact the quality of materials used at different phases, including proximity of the raw material to the patient and route of administration. Users should thoroughly analyze their drug product program requirements to determine the best options to choose.” because it is not necessary to have this paragraph in the text.
Response: Comment incorporated. The paragraph was deleted.

5. DNA Starting Material Quality
Comment Summary #297: The commenter suggested to remove Section 5.0 and cross-referencing applicable guidance. The commenter also suggested to remove electron microscopy from Table 4.
Response: Comment not incorporated. This section provides information on testing and characterization of the plasmid DNA, which is useful for the users of the chapter. Electron microscopy was not removed from the table, but the title of the table and column headings were changed to clarify that characterization tests are also included in the table. **Comment Summary #298:** The commenter suggested to add “Poly(A) tail length as a quality attribute, if a poly(A) tail is encoded”.
Response: Comment incorporated. Poly A tail length was added to Table 3.
Comment Summary #299: The commenter suggested to explain that Table 4 is complementary to Table 3 for linear plasmid.
Response: Comment incorporated. The statement was added to clarify that Table 4 is complementary to Table 3 for linear plasmid.
Comment Summary #300: The commenter requested to add footnotes to attributes applicable depending on the intended application. The commenter requested more guidance be provided to inexperienced developers of ATMP by clarifying which quality attribute should be looked at depending on the intended application.
Response: Comment not incorporated. There are too many applications of plasmid DNA and the attributes tested may or may not be specific to each case. Adding specific attributes would be too specific for this chapter.
Comment Summary #301: The commenter suggested to add a sentence such as, “Manufacturing may either start with DNA plasmids that have been produced in bacteria and then linearized, or with linear DNA templates that have been produced enzymatically using PCR or other synthetic methods" (see WHO_TRS no.1039_Annex 3).” To differentiate between Table 3 and Table 4.

Response: Comment partially incorporated. Synthetic DNA is out of the scope of the chapter; therefore, the suggested revision is out of the scope of the chapter. The text has been added to the chapter to differentiate between Table 3 and Table 4.
Comment Summary #302: The commenter suggested to add the statement in bold: "Table 3 represents a comprehensive overview of all possible attributes that might be of interest depending on the application. The User should select a subset of these tests ****as release specification testing or as extended characterization testing**** or other procedures not listed as appropriate for the intended application. that extended characterization tests, in addition to release testing, should be considered as part of the overall control strategy.
Response: Comment partially incorporated. Text has been added to the Table titles and column titles to include characterization tests. Moreover, "all" and "comprehensive" were removed to allow for the development of new technologies.
Comment Summary #303: The commenter indicated that many of the suggested acceptance criteria in Table 3 are "End Use Specific" while values are provided in some cases. The commenter requested that more values be provided for the suggested acceptance criteria, and that more information and context as to why these quality attributes and acceptance criteria are important.
Response: Comment not incorporated. The values for the acceptance criteria would be nice to have but is not possible due to every situation being unique.
Comment Summary #304: The commenter suggested to consider also DNA purity (A_{260}/A_{280} ratio) and osmolality as quality attribute.
Response: Comment incorporated. A_{260}/A_{280} and Osmolality were added to Table 3.
Comment Summary #305: The commenter suggested to also include A_{260}/A_{280} method as a purity test.
Response: Comment incorporated. A_{260}/A_{280} was added to Table 3.
Comment Summary #306: The commenter suggested to change the heading of the second column of Table 3 to "Exemplary Analytical Procedures".
Response: Comment incorporated.
Comment Summary #307: The commenter suggested adding the reference Ph. Eur. 2.2.1 to the Appearance test to provide a reference for clarity.
Response: Comment incorporated.
Comment Summary #308: The commenter suggested to only include references to Ph.Eur. only for procedures that have been harmonized. The commenter indicated that the citation of Ph. Eur. and USP citations are not consistent in the table.
Response: Comment not incorporated. The references to Ph Eur. are helpful additional references for the user and will help users who also intend to release products in Europe.
Comment Summary #309: The commenter suggested to include particulates as part of appearance.
Response: Comment incorporated.
Comment Summary #310: The commenter recommended to remove attribute, including the references to chapters about particulates in this General Chapter because the scope is starting material, not injectable product.
Response: Comment partially incorporated. Particulates were not removed from Table 3 because Table 3 is a menu of tests that can be used but not required. It is up to the manufacturer to determine which tests are appropriate for their process. The references were removed except for Ph. Eur. 2.9.20 because these references referred to drug products that are out of the scope of the chapter.
Comment Summary #311: The commenter recommended to remove particulates as a quality attribute because particulates may not be a critical quality attribute if the material is filter sterilized as part of downstream processing.
Response: Comment not incorporated. Table 3 is a menu of tests that can be used but are not required. It is up to the manufacturer to determine which tests are appropriate for their process.

Comment Summary #312: The commenter recommended removing references to sub-visible particles such as Ph. Eur. 2.9.19. The commenter suggested adding a reference: Ph. Eur. 5.17.2 as a reference, since this is progress toward harmonization between USP and EP.
Response: Comment partially incorporated. The reference to Ph. Eur. 2.9.19 for sub visible particles has been removed. References to <787> and <788> are not required since sub-visible particles are not tested; therefore, they were also removed. The reference to Ph. Eur. 5.17.2 was added.
Comment Summary #313: The commenter suggested removing the reference to Ph. Eur 2.9.19 because it refers to sub-visible particulates. The reference to Ph. Eur 2.9.20 is sufficient.
Response: Comment incorporated.
Comment Summary #314: The commenter suggested to remove the reference to Ph. Eur. 2.9.19 in Table 3 because the reference is for sub-visible particles and is not relevant here.
Response: Comment incorporated.
Comment Summary #315: The commenter recommended removing visible particulates as a quality attribute because it is usually only applicable to drug substance and drug product.
Response: Comment not incorporated. Table 3 is a menu of tests that can be used but do not need to be used. It is up to the manufacturer to determine which tests are appropriate for their process. Testing for visible particulates is a best practice for the starting materials used in cell and gene therapy products; therefore, particulates should remain listed as test options in Table 3.
Comment Summary #316: The commenter suggested to remove the reference to Ph.Eur. 2.9.19 because it is about subvisible particles, but if reference is retained, then add in reference to USP<788> which is harmonized with 2.9.19. Also change the section title to Particulates or something to reflect that it is not just visible particulates.
Response: Comment partially incorporated. Sub-visible particulate testing and the associated references were removed.
Comment Summary #317: The commenter recommends removing Visible Particulates as a Quality Attribute for this General Chapter because the scope is starting material, not injectable product.
Response: Comment not incorporated. Table 3 is a menu of tests that can be used, but do not need to be used. It is up to the manufacturer to determine which tests are appropriate for their process. Testing for visible particulates is a best practice for the starting materials used in cell and gene therapy products; therefore, particulates should remain listed as test options in Table 3.
Comment Summary #318: The commenter suggested to remove the quality attribute “Visible Particulates” from the suggested testing in Table 3 because the visible particulates is not a quality attribute for DNA plasmids used as starting material.
Response: Comment not incorporated. Table 3 is a menu of tests that can be used, but do not need to be used. It is up to the manufacturer to determine which tests are appropriate for their process. Testing for visible particulates is a best practice for the starting materials used in cell and gene therapy products; therefore, particulates should remain listed as test options in Table 3.
Comment Summary #319: The commenter requested to have separate lines for visual inspection, appearance and visible particulates. The acceptance criteria should be clear as to what part of <1790> is applicable.
Response: Comment partially incorporated. Visual inspection and appearance are listed together in the same USP chapter; therefore, they are not separated in Table 3. The reference to <1790> was removed because the chapter covers injections, and injections are not in the scope of the chapter.
Comment Summary #320: The commenter suggested to align with USP or EP on terminology for the Example Acceptance Criteria. To align with USP, the acceptance criteria should be changed to “essentially free of visible particulates”.

Response: Comment incorporated.
Comment Summary #321: The commenter suggested to add a reference <857> *Ultraviolet Visible Spectroscopy* for the Concentration A_{260} in Table 3.
Response: Comment incorporated.
Comment Summary #322: The commenter suggested to add detection by fluorescent labelling of DNA as an analytical procedure for determining DNA concentration.
Response: Comment incorporated. The procedure was added.
Comment Summary #323: The commenter suggested to use “UV absorption 260nm” instead of “Concentration A260”.
Response: Comment partially incorporated. UV was added before A_{260} to clarify UV absorption.
Comment Summary #324: The commenter suggested to add a footnote e.g. "various HPLC methods may be applicable". The commenter suggested to add references to USP chapters: <621>, <736>, <1736>.
Response: Comment partially incorporated. Size exclusion was removed to allow for the use of other HPLC methods. The references to the USP chapters for Chromatography and Mass Spectrometry were not included because they do not describe how to measure plasmid DNA concentration.
Comment Summary #325: The commenter suggested to add references to USP chapters <1125> Nucleic Acid-Based Techniques-General, <1126> Nucleic Acid-Based Techniques-Extraction, Detection, and Sequencing, <1129> Nucleic Acid-Based Techniques-Genotyping in the Identity Restriction Map in Table 3.
Response: Comment not incorporated. These USP chapters do not have methods for the mapping restriction of plasmid DNA and would not add value to the chapter.
Comment Summary #326: The commenter suggested to clarify the restriction map is restriction digest by DNA Agarose Gel Electrophoresis (AGE). The commenter also suggested to change to DNA sequencing for consistency with table 2. It may be helpful to specify the type of DNA sequencing (e.g. Sanger).
Response: Comment partially incorporated. The use of Agarose gel (AGE) is not specified because there are other ways of analyzing the restriction digest. The terminology in Table 2 and Table 3 were harmonized. The type of DNA sequencing was not specified to allow for different methods to be used.
Comment Summary #327: The commenter suggested to add references to USP chapters <1125> Nucleic Acid-Based Techniques-General, <1126> Nucleic Acid-Based Techniques-Extraction, Detection to the Identity Sequencing in Table 3.
Response: Comment not incorporated. These USP chapters do not have methods for the sequencing of plasmid DNA and would not add value to the chapter.
Comment Summary #328: The commenter suggested to add PCR to the Analytical Procedures column (in addition to Restriction map and Sequencing), to align the analytical procedures of Identity of homologous regions.
Response: Comment incorporated.
Comment Summary #329: The commenter suggested to add a point that sequencing for plasmid release on linearized plasmid.
Response: Comment partially incorporated. A statement was added to clarify that the identity testing in Table 3 can be performed on linearized plasmid.
Comment Summary #330: The commenter suggested to add references to USP chapters <1125> *Nucleic Acid-Based Techniques-General*, <1126> *Nucleic Acid-Based Techniques-Extraction, Detection, and Sequencing*, <1129> *Nucleic Acid-Based Techniques-Genotyping*.
Response: Comment not incorporated. These USP chapters do not have methods for these attributes of plasmid DNA and would not add value to the chapter.
Comment Summary #331: The commenter suggested to add these references to the row for Identity of homologous regions including long terminal repeats (LTRs) and Poly (A) tails in Table 3: <1125> *Nucleic Acid-Based Techniques-General*, <1126> *Nucleic Acid-Based Techniques-*

Extraction, Detection, and Sequencing, <1127> *Nucleic Acid-Based Techniques-Amplification*. The commenter suggested to add a footnote e.g. various PCR methods may be applicable.
Response: Comment not incorporated. These USP chapters do not have methods for these attributes of plasmid DNA and would not add value to the chapter. There are several PCR methods that can be used, and using "PCR" in the table covers the different types. It is not necessary to say that various PCR methods may be applicable.
Comment Summary #332: The commenter suggested to add references to USP chapters <1125> *Nucleic Acid-Based Techniques-General* and <1126> *Nucleic Acid-Based Techniques-Extraction, Detection* to the row for Identity of homologous regions including long terminal repeats (LTRs) and Poly (A) tails in Table 3.
Response: Comment not incorporated. These USP chapters do not have methods for these attributes of plasmid DNA and would not add value to the chapter.
Comment Summary #333: The commenter suggested to add <1126> *Nucleic Acid- Based Techniques-Extraction, Detection* to the Plasmid DNA topology row in Table 3.
Response: Comment not incorporated. These USP chapters are high level, do not have methods for the topology of plasmid DNA, and would not add value to the chapter.
Comment Summary #334: The commenter suggested to add footnote to the Plasmid DNA topology row in Table 3 to state that Capillary Gel Electrophoresis (CGE) and Anion Exchange HPLC (AEX) are preferred methods for topology for pivotal.
Response: Comment not incorporated. Currently, there are no preferred methods for plasmid topology.
Comment Summary #335: The commenter suggested to add <1065> *Ion Chromatography* to the row of Plasmid DNA Topology Table 3.
Response: Comment not incorporated. These USP chapters are high level, do not have methods for the topology of plasmid DNA, and would not add value to the chapter.
Comment Summary #336: The commenter suggested to add these references to the Plasmid DNA topology row in Table 3: <1053> *Capillary Electrophoresis*, <1056> *Biotechnology-Derived Articles-Polyacrylamide Gel Electrophoresis* and <1126> *Nucleic Acid-Based Techniques-Extraction, Detection*. The commenter to add a footnote that states, "CE or equivalent may be used to analyze wide range of samples including linearized plasmid DNA."
Response: Comment not incorporated. The USP chapters are high level and do not have methods for the topology of plasmid DNA and would not add value to the chapter. Capillary Gel Electrophoresis is already included in Table 4 for analysis of linearized DNA.
Comment Summary #337: The commenter suggested adding another methodology to the table for analysis of Plasmid DNA topology. The assay involves the radius of gyration by a multiple angle light scattering system.
Response: Comment partially incorporated. Light scattering was added as method to for Plasmid DNA topology in Table 3.
Comment Summary #338: The commenter suggested to use "DNA homogeneity" instead of Plasmid DNA topology in Table 3.
Response: Comment partially incorporated. Homogeneity was added to the Plasmid DNA topology in Table 3.
Comment Summary #339: The commenter requested confirmation that electron microscopy and ultracentrifugation are methods used to assess DNA topology.
Response: Comment partially incorporated. Analytical ultracentrifugation and electron microscopy have been used for characterization. One of the headings in Table 3 was revised to indicate that some test methods are characterization.
Comment Summary #340: The commenter suggested to delete electron microscopy as a test for topology because they have not found electron microscopy for quantifying plasmid topology to be supported in the literature as an analytical method for this purpose and, as such, application may be limited and not as effective as other recognized methods.

Response: Comment not incorporated. Electron microscopy has been used for characterization. One of the headings in Table 3 was revised to indicate that some test methods are characterization.
Comment Summary #341: The commenter suggested to add a reference to <1103> *Immunological Test Methods—Enzyme-Linked Immunosorbent Assay (ELISA)* to the Residual Host protein row in Table 3.
Response: Comment not incorporated. This USP chapter is high level and do not have methods for ELISA and would not add value to the chapter.
Comment Summary #342: The commenter suggested to add references to <1125> *Nucleic Acid-Based Techniques—General* "(qPCR), <1126> *Nucleic Acid-Based Techniques—Extraction, Detection, and Sequencing* and <1127> *Nucleic Acid-Based Techniques—Amplification* to the Residual host DNA row in Table 3.
Response: Comment not incorporated. These USP chapters are high level, do not have validated methods for nucleic acid quantification, and would not add value to the chapter.
Comment Summary #343: The commenter suggested to change the expected acceptance criteria for residual host DNA and residual host RNA to end use-specific; < 1 – 5%. The reason for the change is to align with current regulatory expectations as high levels of these impurities would likely have an impact on downstream processes (e.g., transfection, nuclease digestion).
Response: Comment partially incorporated. The acceptance criteria was changed to “end use-specific.” The specific range was removed as the limits vary.
Comment Summary #344: The commenter is suggesting to only have an upper limit for the acceptance criteria for residual host cell protein, so a batch would not fail for having too little residual host cell protein.
Response: Comment incorporated. “No more than (NMT)” was added to make the limit an upper limit only.
Comment Summary #345: The commenter requested to consider if reverse transcription-qPCR (RT-qPCR) could be considered as another possible analytical method for residual host RNA.
Response: Comment partially incorporated. PCR was added as a method to measure residual host RNA. RT-PCR was not specified to provide flexibility in the type of PCR method used.
Comment Summary #346: The commenter suggested to only have an upper limit for the acceptance criteria for residual host DNA because a batch should not fail for having too little residual host DNA.
Response: Comment partially incorporated. The example acceptance criteria was removed in response to other comments and is now “end-use specific.”
Comment Summary #347: The commenter is suggesting to only have an upper limit for the acceptance criteria for residual host RNA, so a batch would not fail for having too little residual host RNA.
Response: Comment partially incorporated. The example acceptance criteria was removed in response to other comments and is now “end-use specific.”
Comment Summary #348: The commenter suggested to add a reference to <1126> *Nucleic Acid-Based Techniques—Extraction, Detection* for residual host RNA.
Response: Comment not incorporated. The reference to USP chapter is too general and does not provide a specific method.
Comment Summary #349: The commenter recommended including RT-PCR as a test method option for detection of residual RNA.
Response: Comment partially incorporated. PCR was added as a method to analyze residual host RNA. RT-PCR was not specified to provide flexibility in the type of PCR method used.
Comment Summary #350: The commenter suggested to add qPCR to the list of test methods for Residual RNA as this method is more accurate than the described methods.

Response: Comment partially incorporated. PCR was added as a method to measure residual host RNA. RT-PCR was not specified to provide flexibility in the type of PCR method used. Spectrometry was also added as a method to measure RNA.
Comment Summary #351: The Commenter suggested to add a reference to <853> Fluorescence Spectroscopy to the Residual host RNA row of Table 3.
Response: Comment incorporated.
Comment Summary #352: The commenter requested that “ddPCR” be added as an analytical procedure for residual host RNA in Table 3.
Response: Comment partially incorporated. PCR for residual RNA was added to the table, but qPCR and ddPCR are not specifically called out. PCR is used to cover all types of PCR.
Comment Summary #353: The commenter suggested to add Reverse phase HPLC to Residual host RNA in Table 3. The commenter also suggested to add a footnote that “"various HPLC methods may be applicable" (for detection of residual host RNA carryover or potential RNA impurities". The commenter also suggested to add references <621> Chromatography, <736> Mass Spectrometry and <1736> Applications of Mass Spectrometry.
Response: Comment partially incorporated. HPLC was added to the residual RNA row in Table 3. HPLC was used to indicate that other HPLC methods in addition to RP-HPLC can be used to measure RNA. The USP chapters were not included because <736> and <1736> refer to mass spectrometry, which are not listed as methods in Table 3. <621> was not included because a specific HPLC method is not described in that chapter.
Comment Summary #354: The commenter suggested to add nucleic acid/protein ratio (A_{260}/A_{280}) should be included with a recommended acceptance criterion of 1.8 - 2.0.
Response: Comment incorporated.
Comment Summary #355: The commenter suggested to add RT-PCR to residual host RNA in Table 3 as this is an option provided in EP 15.14.
Response: Comment partially incorporated. PCR for residual host RNA was added to the table, but RT-PCR was not specifically called out. PCR is used to cover all types of PCR.
Comment Summary #356: The commenter suggested adding ddPCR as option for a method for residual host RNA testing.
Response: Comment partially incorporated. PCR for residual host RNA was added to the table, but dd-PCR was not specifically called out. PCR is used to cover all types of PCR, including ddPCR.
Comment Summary #357: The commenter suggested to incorporate another type of detection (UV/Vis) with special deconvolution software to analyze the residual host DNA and residual host proteins.
Response: Comment not incorporated. The table is not inclusive of all methods that can be used for detection. The use of UV/Vis with a deconvolution software could be used but would require validation.
Comment Summary #358: The commenter suggested to add Sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) and western blot to Table 3 as a test for Residual host proteins.
Response: Comment incorporated.
Comment Summary #359: The commenter suggested that there may not need to be a test for residual antibiotics if there is a clearance model, and this information could be included in a footnote.
Response: Comment not incorporated. Table 3 is meant to present possible tests that can be performed. Table 3 does not describe justifications for not including tests because the user does not need to perform all tests in Table 3.
Comment Summary #360: The commenter suggested to add a reference to <853> Fluorescence Spectroscopy to the “Residual Antibiotics, ELISA” in Table 3.
Response: Comment incorporated.
Comment Summary #361: The commenter suggested to add footnote that antibiotic

residue testing is optional depending on whether antibiotics are used in the process.
Response: Comment not incorporated. Table 3 is meant to present possible tests that can be performed. Table 3 does not describe justifications for not including tests because the user does not need to perform all tests in Table 3.
Comment Summary #362: The commenter recommended to add a footnote that the Residual antibiotic test is optional, depending on process used.
Response: Comment not incorporated. Table 3 is meant to present possible tests that can be performed. Table 3 does not describe justifications for not including tests because the user does not need to perform all tests in Table 3.
Comment Summary #363: The commenter recommended to remove reference to environmental factors (not relevant to acceptance criteria). The commenter requested additional clarity desired for end-use specific. In the places where there is no acceptance criteria, could historically acceptable ranges be provided?
Response: Comment partially incorporated. Environmental safety was not removed from Table 3, but a statement was added to the text to address the environmental concerns about antibiotics, “In addition, environmental concerns related to manufacturing waste may also need to be considered during process development.” End-use specific was used to describe criteria that are specific to the process (end use-specific) and that the user must determine the specification for their own product. The places where acceptance criteria are not provided is because there is not a general acceptable criteria to provide.
Comment Summary #364: The commenter indicated that the limited total amount of plasmids limits the testing volume for Sterility. The commenter suggested a percent-wise approach of total batch as a mitigation.
Response: Comment not incorporated. The information provided by the commenter is already included in Section 5.1.12 Bioburden and Sterility, which is cited in Table 3.
Comment Summary #365: The commenter suggested that testing for other potential process related residuals (e.g., solvents, etc.) should be included.
Response: Comment not incorporated. The sentence in the text before Table 3 states “The User should select a subset of these tests or other procedures not listed as appropriate for the intended application.” This statement specifies that other testing may be needed for the intended application, and this would include residual solvents.
Comment Summary #366: The commenter suggested to propose between ≤ 1 CFU/10mL and < 5 CFU/10mL for bioburden with a note that limit should consider the specific process capability and risks.
Response: Comment not incorporated. The acceptance criteria are described as “end use specific” which is defined as specific to the process. The acceptance criteria of ≤ 1 CFU/10mL was just provided as an example.
Comment Summary #367: The commenter recommended to set the specification for the bioburden test to between ≤ 1 CFU /10mL and < 5 CFU/10 mL for TAMC and for TYMC completed by a microbial identification and investigation of the impact if at least one CFU been detected.
Response: Comment not incorporated. The acceptance criteria are described as “end use specific” which is defined as specific to the process. The acceptance criteria of ≤ 1 CFU/10mL was just provided as an example. The bioburden test is described with end-use specific criteria. If the user wants to test the identity of a detected microbial organism, this can be done.
Comment Summary #368: The commenter suggested to specify that the suggested bioburden acceptance criteria are for both TAMC and TYMC.
Response: Comment not incorporated. Bioburden is usually reported as CFU/mL for both TAMC and TYMC. Because the acceptance criteria are described as “end use specific,” which means it is specific to the process, the user can report the result for TAMC and TYMC.

Comment Summary #369: The commenter recommended the removal of the Sterility testing from the chapter because sterility would be tested on the final dosage form and not the DNA as a starting material.
Response: Comment not incorporated. There are some products that require sterility testing of the plasmid. Some facilities require sterility testing before the plasmid can be brought into the facility. Because sterility testing of the plasmid is required in some cases, it will not be removed from the chapter.
Comment Summary #370: The commenter suggested changing the acceptance criteria for the Mycoplasma test to "Should not be present" to emphasize that Mycoplasma should not be present.
Response: Comment incorporated. The acceptance criteria for mycoplasma was changed to "Not detected."
Comment Summary #371: The commenter suggested adding an additional column to Table 3 or adding footnotes to indicate the critical minimum tests that should be provided: appearance, concentration, identity, full sequence, plasmid purity, endotoxin, sterility and/or bioburden, residual host cell protein,
Response: Comment not incorporated. The purpose of Table 3 is to provide a list of tests that can be used. The user must determine which tests apply to their application. The critical minimum tests may be different for different plasmid applications.
Comment Summary #372: The commenter suggested to add the following USP chapter resources: ⟨621⟩ *Chromatography*, ⟨736⟩ *Mass Spectrometry*, ⟨853⟩ *Fluorescence Spectroscopy*, ⟨857⟩ *Ultraviolet-Visible Spectroscopy* ⟨1053⟩ *Capillary Electrophoresis* ⟨1056⟩ *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis,* ⟨1065⟩ *Ion Chromatography,* ⟨1103⟩ *Immunological Test Methods—Enzyme-Linked Immunosorbent Assay (ELISA),* ⟨1104⟩ *Immunological Test Methods—Immunoblot Analysis,* ⟨1125⟩ *Nucleic Acid-Based Techniques—General*, ⟨1126⟩ *Nucleic Acid-Based Techniques—Extraction, Detection, and Sequencing*, ⟨1127⟩ *Nucleic Acid-Based Techniques—Amplification*, ⟨1129⟩ *Nucleic Acid-Based Techniques—Genotyping*, ⟨1132⟩ *Residual Host Cell Protein Measurement in Biopharmaceuticals* and ⟨1736⟩ *Applications of Mass Spectrometry*.
Response: Comment not incorporated. Adding the references would not add value to the chapter as the USP chapters do not have specific methods that are relevant to the uses with testing plasmid DNA.
Comment Summary #373: The commenter suggested to include more USP chapters and resources.
Response: Comment not incorporated. Adding the references would not add value to the chapter as the USP chapters do not have specific methods that are relevant to the uses with testing plasmid DNA.
Comment Summary #374: The commenter suggested to add the poly(A) tail length as quality attribute to be tested if the poly(A) tail is encoded. The poly (A) tail length would be added as a new row in Table 3.
Response: Comment partially incorporated. Poly A tail length was added to Table 3 but not in a new row.
Comment Summary #375: The commenter suggested to include the patient safety guidelines mentioned in footnote f of Table 3.
Response: Comment partially incorporated. There is no specific patient safety guideline to follow; therefore, the word guideline was removed.
Comment Summary #376: The commenter suggested adding the heading "Exemplary Analytical Procedures" to the second column of Table 4 to clarify that alternative methods can be used.
Response: Comment partially incorporated. The heading of the second column in Table 4 was changed to "Exemplary Analytical Procedures for Characterization, Release and/or Stability."

Comment Summary #377: The commenter suggested to add a sentence between Table 3 and Table 4 to explain how testing is employed for linear DNA template in relation to the testing for the Plasmid DNA.
Response: Comment partially incorporated. The sentence, “Table 4 is complimentary to Table 3 for linear specific attributes” was added for the clarification.
Comment Summary #378: The commenter suggested adding another technology and assay method (e.g. radius of gyration (Rg) by multiple angle light scattering) for analyzing linearity or linearization efficiency.
Response: Comment partially incorporated. Light scattering as a more general category of tests was added to the table. Radius of gyration is covered under this general category.
Comment Summary #379: The commenter suggested to remove the analytical ultracentrifugation, electron microscopy and PCR from the list of tests for Linearization efficiency in Table 4.
Response: Comment not incorporated. These test methods can be used to test the linearization efficiency. Some tests are used for release and some for characterization. Clarification has been added to Table 4 that some tests are for characterization.
Comment Summary #380: The commenter suggested to add a reference to <1126> *Nucleic Acid-Based Techniques—Extraction, Detection, and Sequencing* in the linearization efficiency row of Table 4.
Response: Comment not incorporated. Adding the references would not add value to the chapter. The USP chapters do not have specific methods that are applicable to the testing of plasmid DNA.
Comment Summary #381: The commenter suggested to add a reference to <1065> Ion Chromatography in the row 'Linearization efficiency ' Anion exchange (AXE) HPLC in Table 4.
Response: Comment not incorporated. Adding the references would not add value to the chapter. The USP chapters to not have specific methods that are applicable to the testing of plasmid DNA.
Comment Summary #382: The commenter suggested to add references to <1125> *Nucleic Acid-Based Techniques—General*, <1126> *Nucleic Acid-Based Techniques—Extraction, Detection, and Sequencing*, <1127> *Nucleic Acid-Based Techniques—Amplification*. The commenter also suggested to add footnote e.g. "various PCR methods may be applicable".
Response: Comment not incorporated. Adding the references would not add value to the chapter. The USP chapters do not have specific methods that are applicable to the testing of plasmid DNA. The footnote indicating that various PCR methods may be applicable was not needed because the test method is listed as PCR, which would include various PCR test methods.
Comment Summary #383: The commenter suggested to add references to <1053>, <1056> *Biotechnology-Derived Articles-Polyacrylamide Gel Electrophoresis* and <1126> *Nucleic Acid-Based Techniques—Extraction, Detection, and Sequencing* to the Linearization efficiency row of Table 4.
Response: Comment not incorporated. Adding the references would not add value to the chapter. The USP chapters do not have specific methods that are applicable to the testing of plasmid DNA.
Comment Summary #384: The commenter suggested to add a reference to <1056> *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* to the row 'Residual enzymes and carrier proteins' Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in Table 4.
Response: Comment not incorporated. Adding the references would not add value to the chapter. The USP chapters do not have specific methods that are applicable to the testing of plasmid DNA.
Comment Summary #385: The commenter suggested to add clarification, including pros and cons and different use cases of the various methods provided is suggested. The clarification

can be added to 5.1.15. The commenter indicated that many tests are listed for reporting residual enzymes, but the methods do not all match to the example acceptance criteria.
Response: Comment partially incorporated. The example acceptance criteria were removed and “end use specific” was retained. The intention of the table in the General Chapter was to provide examples of tests that can be used. Describing pros and cons is specific to the use cases, and that is beyond the scope of the chapter.
Comment Summary #386: The commenter suggested to add <1104> *Immunological Test Methods—Immunoblot Analysis* to “Residual enzymes and carrier proteins in Table 4.
Response: Comment not incorporated. Adding the references would not add value to the chapter. The USP chapters do not have specific methods that are applicable to the testing of plasmid DNA.
Comment Summary #387: The commenter requested clarification on whether Capillary Gel Electrophoresis- Sodium dodecyl sulfate-polyacrylamide gel (CGE-SDS) would be considered as a variation of the SDS-PAGE analytical method.
Response: Comment partially incorporated. CGE-SDS and SDS-PAGE are not the same methods. Capillary Gel Electrophoresis was added to Table 4.
Comment Summary #388: The commenter suggested to add a reference to <1103> *Immunological Test Methods—Enzyme-Linked Immunosorbent Assay (ELISA)* to Table 4.
Response: Comment not incorporated. Adding the reference would not add value to the chapter. The USP chapter <1103> does not have specific methods that are relevant to the uses with testing plasmid DNA.
Comment Summary #389: The commenter suggested to add a footnote "various activity assays may be applicable" in Residual Enzymes and carrier proteins Activity Assay.
Response: Comment not incorporated. The chapter describes the tests in the tables as an overview of test methods that can be used. The tests described are not the only tests that can be used. The user must determine which tests are appropriate for their plasmid DNA application.
Comment Summary #390: The commenter suggested to add a footnote e.g. ""various HPLC methods may be applicable " to the row “Residual enzymes and carrier proteins in Table 4. The commenter also suggested to add references to <621> *Chromatography*, <736> *Mass Spectrometry*, <1736> *Applications of Mass Spectrometry*.
Response: Comment not incorporated. HPLC is listed as a method in Table 4 and would encompass all types of HPLC. Adding the references would not add value to the chapter. The USP chapters do not have specific methods that are applicable to the testing of plasmid DNA.
Comment Summary #391: The commenter suggested to add references to <621> *Chromatography*, <736> *Mass Spectrometry*, <1736> *Applications of Mass Spectrometry*.
Response: Comment not incorporated. Adding the references would not add value to the chapter. The USP chapters do not have specific methods that are applicable to the testing of plasmid DNA.
Comment Summary #392: The commenter suggested to include the patient safety guidelines mentioned in footnote a of Table 4.
Response: Comment partially incorporated. The reference to safety guidelines was removed. The safety guidelines were replaced with “patient safety” as no specific guidelines were meant to be cited.

5.1.1 Appearance - Color and Clarity
Comment Summary #393: The commenter suggested to note that not all products can be colorless.
Response: Comment not incorporated. The following text exists in Table 3 to allow for flexibility in the color requirement. "Clear, colorless solution but may be molecule and/or process specific”.

Comment Summary #394: The commenter recommended to edit as follows, “Use of a compendial method, such as the visual methods from Ph. Eur. 2.2.2 (17) or ⟨631⟩ for color and clarity, helps to reduce variability from **vial to vial, operator to operator and** test site to test site for this qualitative method.”
Response: Comment incorporated.
Comment Summary #395: The commenter suggested to allow for the use of methods other than the compendial methods because the batch size and fill volume per aliquot might not fit the requirements of the compendial method. The change should also be reflected in Table 3.
Response: Comment not incorporated. The test methods listed in the table and in 5.1.1 are not required; therefore, there is flexibility to use another method.

5.1.2 Visible Particulates
Comment Summary #396: The commenter suggested to delete Ph.Eur. 2.9.19 because it is for sub-visible particles only if the chapter refers to visible particles only. If sub-visible and visible particles are included, then the General Chapter should reference USP chapters <787> and <788>.
Response: Comment partially incorporated. The testing is for visible particles only; therefore, the references were aligned to reflect this.
Comment Summary #397: The commenter recommends removing the reference to Ph. Eur. Methods 2.9.19 because it is for sub-visible particles.
Response: Comment incorporated. The reference was removed.
Comment Summary #398: The commenter indicated that the impact of visible particulates may be minimal for plasmid DNA starting material if it is sterile filtered downstream.
Response: Comment not incorporated. This statement is true, but the particulates may still need to be measured. The chapter includes a list of attributes that could be tested, including visible particles, but it is not a requirement.
Comment Summary #399: The commenter suggested that the analytical procedures provided are for visible particulates in injectable drug products which is not appropriate for plasmid as a starting material for a CGT product; therefore, the test/attribute should be removed.
Response: Comment not incorporated. Testing for particulates is not a requirement but rather a suggestion of a possible attribute to test in this chapter. Because testing of particulates is not a requirement but an item on a list of tests that can be performed on plasmid starting material, the test will not be removed.
Comment Summary #400: The commenter recommended splitting Section 5.1.2 into two different methods in alignment with Table 3 above <790> Visible Particulates and <1790> Visual Inspection.
Response: Comment partially incorporated. The references to <790> and <1790> have been removed from Section 5.1.2 because the chapter pertains to injectable products and not a plasmid DNA starting material.
Comment Summary #401: The commenter suggested to remove visible particulates as a quality attribute as the particulates can be prevented in the manufacturing process, and particulates do not have an influence on plasmid quality as long as other quality attributes have been fulfilled.
Response: Comment not incorporated. Testing for particulates is not a requirement but rather a suggestion of a possible attribute to test in this chapter. Because testing of particulates is not a requirement but an item on a list of tests that can be performed on plasmid starting material, the test will not be removed.

5.1.4 Concentration
Comment Summary #402: The commenter recommended adding the A_{260}/A_{280} ratio measurements, which will provide insight on purity as well. A_{260}/A_{230} should also be added for process-related components and impurities (e.g. HCL).

Response: Comment incorporated. The $A_{260}/_{280}$ ratio and the A_{260}/A_{230} ratio were added to Table 3 and to the text to test purity and measure the amount of impurities.
Comment Summary #403: The commenter suggested to change the text to "in-process crude samples" for clarification and to correct a typo.
Response: Comment partially incorporated. The word "impure" was removed; therefore, the text would be "In-process samples" as these samples are impure, but removing the word "impure" will provide clarity.
Comment Summary #404: The commenter suggested to add information about the HPLC measurement that is mentioned in Table 3.
Response: Comment not incorporated. The text in the sections below Tables 3 and 4 are meant to discuss the quality attribute in general. The tables list tests that can be used to test the attribute. Therefore, the sections below the tables are not meant to restate all of the test methods listed in the table.
Comment Summary #405: The commenter suggested to delete the statement that plasmid products are increasingly difficult to manage at greater than 5 mg/mL.
Response: Comment partially incorporated. The text was edited as follows: "As the concentration exceeds 5mg/mL, the viscosity may increase to such a point that specialized handling is necessary to accurately measure the concentration including positive displacement pipettes, etc."

5.1.5 Identity
Comment Summary #406: The commenter suggested to add allowance for Next Generation Sequencing (deep sequencing) as a characterization test to supplement other validated identity tests in order to detect low-level contaminating sequences.
Response: Comment partially incorporated. A statement was added to include next-generation sequences as a method used for long read sequencing.
Comment Summary #407: The commenter suggested to add footnote to state "if Sanger not appropriate for (e.g., ITR containing plasmids) suggest Next Generation Sequencing as alternative.
Response: Comment partially incorporated. DNA sequencing is used in the chapter to mean any kind of sequencing that is appropriated (Sanger, NGS etc.). A note has been added to clarify that sequencing includes all kinds of sequencing.
Comment Summary #408: The commenter suggested to consider adding a comment to say receipt identity test not required for non-pivotal (and not commercial) or only use non-GMP NGS for nonpivotal receipt testing.
Response: Comment not incorporated. Receipt testing quality requirements are defined by each user's quality management system and cannot be specified in the chapter.
Comment Summary #409: The commenter requested to add more details in the second paragraph of Section 5.1.5 Identity.
Response: Comment not incorporated. There is sufficient information provided in the Identity section.

5.1.6 Plasmid DNA Topology
Comment Summary #410: The commenter suggested the following edits, "Analyses of plasmid DNA topology and its influence on viral vectors or mRNA product quality is likely one of the most…. […]"adding at the end of the section the sentence: **"Plasmid DNA topology is of less importance for plasmid DNA used for mRNA production, as this is linearized prior to use in vitro transcription, therefore consider section 5.1.14 Linearization Efficiency."**
Response: Comment not incorporated. The impact of plasmid topology on each end user's process may be different. A statement that topology is less important for mRNA may not be true for some end users; therefore, this statement cannot be added to the chapter.

Comment Summary #411: The commenter suggested changing the first sentence of the section for clarification. The commenter suggested the following text "**Plasmid DNA topology is likely to be one of the most significant attributes as it impacts the final product quality viral vectors or mRNA when defining a plasmid DNA starting material control strategy**"
Response: Comment partially incorporated. The first sentence was revised to "Plasmid DNA topology may be an attribute as it impacts the final product quality viral vectors or mRNA when defining a plasmid DNA starting material control strategy."

5.1.7 Residual Host Protein
Comment Summary #412: The commenter indicated that the methods listed under the residual host protein section are not specific for host protein, although it can be inferred that the host cell is the source of residual protein.
Response: Comment not incorporated. No revision to the text or action was requested.
Comment Summary #413: The commenter recommended to add "If necessary, more sensitive techniques like host protein-specific ELISA are available."
Response: Comment not incorporated. The statement "If necessary, more sensitive techniques like host protein-specific ELISA are available" is already included in the chapter; therefore, it does not need to be added.

5.1.8 Residual Host DNA
Comment Summary #414: The commenter suggested to change text to, "~~Because of its similarity in molecular composition to plasmid DNA,~~ Targeted PCR-based technology is often used to selectively quantify the amount of residual host cell DNA." As the prepositional first part of sentence is not necessary.
Response: Comment not incorporated. The text adds value to highlight the reason for using targeted PCR to quantify residual host cell DNA; therefore, the text will not be deleted.

5.1.9 Residual Host RNA
Comment Summary #415: The commenter recommended, "Reversed phase HPLC, which is not subject to this limitation of a diffuse banding pattern, should be used. Alternatively, Host RNA can be resolved from DNA by AGE and quantified with a fluorescent stain. However, host RNA size heterogeneity results in a diffuse banding pattern making measurement uncertain."
Response: Comment not incorporated. The EC finds the current wording and order of tests listed in the General Chapter to be sufficient.
Comment Summary #416: The commenter suggested to reference the use of RNA-specific fluorescent dye and measurement by fluorimetry, as mentioned in the Table 3.
Response: Comment incorporated.
Comment Summary #417: The commenter recommended adding RT-qPCR as one of the possible residual host RNA methods.
Response: Comment partially incorporated. RT-PCR was added to Table 3 and the Residual Host RNA section.
Comment Summary #418: The commenter suggested to add that "various electrophoretic methods other than agarose gel may be applicable" for residual host RNA.
Response: Comment incorporated. Text was added to indicate that various electrophoretic methods may be applicable.
Comment Summary #419: The commenter suggested that reversed phase HPLC could be used.
Response: Comment incorporated. Text was added to include RP-HPLC as a method used for residual host RNA.

5.1.10 Residual Antibiotics

Comment Summary #420: The commenter suggested to include a clearance model as an option for residual antibiotics.
Response: Comment incorporated. A statement about the clearance model was added as an option for residual antibiotics. This statement was added to the Residual Antibiotics section: "Validation of clearance may alleviate the necessity for lot-to-lot release testing."
Comment Summary #421: The commenter recommended that the statement, "The use of antibiotics should be justified" should be included in the chapter.
Response: Comment not incorporated. The statement was not included because residual antibiotics is a concern for a drug product and would require justification. For plasmid used as a starting material, which is the scope of this chapter, the use of antibiotics does not need to be justified.
Comment Summary #422: The commenter recommended not to require residual antibiotic testing and rather allow risk-based approach based on application.
Response: Comment partially incorporated. Text has been added to discuss a clearance model where residual antibiotic testing may not be necessary. There are no requirements in this general information chapter. Options for attributes to test are presented but not required.
Comment Summary #423: The commenter suggested to add that the limit of detection (LOD) for the assay should be rationalized based on the amount of antibiotic that could be sensitizing in the final product.
Response: Comment partially incorporated. The statement about an appropriate LOD has been added, "the chosen method should have a limit of detection relevant to the chosen specification which may vary depending on the antibiotic implemented, the clearance during the plasmid and DS processing as well as the overall drug product dose."

5.1.11 Endotoxins
Comment Summary #424: The commenter recommended that acceptance criteria for Endotoxins should be established per regulatory guidelines.
Response: Comment partially incorporated. Regulatory guidelines are not clear on the endotoxin requirements for plasmid DNA starting material. A statement that patient safety should be considered in endotoxin acceptance criteria was added.
Comment Summary #425: The commenter requested clarification on which process is referred to for "manufacturing process" in Section 5.1.11 Endotoxins or the commenter suggested to change the language to, "any manufacturing process in which plasmid is used as a starting material" or end-to-end.
Response: Comment partially incorporated. Drug substance was added for clarification on the manufacturing process.

5.1.12 Bioburden and Sterility
Comment Summary #426: The commenter suggested the following edits:
"For example, plasmid DNA going directly into a bioreactor for adeno-associated virus production ~~might have different~~ **may have a higher sterility requirements** than the linear template used in an in vitro transcription reaction to create mRNA."
Response: Comment incorporated.
Comment Summary #427: The commenter requested clarification on what is expected of a reduced sampling plan for Sterility.
Response: Comment partially incorporated. A statement was added to indicate that ICH Q5D or other justifications can be used for a reduced sampling plan.
Comment Summary #428: The commenter recommended citing <1119>, and that limits should be established for bioburden based on efficiency of downstream processing steps. More explanation is needed on when sterility testing verses bioburden testing is needed.

Response: Comment not incorporated. <1119> is not an official USP chapter and therefore cannot be cited. Text in the General Chapter specifies that choosing between sterility or bioburden testing is justified based on the downstream application.
Comment Summary #429: The commenter suggested to use other references or approaches to Sterility since Sterility is sometimes impractical at the scale required for large numbers of gene therapy and RNA vaccine applications.
Response: Comment not incorporated. Sterility is listed as an option for testing in this General Chapter. Suggestions for reduced sampling plans for sterility testing with references have been provided. There are no other references to include in the chapter.
Comment Summary #430: The commenter suggested to clarify and expand this sentence, "Additionally, a distinction should be made about whether the DNA starting material is a sterile product and/or tested for sterility as a sterile product claim goes beyond testing and is based on facility controls."
Response: Comment partially incorporated. The sentence was deleted for clarity. The plasmid can be tested for sterility, but there is no risk to patients if the material is not sterile because the material is not intended for administration to humans..
Comment Summary #431: The commenter requested clarification on the citation of <71> Sterility with a reduced sampling plan, and why bioburden was not the only method cited.
Response: Comment not incorporated. The General Chapter states that bioburden or sterility can be tested. There are still some cases where sterility needs to be tested. The chapter provides the flexibility to use either method.

5.1.13 Mycoplasma
Comment Summary #432: The commenter suggested to add this wording, "unlike mammalian cells, bacterial cells cannot be infected by mycoplasma and therefore, routine testing for the presence of mycoplasma is not required."
Response: Comment partially incorporated. The sentence was revised to "Mycoplasma are not associated with prokaryotic-derived products."
Comment Summary #433: The commenter requested to consider if a reference to the appropriate USP chapter, <63> *Mycoplasma Tests* for testing.
Response: Comment incorporated.

5.1.15 Residual Enzymes and Carrier Proteins
Comment Summary #434: The commenter suggested to add a sentence to 5.1.5 to include limitations of QC methods and alternative Next Generation Sequencing (NGS) sequencing for characterization: "Quality control release testing of such sequences can be challenging for state of the art release test methods. Therefore, if they fail to characterize those sequences, extended characterization by NGS is recommended." The commenter requested clarification about testing for release versus characterization as stated in the methods description section.
Response: Comment not incorporated. NGS is used for characterization but can also be validated and used for QC release. The chapter allows both the options.
Comment Summary #435: The commenter suggested to split the section into qualitative and quantitative testing. The purpose of the qualitative would be to know if residual proteins are present. The quantitative testing would be used to determine how much of the protein was present. Maybe reference these chapters: immunological test methods <1102> and <1103> and <1104> for consideration of specific methods to enzymes and proteins.
Response: Comment not incorporated. The methods used will identify the presence of residual proteins and quantify the amount. The quantity of residual enzymes and carrier proteins will need to be determined. The above 1000 chapters do not include specific methods; therefore, citing these chapters would not add value to the chapter.
Comment Summary #436: The commenter suggested to add that materials containing human or animal materials such as bovine serum albumin would need a BSE/TSE statement.

Response: Comment not incorporated. This statement is included earlier in the chapter. BSE/TSE would be evaluated for incoming raw materials.
Comment Summary #437: The commenter suggested to change the wording to not limit to just mRNA vaccines only. For example use mRNA vaccines and therapeutics.
Response: Comment incorporated. In the text, “mRNA vaccines” was changed to “mRNA therapeutics.”

5.2 Stability Testing
Comment Summary #438: The commenter recommended the following edits, “It is recommended that **a phase-dependent** plasmid stability **study be conducted**, and an appropriate use period assigned.” The stability study design may vary between research, preclinical and clinical phases.
Response: Comment not incorporated. The suggested edits would add a level of detail that is beyond the scope of the chapter.
Comment Summary #439: The commenter recommended the following edits, “A plasmid stability protocol may include appearance, pH, concentration, topology, and microbial testing **and must address proposed storage conditions**.” **It should be specified if accelerated studies can be appropriate.**
Response: Comment partially incorporated. The following text was added to include other stability conditions: “Additionally, one or more of the attributes analyzed should be stability-indicating if possible. The stability study should address the recommended storage condition and could include other stressed conditions as appropriate.”
Comment Summary #440: The commenter requested to edit the sentence, “A plasmid stability protocol may **should include testing of relevant stability-indicating critical quality attributes such as** appearance, pH, concentration, or topology, and microbial testing **to support assessment of plasmid quality during long-term storage.”**
Response: Comment partially incorporated. The text was edited as follows: “A plasmid stability protocol **may include** appearance, pH, concentration, topology, and microbial testing. Additionally, one or more of the attributes analyzed should be stability-indicating if possible. The stability study should address the recommended storage condition and could include other stressed conditions as appropriate.”
Comment Summary #441: The commenter requested to reference an ICH guidance to support stability protocol development and frequency of testing for plasmids.
Response: Comment incorporated.
Comment Summary #442: The commenter requested clarification regarding the intended plasmid usage when the topology acceptance criteria are crucial. The impact of topology for accessibility during a PCR process is unknown, which might be different compared to direct transfection of plasmid DNA for CGT manufacturing. Setting acceptance criteria on that is impossible (Mitigation by Risk Analysis might be possible).
Response: Comment not incorporated. The specifications are discussed in Table 3 and are described as end-use specific meaning that the specifications are determined by the use of the plasmid.
Comment Summary #443: The commenter recommended providing guidance around time points of stability testing for plasmid.
Response: Comment partially incorporated. Guidance on timepoints was not added, but a reference to the ICH guidelines has been added.

5.3 Performance Testing
Comment Summary #444: The commenter requested that the wording in this text, “In general, sponsors should have a small scale, representative drug substance process in which the performance of the DNA starting materials can be evaluated.” be changed to indicate that this is a recommendation only.

Response: Comment partially incorporated. The sentence was changed to “In general, users should consider having a representative, small scale process in which the performance of the DNA starting materials can be evaluated.”
Comment Summary #445: The commenter recommended to align “users” and “sponsors”. The commenter recommends using “sponsors”.
Response: Comment partially incorporated. The chapter defines the use of suppliers as the manufacturer of the plasmid, and the user is the drug substance or drug product developer. It is also possible for the supplier to be the end user. Sponsor was not intended to be used in the chapter; therefore, it has been changed to user.
Comment Summary #446: The commenter requested that small scale processes be fully defined and justified.
Response: Comment not incorporated. The representative, small-scale drug substance implies that it is defined and justified. It is beyond the scope of the General Chapter to define these terms and justify for an individual process.
Comment Summary #447: The commenter suggested to remove or change wording to allow for more flexibility on at scale evaluation for Performance Testing.
Response: Comment partially incorporated. The sentence was edited to “In general, ~~sponsors~~ users should **consider having** a **representative**, small scale ~~drug substance~~ process in which the performance of the DNA starting materials can be evaluated.”
Comment Summary #448: The commenter requested clarification if PCR can be used for as a functional test.
Response: Comment partially incorporated. The small-scale analysis is optional. The drug substance was removed from the sentence for clarification. The sentence was edited to “In general, ~~sponsors~~ users should **consider having** a **representative**, small scale ~~drug substance~~ process in which the performance of the DNA starting materials can be evaluated.”
Comment Summary #449: The commenter requested clarification and further guidance on defining acceptance criteria of plasmid DNA and end-use performance.
Response: Comment partially incorporated. This sentence was edited to allow flexibility for the user: “**In some situations**, these small-scale models **can be** value added for assessment of release and stability acceptance criteria along with comparability evaluation.”

5.4 Plasmid to Plasmid Cross-Contamination
Comment Summary #450: The commenter suggested to revise wording to avoid "only" and definitely", as in addition to sequencing there are also PCR-based methods possible to detect low level plasmid contamination (e.g. qPCR, or PCR-based replicon typing, targeting the conserved replicon sites of plasmid).
Response: Comment incorporated. The sentence was revised to remove “only” and “definitely.”
Comment Summary #451: The commenter suggested to include restriction digest for cross contamination and not include Sanger sequencing.
Response: Comment not incorporated. The term “sequencing” was used, but Sanger sequencing was not specified in the General Chapter. Restriction digest is not sensitive enough to detect cross contamination.
Comment Summary #452: The commenter suggested to add text to say equipment should also be assessed for potential plasmid to plasmid cross-contamination and potentially limited to a specific plasmid.
Response: Comment not incorporated. The suggestion to include equipment assessment and dedicating equipment for a specific product is specific to a user and is more detailed than the scope of the chapter.

Comment Summary #453: The commenter recommended moving this sentence “The primary control should be built into facility design and procedures to prevent cross-contamination during the manufacturing process.” to the beginning of the paragraph.
Response: Comment not incorporated. The sentence fits better at the end of the paragraph because it connects with the preceding sentence in the paragraph.
Comment Summary #454: The commenter recommended the following edits, ““Facilities must be able to uniquely identify all plasmid DNA starting materials produced at the site by the identity testing employed at release.”
Response: Comment not incorporated. The intent is to recommend and not to mandate the identity of all plasmid DNA starting materials.
Comment Summary #455: The commenter suggested to add the allowance for Next generation sequencing to be supplement other identity tests in order to detect low-level contaminating sequences; aligns to Biophorum defining the required critical quality attributes (CQAs) and phase requirements for mRNA/LNP product development and manufacture 2023. The following edits are suggested: “It is recommended that facilities be able to uniquely identify all plasmid DNA starting materials produced at the site by the identity testing employed at release. … However the **validated** sequencing procedures **(e.g. Sanger)** are not designed to specifically detect low level plasmid to plasmid cross-contamination. **Next generation sequencing (NGS) can be used for characterization testing of plasmids to identify low-percentage variants in the population which are not detectable by the validated method.”**
Response: Comment not incorporated. The statement in the chapter does not refer to a specific sequencing technique (e.g., Sanger or NGS). The sentence was edited for clarification but does not mention specific sequencing techniques: “However, the identity sequencing procedures are often not designed to specifically detect low level plasmid to plasmid cross-contamination.”

5.5 Receipt Testing
Comment Summary #456: The commenter suggested to change “Receipt Testing” to “Acceptance Testing”. Defining the specification for the acceptance test should be risk based, hence decisions on the specification and acceptance criteria is justified following risk assessment.
Response: Comment not incorporated. Acceptance testing is not commonly used for receipt testing and may be confused with release testing.
Comment Summary #457: The commenter suggested the following edits, “5.5 Receipt Testing **of Plasmid Starting Material** users may require receipt testing of **plasmid** materials from an external supplier (vendor). Receipt testing should at a minimum include identity determination.”
Response: Comment partially incorporated. The title of section 5 is Plasmid Quality; therefore, the subheading of section 5.5 does not need to be changed. The text was edited to specify the plasmid starting material.
Comment Summary #458: The commenter recommended the following edits, “Users may require **Acceptance** testing of materials from an external supplier (vendor). **Acceptance** testing should at a minimum include identity determination. The scope and frequency of **Acceptance** testing can be adjusted as the relationship with the vendor reaches various stages of maturation. For example, the User might verify all of the testing results in the Supplier’s Certificate of Analysis (COA) during the initial vendor qualification; once the vendor becomes a qualified supplier, **Acceptance** testing might be reduced to identity testing only or other subset of tests.”
Response: Comment not incorporated. Acceptance testing is not commonly used for receipt testing and may be confused with release testing.

Comment Summary #459: The commenter requested clarification that the Receipt Testing section is discussing receipt testing of plasmid itself and not receipt testing of raw materials used to make plasmid.
Response: Comment incorporated. The text was edited to specify the plasmid starting material.
Comment Summary #460: The commenter suggested to add a comment that "receipt testing may not always require chemical test. An option of a supplier CoA can be considered if sponsor can justify".
Response: Comment not incorporated. The chapter text states, "Users may require receipt testing," which indicates that testing of received materials is optional.

5.6 General Acceptance Criteria and Manufacturing Considerations
Comment Summary #461: The commenter suggested to move the first and last sentence of the paragraph of this section to the "SCOPE" section of this chapter, and delete the remainder of the text in this section.
Response: Comment not incorporated. The text is necessary in this location to make a statement about the plasmid quality acceptance criteria. As section 5 discusses plasmid quality, this is the best location for this text.

6.0 Conclusions
Comment Summary #462: The commenter suggested adding a statement to clarify if GMP does not apply to early phase products and applies to late-stage pivotal studies.
Response: Comment not incorporated. This statement in the Conclusion addresses compliance with GMPs, "This chapter shares common aligned understanding of this starting material's manufacture in compliance with GMPs." The GMP compliance is further expanded on in Figure 2 and Section 4.1 of the chapter.
Comment Summary #463: The commenter suggested that an additional chapter could be written on changes to the plasmid manufacturing process and comparability study design.
Response: Comment not incorporated. There are no plans to write another chapter on plasmid at this time, but this could be considered in the future.
Comment Summary #464: The commenter suggested deleting the Conclusion section, and moving the text to the beginning of the Introduction.
Response: Comment partially incorporated. The first sentence was changed to remove 'The goal of the chapter", so the text was more like a conclusion and less like an introduction.

References
Comment Summary #465: The commenter suggested adding references that are technical in nature.
Response: Comment not incorporated. The technical literature is constantly evolving, and the intent of this chapter is to describe existing regulatory guidelines and quality best practices.
Comment Summary #466: The commenter suggested adding these references:
21. USP, Analytical Procedures for mRNA Vaccine Quality (Draft Guidelines)- 2nd Edition
22. USP COVID-19 Vaccine Quality Assessment Toolkits, March 2023, 23.WHO TRS no 1039, Annex 3 Evaluation of the quality, safety and efficacy of messenger RNA vaccines for the prevention of infectious diseases: regulatory considerations.
Response: Comment partially incorporated. The first two references are online guides provided by USP, and not official text that can be cited in a chapter. A reference to the WHO TRS no 1039, Annex 3 has been added to the chapter.
Comment Summary #467: The commenter suggested to reference and alignment with

EMA guidance (Q&A on principals of GMP 24 Feb 2021).
Response: Comment not incorporated. The guidance is already referenced in the chapter.

General Chapter/Section(s):	<1085> GUIDELINES ON ENDOTOXINS TEST
Expert Committee:	General Chapters - Microbiology 2022
No. of Commenters:	12

General
Comment Summary #1: The commenter indicated their appreciation for the effort to streamline the text of the general informational chapter and include recombinant technologies. They also mentioned that their comments aim to further streamline the chapter and add clarification in some areas. They emphasized the importance of USP <1085> for global stakeholders in bacterial endotoxins testing.
Response: Comment acknowledged. The Expert Committee appreciates the recognition of the efforts to streamline the text and include recombinant technologies as described in the new USP chapter <86>.
Comment Summary #2: The commenter suggested that while the aim to reduce complexity and provide more freedom for interpretation in BET analysis is valuable, certain chapters have had important information removed that should guide what is expected by health authorities. They also noted that some restructured or extended chapters lack necessary detail. They recommend addressing these aspects in the new version to ensure clarity for those unfamiliar with the method.
Response: Comment acknowledged.
Generally, the chapter was streamlined where previous information could be construed as general cGMP and/or specific guidance found in existing regulatory guidances and/or general endotoxin texts.
Comment Summary #3: The commenter inquired whether Tachypleus amebocyte lysate (TAL) is acceptable for use.
Response: Comment not incorporated. TAL is implicitly permitted in <85> but is not applicable to <86>. This topic is outside the scope of <1085>.
Comment Summary #4: The commenter suggested clarifying references to *BET* throughout the document by specifying *BET techniques*, assuming the intent was to reference both USP <85> and <86>.
Response: Comment not incorporated. *BET* inherently refers to the methods described in both <85> and <86>, encompassing all relevant techniques. Therefore, additional clarification is not necessary.
Comment Summary #5: The commenter suggested replacing the term *assay* with *test* throughout the chapter to align with the title of USP <85> Bacterial Endotoxins Test, Ph. Eur. 2.6.14, and to avoid connotations of potency determinations associated with *assay*.
Response: Comment not incorporated. The term *assay* has historical significance in BET terminology and appears in approximately 30 references throughout the text. Maintaining consistency with the established precedent was deemed the preferred approach at this time.

Title
Comment Summary #6: The commenter suggested revising the title to better align with the titles of USP <*85*> and USP <*86*>.
Response: Comment Incorporated. The title has been changed to *"Guidelines for Bacterial Endotoxins Testing"* to improve consistency with related chapters.

Introduction

Comment Summary #7: The commenter suggested including a sentence on the addition of USP <86> for full context in the introduction.
Response: Comment partially incorporated. A reference to USP <86> will be included, but details on the lack of harmonization will not be added, as the PDG process addresses harmonization and it is assumed that USP chapters are US-specific unless otherwise mentioned.

Background
Comment Summary #8: The commenter suggested a change for improved readability in the background section. They proposed rephrasing the sentence to clarify that endotoxins from cell fragments may remain in sterile material and retain their ability to elicit a pyrogenic response.
Response: Comment partially incorporated. The proposed change was accepted, but the wording will remain "may not be" instead of stating definitively "are not," to reflect that endotoxins can be retained by charged sterilizing filters.
Comment Summary #9: The commenter suggested re-adding the "Endotoxins" section from the previous version, as it provides the basics of what pyrogens and endotoxins are, which is informative for those new to this testing.
Response: Comment not incorporated. Much of the information is retained in RSE:CSE Calibration, and endotoxin definitions have been moved to *<1117.1> Microbiological Chapters – Glossary*.
Comment Summary #10: The commenter suggested correcting the capitalization of "Gram-negative" to "gram negative" to align with CDC preferred usage guidelines, noting that "Gram" is only capitalized when part of a proper noun.
Response: Comment not incorporated. The current formatting follows USP style guidelines.
Comment Summary #11: The commenter suggested updating the terminology from "assay suitability" to "method suitability" to avoid future confusion.
Response: Comment not incorporated. The terms "method" and "assay" describe the test itself and are considered interchangeable in this context.
Comment Summary #12: The commenter suggested adding missing wording to clarify that endotoxins are an integral part within the outer cell membrane of Gram-negative bacteria (GNB). They also recommended including a sentence on how endotoxins are likely introduced into the manufacturing process and proposed inserting this information at the end of the first paragraph.
Response: Comment incorporated. The text was revised to include the suggested wording:
“Endotoxins are the major constituent of the outer membranes of Gram-negative bacteria composed of lipid A, the core polysaccharide, and the O-antigen polysaccharide; endotoxin is also known as lipopolysaccharide (LPS), see Microbiological Chapters—Glossary⟨1117.1⟩”
Comment Summary #13: The commenter suggested updating the terminology to align with the terms used in <85>, <86>, and <161> to avoid future confusion.
Response: Comment incorporated. The text was revised as follows:
“All laboratories must demonstrate that the chosen bacterial endotoxins test (BET) technique (<85> or <86>) is suitable for a specific product or material tested according to the applicable BET test for interfering factors (also referred to method suitability, method verification, or suitability). All are consistent with Verification of Compendial Procedures⟨1226⟩ …”
Comment Summary #14: The commenter suggested using a general term to describe both <85> and <86> by updating the text.
Response: Comment incorporated. The text will be revised as follows:
“(e.g., the reagent and/or endotoxins source…”
Comment Summary #15: The commenter suggested changing “BET test” to “BET” to avoid redundancy. This comment pertains to the terminology used in the relevant section. The commenter’s intention is to streamline the language for clarity.

Response: Comment partially incorporated. The decision is to delete "BET" and retain "test," as "BET" refers to the chapter while "test" specifies the section for interfering factors.

RSE:CSE Calibration

Comment Summary #16: The commenter questioned whether the provided definition of *Control Standard Endotoxin (CSE)* could encompass non-conventional CSEs or even naturally occurring endotoxins (NOEs) if prepared in a defined and controlled manner. They noted that the FDA has historically pushed back on such interpretations and suggested including a definition specifying the parameters that must be described or controlled.

Response: Comment not incorporated. The current definition aligns with established regulatory expectations. While non-conventional endotoxins may be used in certain research or development contexts, their acceptance as *CSE* would require regulatory endorsement. The parameters for CSE preparation are not further defined here, as existing regulatory guidance documents already outline relevant expectations.

Comment Summary #17: The commenter suggested revising the definition of *Control Standard Endotoxin (CSE)* to specify that it is produced under defined and controlled conditions, as well as calibrated against the *Reference Standard Endotoxin (RSE)*. They also noted that key parameters for CSE preparation, such as Gram-negative bacteria (GNB) source, growth medium, purification (if performed), and stabilization reagents, are missing.

Response: Comment incorporated. The text has been revised to state:

“CSE are any endotoxin preparation, solid or aqueous, produced under defined and controlled conditions, that are calibrated as a secondary reference standard against the primary RSE.”

Comment Summary #18: The commenter suggested removing the redundant use of *"assay"* in the phrase *"…parameters such as standard curves and assay controls…"* since controls are already identified as an *assay* parameter.

Response: Comment incorporated. The text has been revised to remove the redundant word for improved clarity.

Comment Summary #19: The commenter suggested changing “USP Endotoxin Reference Standard” to “USP Reference Standard Endotoxin” to align with the RSE acronym.

Response: Comment incorporated. The text now reads "USP Reference Standard Endotoxin (RSE)."

Comment Summary #20: The commenter questioned the intent of the new text stating that "all CSE should be adequately characterized, e.g., for uniformity and stability." They suggested specifying that this applies to *liquid CSEs* if the intent is to distinguish them from those provided by qualified vendors.

Response: Comment partially incorporated. Sentence was revised:

“All CSE should be adequately characterized because purified endotoxins are generally more susceptible to environmental and matrix effects than endotoxins in nature (3).”

Comment Summary #21: The commenter indicated that the sections on gel clot and quantitative assays lack clarity regarding the reliance on vendor-supplied reagent kits for CSE calibration and potency determination. They suggested adding language to clarify that verification provided by a qualified supplier exempts users from repeating these assays for the specified combination of lysate and CSE lots.

Response: Comment incorporated. The following sentence was added to address this clarification: "If a kit is purchased from a reagent vendor, the vendor will conduct the lot-specific standardization and provide a lot-specific certificate of analysis (CoA) in the kit, which should be retained for reference in the laboratory."

Comment Summary #22: The commenter suggested updating the text to use the more general term *BET test kits* instead of *LAL test kits* to encompass both <*85*> and <*86*>.

Response: Comment incorporated. The text has been updated to: "CSE may be included in test kits purchased from reagent manufacturers."

Comment Summary #23: The commenter suggested reinstating removed text emphasizing the biological variability of BET reagents and the necessity of calibrating each unique combination of reagent lot and *Control Standard Endotoxin (CSE)* lot against the *Reference Standard Endotoxin (RSE)*.
Response: Comment partially incorporated. Instead of fully restoring the original text, the following statement has been added: "Prior to the use of a CSE, it should be calibrated against the current RSE lot."
Comment Summary #24: The commenter suggested updating the text to clarify that gel-clot reagents have an associated sensitivity and that lysate reagents described in *<85>* are FDA-licensed in the United States. They also noted that FDA does not license *Control Standard Endotoxin (CSE)* preparations.
Response: Comment not incorporated. The addition does not provide clarity.

CSE Calibration and Potency Determination Using Quantitative Kinetic and Endpoint Assays
Comment Summary #25: The commenter suggested adding additional clarity regarding the impact of the method on potency determination when using a CSE lot with a different reagent lot and/or method.
Response: Commenter incorporated. The text will be revised as follows:
“If the lab wants to use this CSE lot with a different reagent lot and/or method – be it kinetic, endpoint, or gel-clot – the calibration must be repeated because potency determination references a specific combination of reagent lot, CSE lot, and method.”
Comment Summary #26: The commenter suggested changing "the" to "each" to clarify that multiple dilutions of CSE are tested. They also recommended replacing "interpolated" with "calculated" as interpolation is a less precise determination of a value from a standard curve.
Response: Comment partially incorporated. The text was revised as follows:
"...from dilutions of the CSE are calculated from an RSE standard curve to yield the activity of each CSE dilution in EU/mL."
Comment Summary #27: The commenter suggested edits for readability to clarify the transition from EU/vial to EU/mL and ng/mL, explaining that the RSE vial is rehydrated in Water for BET and the CSE is filled by weight and typically rehydrated in Water for BET.
Response: Comment not incorporated. The intended audience are expected to be familiar with the preparation procedures.
Comment Summary #28: The commenter suggested adding a new bullet point to provide more clarity for end users regarding modern endotoxin analysis software.
Response: Comment not incorporated. The Expert Committee would need to address additional concerns to add this bullet point. Such concerns include software availability for all potential users, validation of the application, the accessibility of the application preventing modification by end-users, data integrity considerations, etc.
Comment Summary #29: The commenter suggested making changes for readability and accuracy by updating the text to clarify that the analyst dilutes the CSE to known concentrations.
Response: Comment incorporated. The text was revised as follows:
“Next, the analyst dilutes the CSE to known concentrations in EU or ng/mL and tests the dilutions as unknowns (see Table 1).”
Comment Summary #30: The commenter suggested updating the text to fix typos and include *Vmean* as another validated output of the BET test, alongside onset (reaction) times and relative fluorescence units (RFU).
Response: Comment not incorporated. The term *Vmean* has limited broad applicability to current BET technologies and is specific to certain manufacturers. To maintain general applicability, the existing terminology was retained.

Screening and Qualification of Labware

Comment Summary #31: The commenter requested reinstating a removed paragraph about controls with plastic materials after molding, emphasizing that it provided important guidance on consumables and vendor Certificates of Analysis (CoAs) to ensure end users select appropriate labware for BET.

Response: Comment not incorporated. The background information on plastic molding and its impact on endotoxins was removed because similar information is available in general endotoxin references. However, vendor CoA interpretation scenarios were retained, and USP *<85>* and *<86>* already define the appropriate labware required for BET.

Comment Summary #32: The commenter noted that *in vivo* testing is not performed on consumables and suggested that the term *non-pyrogenic* may be misleading in this context.

Response: Comment not incorporated. The term *non-pyrogenic* is commonly used in vendor specifications for labware, and while *in vivo* testing is not standard for consumables, the existing text reflects how vendors describe their products. However, further clarification may be considered in future revisions.

Comment Summary #33: The commenter recommended not adding a requirement that labware used for the Bacterial Endotoxins Test (BET) be explicitly free of interfering factors, arguing that this would be a more stringent requirement than what is currently stated in USP <85>. They also expressed concerns about the increased complexity and cost of testing labware without a clear safety benefit.

Response: Comment partially incorporated. The statement was revised to clarify that BET chapters require users to ensure that labware does not interfere with the test. However, the other suggested changes were not incorporated, as the requirement aligns with ensuring test accuracy and reliability, and users remain responsible for verifying potential interferences in their specific testing conditions.

Comment Summary #34: The commenter recommended re-wording the section for conciseness while maintaining its essential meaning.

Response: Comment incorporated. The revised text ensures that critical details regarding qualification and storage validation are retained.

Comment Summary #35: The commenter suggested breaking up the discussion between glass and plastic labware and expanding the guidance on storage conditions, noting that storage considerations apply not only to *Reference Standard Endotoxin (RSE)* and *Control Standard Endotoxin (CSE)* but also to samples containing endotoxin.

Response: Comment incorporated. The text has been revised to distinguish between glass and plastic labware by adding: "There are typically two types of labware used for BET: glass and plastic labware." Additional details have been provided, including a reference to *Depyrogenation <1228>* for glassware. The discussion on storage conditions remains, with clarification that it applies to both RSE/CSE and endotoxin-containing samples.

Comment Summary #36: The commenter recommended moving the final sentence regarding the storage of RSE and CSE dilutions to the *RSE:CSE Calibration* section, stating that it is more relevant to storage guidelines than to labware qualification.

Response: Comment not incorporated. The Expert Committee determined that the impacts of storing CSE/RSE are directly correlated to the material of construction in which they are stored, making this guidance appropriate within the *Screening and Qualification of Labware* section.

Comment Summary #37: The commenter suggested clarifying the storage statement by specifying a defined limit, such as "past assay execution" or another appropriate term, to ensure clear guidance on storage restrictions.

Response: Comment incorporated.

Analyst Qualification

Comment Summary #38: The commenters suggested re-adding detailed sections on Analyst Qualification, emphasizing that this section is key to bacterial endotoxin testing and ensuring an

analyst can adequately construct and test a standard curve. They noted that the section was valuable in offering guidance on how to perform this aspect of the testing and that the majority of lab errors are operator errors, making the full section useful. They also mentioned that the minimum requirement for qualification of analysts on BET testing is provided, but on-the-job training details are missing, which would be helpful for newly trained analysts.
Response: Comment not incorporated. The section was simplified for clarity as much of the previous text is general cGMP guidance not specific to endotoxin; guidance can be found in general endotoxin texts and regulatory guidance documents.
Comment Summary #39: The commenter suggested changing the term 'assay sensitivity' to 'sensitivity' in the Analyst Qualification section.
Response: Comment not incorporated. 'Assay sensitivity' is historical BET language and is referenced 30 times throughout the document.
Comment Summary #40: The commenter suggested that if the laboratory uses a system with an archived standard curve, it is recommended that the laboratory devise another method of ensuring that the analyst's technique in sample preparation and dilution is sound. They emphasized the importance of distinguishing between cartridge-based systems and other systems where the archived curve is initially determined by a qualified analyst. They suggested replacing the current text with: "If the laboratory uses a cartridge system, it is recommended that the laboratory devises another method of analyst qualification and proficiency testing. Competency and proficiency assessment should require analysts to prepare and dilute endotoxin standards and samples." Alternatively, they suggested removing the sentence.
Response: Comment partially incorporated. The sentence regarding the use of archived standard curves in laboratory systems has been removed.
Comment Summary #41: The commenter suggested that the term "incorrect" is relative and recommended specifying that values should be within a 2-fold range from the known value. If the calculated values are incorrect outside of this range, it may raise concerns about the analyst's technique.
Response: Comment partially incorporated. The last sentence in the paragraph has been removed.
Comment Summary #42: The commenter indicated that the reference to General Chapter <1117> is factually incorrect, as it contains no requirements. They pointed out that the current good manufacturing requirements for training are found in 21 CFR 211.25. To address this, the commenter recommended either removing the reference to General Chapter <1117> or revising the sentence to clarify the relationship between general laboratory training for analysts and the relevant regulations.
Response: Comment incorporated. The sentence will be revised as proposed to read: "General laboratory training for analysts is good laboratory practice (see Microbiological Best Laboratory Practices <1117>), as well as a current good manufacturing practice requirement (21 CFR 211.25(a))."

Equipment and Instrumentation Calibration and Qualification
Comment Summary #43: The commenters requested to re-add sections that were removed, which included information on calibrating/qualifying per the vendor, ensuring uniformity of heating, validating dry heat ovens, and compliance of computer software with federal regulations and standards.
Response: Comment not incorporated. The sections were simplified for clarity as much of the previous text is general cGMP guidance not specific to endotoxin. Guidance on these topics can be found in regulatory guidance documents. The decision to simplify the text was made to focus on endotoxin-specific information and avoid redundancy with existing regulatory guidance.
Comment Summary #44: The commenter suggested updating the text to include depyrogenation ovens, which are quite common, in the list of equipment that should be qualified.

Response: Comment incorporated. Text was revised as follows: “All instrumentation and equipment used in the BET, including (but not limited to) pipettes, water baths, heat blocks, incubating plate readers, and depyrogenation ovens should be qualified using proper scientific standards and according to approved protocols and maintenance schedules.”
Comment Summary #45: The commenter requested to re-add a paragraph that was removed, which included references to the performance of CSV and controls for temperature-critical equipment for the test, as they believe these are important to be included.
Response: Comment not incorporated. The section was simplified for clarity as much of the previous text is general cGMP guidance not specific to endotoxin; guidance can be found in regulatory guidance documents.

Calculating Endotoxin Limits for Drug and Biological Products
Comment Summary #46: The commenter suggested that the abbreviation HIMA should be explained or written out in the context of the pyrogenic threshold limit.
Response: Comment incorporated. The text has been updated to read: "The 5 EU/kg pyrogenic threshold limit incorporates safety factors, as the HIMA (Health Industry Manufacturers Association) study determined this level to attain 50% pass-fail with a 95% confidence interval (ED50)."
Comment Summary #47: The commenter suggested moving the entire text from the section on calculating endotoxin limits to the BACKGROUND section and updating the text to remove "the worst case, i.e." and maintain that the lowest body weight is considered.
Response: Comment incorporated. Text was moved to the "Common Test Interferences" section.
Comment Summary #48: The commenter suggested shortening the text to: “5 EU/kg pyrogenic threshold is the current public standard; any other number must be justified by supporting clinical context and data,” arguing that providing further information may seem to justify going higher.
Response: Comment not incorporated. The intent was not to address going higher but to provide education for health authorities asking for lower limits (e.g., 2X cuts to 2.5 EU/kg) without justification against the public health standard. Based on committee discussion, it was decided to provide education on the basis of the establishment of the 5 EU/kg pyrogenic threshold.
Comment Summary #49: The commenter suggested updating the text to remove the phrase "according to current understanding and experimental evidence" as it may be misleading.
Response: Comment incorporated. The text will be revised as follows:
"An endotoxin limit specification for a compendial article is the allowable amount of endotoxin activity that can be safely contained in a parenteral product."
Comment Summary #50: The commenter suggested that the lowest body weight might not necessarily be the worst case and recommended removing "the worst case, i.e." and maintaining that the lowest body weight is considered. They also questioned why the dose is specifically outlined in mg/kg when it can also be in mL/kg.
Response: Comment incorporated. The text was revised as follows:
“The body weight factor selected for pediatric and other special category patients should be considered, i.e., lowest body weight in targeted patient populations that can receive the greatest recommended dose and/or the most conservative dose in milligrams per kilogram.”
Comment Summary #51: The commenter suggested that for products diluted for infusion, the endotoxin contribution from both the product and the diluent should be considered when calculating the endotoxin specification. They noted that while this is addressed for compounding intrathecal products in the section “Relevance of Limits for Compounded Sterile Preparations,” it is not mentioned for non-compounded intrathecal or intravenous infusions.
Response: Comment not incorporated. The inclusion of a third-party diluent outside the quality system of a drug product manufacturer involves complex and debated endotoxin considerations

within the industry. It is assumed that drug products and diluents typically contain endotoxin levels near their respective limits, which is not supported by available data. The guidelines recommend using compendial grade diluents for compounded sterile preparations, allowing the compounder to test the finished product prior to administration, as both the diluent and compound are under their control.
Comment Summary #52: The commenter suggested changing “endotoxin limit specification” to “endotoxin limit” for consistency with the terms used in General Chapters *<85> Bacterial Endotoxins Test* and *<161> Medical Devices – Bacterial Endotoxin and Pyrogen Tests*. This change aims to enhance clarity and uniformity in terminology across relevant documents.
Response: Comment Incorporated. The Expert Committee accepted the suggestion and replaced seven instances of the term to ensure consistency throughout the text.
Comment Summary #53: The commenter suggested deleting the phrase “Because products do not have glucan specifications” from the second paragraph, third sentence, as it was deemed irrelevant to the rest of the sentence.
Response: Comment incorporated. The text was deleted.
Comment Summary #54: The commenter suggested that the content in the second paragraph of the "Calculating Endotoxin Limits for Active Substances and Excipients" section is more relevant to the “Common Test Interferences” section. They recommended moving this paragraph to that section and placing it as the second-to-last paragraph.
Response: Comment incorporated. The Expert Committee has accepted the suggestion, and the content has been moved to the “Common Test Interferences” section as proposed.

Calculating Endotoxin Limits for Medical Devices
Comment Summary #55: The commenter suggested adding clarity and relevant information by stating that the endotoxin limit for an extract is inversely proportional to both the volume of solvent used for the extraction and the number of devices extracted.
Response: Comment not incorporated. The volume is in the denominator, and the device number is in the numerator. The original statement is correct as it stands.
Comment Summary #56: The commenter suggested including the volume of liquid used for extraction when the EU/ml unit is applied, noting a potential conflict with the statement regarding the classification of liquid and solid medical devices. The commenter highlighted that the current wording may create confusion regarding how endotoxin limits are calculated for these products.
Response: Comment partially incorporated. The last sentence in the section has been deleted to enhance clarity regarding the extraction process for medical devices, which generally do not treat them as drug products except in rare cases.
Comment Summary #57: The commenter suggested describing the circumstances under which the 0.2 EU/ml limit is used instead of the 0.2 EU/device limit in the section on Calculating Endotoxin Limits for Medical Devices.
Response: Comment not incorporated. The existing reference to Table 2 provides sufficient context regarding the use of EU/mL for intraocular fluids and EU/device for anterior segment (ocular) devices.

Maximum Valid Dilution
Comment Summary #58: The commenter suggested clarifying the statement regarding product concentration for products administered on a milliliter-per-kilogram basis or for medical device extracts, specifically noting that the endotoxin limit for extraction fluid is already in EU/mL.
Response: Comment partially incorporated. The last sentence of the bullet has been deleted, and the first sentence has been revised to: *"The product concentration equals the concentration per milliliter."*

Comment Summary #59: The commenter suggested adding a bullet point in the *Additional Information* section to clarify that if units are pooled, the *Maximum Valid Dilution (MVD)* must be adjusted accordingly.
Response: Comment not incorporated. This topic is already addressed in the *Pooling* section.
Comment Summary #60: The commenter suggested adding a bullet point under *Maximum Valid Dilution* to provide clarification on rounding, noting that the chapter does not currently address this topic.
Response: Comment not incorporated. Rounding rules are already covered in the *General Notices*, which apply broadly to all USP chapters, including this one.
Comment Summary #61: The commenter suggested defining the acronym MVD in the text as "maximum valid dilution (MVD)."
Response: Comment incorporated. The text has been updated to include the definition of the acronym MVD as "maximum valid dilution (MVD)."
Comment Summary #62: The commenter suggested changes to the section on Maximum Valid Dilution. They recommended eliminating the first sentence due to its confusing nature and lack of informative value. Alternatively, they proposed rephrasing the first sentence to clarify that the MVD does not limit product dilution when determining the true amount of product contamination during an out-of-specification (OOS) investigation. Additionally, they requested an edit to the last sentence to avoid redundancy.
Response: Comment incorporated. The text of the first sentence has been revised to reflect the recommended wording.
Comment Summary #63: The commenter suggested changing the phrase "dilution to extinction" to "dilution to extinction potentially beyond the MVD" in the second-to-last sentence for clarity.
Response: Comment incorporated. The phrase has been replaced with the proposed text.

Method Suitability – Verification Test for Interfering Factors
Interference Screening
Comment Summary #64: The commenter suggested adding a sentence at the end of the *Interference Screening for Drug Products, Including Biological Drugs* section to acknowledge that validated samples may still show interference when tested at a later time. The commenter noted that while manufacturing processes are generally controlled, some products—particularly biologicals—may exhibit lot-to-lot variability that could lead to increased interference.
Response: Comment not incorporated. The chapter assumes that manufacturing processes are sufficiently stable to minimize interference variability over time. While some degree of variability may occur, the existing guidance on interference screening is intended to help detect and address such issues as they arise.
Comment Summary #65: The commenter noted that the PPC criteria discussion applies only to photometric and fluorometric methods, not the gel-clot method, and suggested revising the text to clarify this distinction.
Response: Comment incorporated. The text has been updated to specify that the PPC recovery criteria of 50%–200% applies *only to photometric and fluorometric techniques*. Additionally, the recommendation to optimize assay conditions for a tighter PPC range (e.g., 75%–150%) remains included to help prevent future PPC issues during routine testing.
Comment Summary #66: The commenter noted that the title *Method Suitability – Verification Test for Interfering Factors* suggests that all the terms are synonymous and recommended explicitly clarifying this at the beginning of the *Method Suitability* section.
Response: Comment not incorporated. It is acknowledged that various terms are used interchangeably for the same concept. If any simplification were to be made, *Method Verification* would be the preferred term to align with USP *<1226>*.

Comment Summary #67: The commenter suggested revising the subsection title *Interference Screening for Drug Products, Including Biologic Drugs* to better align with the expectations outlined in USP *<1226>*, proposing *Verification of Suitability* as a possible alternative.
Response: Comment incorporated. The title has been updated.
Comment Summary #68: The commenter suggested reinstating removed text that provided guidance on reviewing endotoxin testing methodology for new raw material vendors, performing site audits, confirming Certificate of Analysis (CoA) accuracy, and managing changes through quality agreements. The commenter recommended moving this content under the *Interference Screening for Drug Products, Including Biologic Drugs* section.
Response: Comment not incorporated. The section was simplified for clarity, as much of the removed text reflects general current good manufacturing practice (cGMP) principles rather than endotoxin-specific guidance. Relevant regulatory guidance documents already provide comprehensive information on vendor qualification, change control, and CoA verification.
Comment Summary #69: The commenter noted that the sentence *"…testing are provided in the BET and derived from…"* is incomplete and suggested specifying *BET* as *USP <85>* for clarity.
Response: Comment partially incorporated. While *BET* inherently refers to both *<85>* and *<86>*, clarifying text has been added to the introduction to ensure this distinction is clear.
Comment Summary #70: The commenter suggested changing "biologic drugs" to "biological products" in the first subheading of the Method Suitability – Verification Test for Interfering Factors section for consistency with the rest of the chapter.
Response: Comment partially incorporated. The section title has been changed to "Interference Screening."

Common Test Interferences
Comment Summary #71: The commenter raised concerns about the term "standard" and the phrase "or both" in the context of product-specific interferences, suggesting that "standard" might be a holdover from an earlier version and that the phrase implies only two targets instead of three.
Response: Comment incorporated. The text was revised to remove "standard, or both" from above Table 3, acknowledging that while it is rare, it is conceivable that someone could be using a product standard.
Comment Summary #72: The commenters suggested re-adding the "interferes with" column to Table 3, as the new "potential causes" column is less informative. They recommended using the term "BET enzymatic reaction" instead of "LAL" or "cascade" to be inclusive of recombinant options.
Response: Comment not incorporated. Table 3 is designed to be inclusive of both LAL and recombinant reagent interference mechanisms. The "potential causes" column provides more comprehensive information than the previous table.
Comment Summary #73: The commenter suggested re-adding the paragraph about dispersing agents and glucan interference, as dispersing buffers are used to mitigate interference and glucan interference is still an important source for traditional LAL reagents.
Response: Comment not incorporated. The glucan discussion is maintained in the section Calculating Endotoxin Limits for Active Substances and Excipients.
Comment Summary #74: The commenter pointed out that the sentence "All BET assays must be conducted at neutral pH" differs from USP <85> and ICH Q4B Annex 14, which allow for pH adjustment if necessary. They asked if this wording will be modified in an upcoming revision to USP <85>.
Response: Comment not incorporated. Making a change to <85> would be assessed through other revisions, which is out of the scope of this chapter.

Comment Summary #75: The commenter suggested replacing "assure" with "ensure" to clarify the meaning, as "ensure" means to make certain, while "assure" means to remove or reduce doubt in someone's mind.
Response: Comment incorporated. The text will be revised to replace "assure" with "ensure."
Comment Summary #76: The commenter suggested including dispersing agents as a potential buffer component to overcome test interferences.
Response: Comment incorporated. The text will be revised as follows:
"A majority of test interferences are overcome by the techniques described in Table 3. Dispersing agents may be added to diluents to further mitigate interference and their value as an additive is typically considered during method development."

Qualifying Test Preparation Methods Other than Dilution
Comment Summary #77: The commenters noted that the sentence *"…heating or ultrafiltration, the BET instructs…"* appears incomplete and suggested specifying *USP <85>* or *<86>* for clarity. Additionally, they recommended providing further clarification on how to challenge the test item with endotoxin and what recovery expectations should be, as this is a common source of confusion for laboratories handling treated samples.
Response: Comment partially incorporated. The sentence has been revised to include *"as required in <85> or <86>."* to clarify the reference. Additional guidance on challenge procedures and recovery expectations was not added at this time.
Comment Summary #78: The commenter noted that the revised text is less detailed than the previous version and suggested reinstating the reference to *Standard Endotoxin* to avoid potential confusion for end users.
Response: Comment not incorporated. While the original version mentioned *Standard Endotoxin*, reintroducing this term would require clarification regarding *Reference Standard Endotoxin (RSE)* versus *Control Standard Endotoxin (CSE)*. The current language is intended to be more general and applicable across different contexts.
Comment Summary #79: The commenter suggested changing the title *"Qualifying Test Preparation Methods Other than Dilution"* to *"Sample Treatment Validation"* for clarity.
Response: Comment partially incorporated. The term *validation* is not appropriate in this context. However, for improved clarity, the title has been revised to *"Qualifying Test Sample Preparation Methods Other than Dilution."*

Sampling of Finished Product
Comment Summary #80: The commenters provided several suggestions related to the *Sampling of Finished Product* section, including relocating content to *Screening and Qualification of Labware*, improving readability, reinstating vendor qualification guidance, and clarifying terminology related to hold times.
Response: Comment not incorporated. The existing placement of the text within *Sampling of Finished Product* is appropriate, as it introduces the discussion on hold time studies.
The suggested readability update (*"in fact, depyrogenation processes require extreme conditions"*) does not significantly improve clarity, so the original wording remains unchanged.
The removed text on vendor qualification and process changes was considered general cGMP guidance rather than endotoxin-specific. Regulatory guidance documents provide detailed expectations for vendor qualification and change control, making its inclusion in USP <1085> unnecessary.
Comment Summary #81: The commenter suggested replacing "adequately mixed" with "adequately vortexed" to emphasize the importance of vortexing in ensuring that endotoxins stuck to surfaces are available in samples.
Response: Comment not incorporated. The decision was made to reject the proposed change because there are multiple methods available for mixing, not just vortexing.

Comment Summary #82: The commenter indicated that the *Sampling* section was shortened, resulting in the removal of relevant information on sampling and raw material management.
Response: Comment not incorporated. The section was revised for clarity, and the key concepts remain available in general endotoxin testing references. Additionally, the section was reorganized to distinguish between raw material and finished product sampling, improving usability without omitting essential guidance.

Pooling
Comment Summary #83: The commenter requested reinstating removed text that highlighted a drawback of pooling—specifically, the potential dilution of endotoxins, which could obscure variability in endotoxin content among pooled units.
Response: Comment not incorporated. This concern is already addressed in the first bullet point of the *Pooling* section, ensuring that the potential risks of pooling remain clearly communicated.
Comment Summary #84: The commenter suggested specifying the circumstances in which the pooling method is applicable and when the direct method is advisable.
Response: Comment not incorporated. The *Pooling* section already provides a reasonable description of appropriate and inappropriate uses of pooling.
Comment Summary #85: The commenter suggested rewording a sentence in the *Pooling* section to clarify that reducing the *Maximum Valid Dilution (MVD)* compensates for potential dilution effects due to pooling, rather than affecting the *Endotoxin Limit Concentration (ELC)*. They also proposed multiplying the measured endotoxin results by the number of pooled units (a "Pooling Factor") to account for possible uneven endotoxin distribution.
Response: Comment not incorporated. While it is acknowledged that reducing the MVD helps protect the ELC, the proposed concept of a *Pooling Factor* is not aligned with established BET principles. Combining samples does not change the validated method's limit of quantification (LOQ), and multiplying results by a pooling factor is not a recognized correction approach in endotoxin testing.
Comment Summary #86: The commenter suggested adding a note after the sentence discussing the adjusted *Maximum Valid Dilution (MVD)* value to clarify how to handle resulting values that are not whole numbers, recommending a worst-case truncation approach.
Response: Comment partially incorporated. A bullet point has been added, stating: *"Truncating of calculated values may be used to establish a conservative (worst-case) MVD value."*
Comment Summary #87: The commenter noted that the reference associated with pooling units was incorrect and should be changed from "19" to "4.
Response: Comment incorporated. The reference has been correctly numbered.

Calculation of Endotoxin Content
Comment Summary #88: The commenter suggested updating the description of raw quantitative bacterial endotoxin test (BET) results to specify that photometric and fluorometric BET results are measured using optical density (OD), Vmean, or relative fluorescence units (RFU) before conversion to EU/mL. The commenter also emphasized the importance of including the rate assay.
Response: Comment not incorporated. The term *Vmean* is specific to one manufacturer and is not broadly applicable across all BET methodologies. The existing language is intended to be inclusive of general measurement principles without favoring proprietary terminology. Additionally, related comments on measurement terminology are being considered collectively.

Out-of-Specification Results and Retesting Considerations
Comment Summary #89: The commenters suggested expanding the *Out-of-Specification Results and Retesting Considerations* section to provide more detailed guidance on investigation steps, including sampling, number of replicates, and whether results should be

averaged. They also expressed concern that a significant portion of the section was removed, noting that end users frequently seek guidance on this topic.
Response: Comment not incorporated. The section was simplified to focus on endotoxin-specific considerations, as much of the removed content reflected general cGMP principles applicable to all OOS investigations. Comprehensive guidance on OOS handling, including investigation frameworks, can be found in regulatory documents, making detailed discussion within this chapter unnecessary.
Comment Summary #90: The commenter noted that the *Out-of-Specification Results and Retesting Considerations* section was shortened, removing relevant information on conducting investigations, including Phase 1 investigations. They recommend maintaining the previous version, as it is commonly used as a reference by end users.
Response: Comment not incorporated. The section was simplified for clarity, as much of the removed content reflected general cGMP principles rather than endotoxin-specific out-of-specification (OOS) guidance. Regulatory guidance documents provide comprehensive instructions on OOS investigations, making the detailed discussion unnecessary within this chapter.
Comment Summary #91: The commenter suggested clarifying the statement in <*85*> regarding the use of the gel-clot test in cases of doubt or dispute, emphasizing that it applies only to conflicts between regulatory agencies and testing laboratories. They also recommended including a similar clarification for <*86*>, assuming it contains a comparable statement.
Response: Comment not incorporated. <*86*> does not include similar language, as the gel-clot test is not considered the gold standard for recombinant bacterial endotoxins tests (rBET). Additionally, conflicts of this nature are typically resolved at the legal or regulatory level between the firm and the health authority, making further clarification in this chapter unnecessary.
Comment Summary #92: The commenter suggested a revision to the second sentence of the first paragraph in the "Out-of-Specification Results and Retesting Considerations" section. They indicated that the term “valid result” could be misinterpreted and proposed a clearer phrasing: “The suspect result is considered valid (i.e. a true OOS result) until a comprehensive investigation clearly demonstrates otherwise.”
Response: Comment incorporated. The proposed text will be replaced as suggested to enhance clarity and prevent misinterpretation.
Comment Summary #93: The commenter suggested strengthening the statement in the second paragraph's last sentence regarding invalid bacterial endotoxin tests. They noted that while invalid tests are a reality, there are manufacturers with unreasonably high invalid test rates who fail to take action. The commenter proposed replacing the sentence with a version that emphasizes the need to track and trend all invalid tests to determine if corrective action is necessary.
Response: Comment partially incorporated. The suggestion to clarify the concern about a high number of invalidated out-of-specification (OOS) tests has been acknowledged, and "OOS" has been added in front of "tests" to enhance clarity. However, the specific definition of what constitutes a high frequency of invalid OOS rates was not included, as it could be subjective and problematic, and is not specified in the referenced 2022 FDA OOS Guideline.

Standard Curve Control
Comment Summary #94: The commenter recommended updating the statement to include *Vmean* for completeness in monitoring standard endotoxins.
Response: Comment not incorporated. The Expert Committee determined that *Vmean* is vendor-specific and should not be included to maintain general applicability across different testing platforms.
Comment Summary #95: The commenter indicated that the statement, *"Because the y-intercept is the point where x = 0, the y-intercept is really at the 1 EU standard,"* is confusing and unnecessary. They recommended its removal.

Response: Comment incorporated. The statement has been removed to improve clarity and avoid potential confusion.
Comment Summary #96: The commenter indicated that if archived standard curves are to be used, the chapter should provide more specific guidance on their effective and safe use. They suggested defining conditions under which archived curves are appropriate, including stricter recovery specifications for the mid-point control.
Response: Comment not incorporated. The published language is deemed acceptable as it already allows end-users to justify the frequency and acceptance criteria for using an archived curve.
Comment Summary #97: The commenter suggested revising the section on standard curves to improve clarity and accuracy by distinguishing between two main types of BET assays based on their standard curve outputs. The proposed update included specifying the proportional and inversely proportional relationships between the measured signal and endotoxin concentration while ensuring consistency in correlation coefficient requirements.
Response: Comment partially incorporated. The proposed text was accepted with modifications, including the removal of "Vmean," as it is addressed in other comments.

Alternative Methods
Comment Summary #98: The commenter is seeking clarification on the validation requirements for methods described in USP <85> and <86> in the updated version of <1085> and suggests that the section be formulated more clearly to avoid misinterpretation.
Response: Comment not Incorporated. Reference <86> FAQ for validation/verification discussion. The proposed Alternative Method section is clear and is meant for orthogonal endotoxin test methods (e.g., chemical, physical, etc.).
Comment Summary #99: The commenter suggested adding the MAT (monocyte activation test) as an alternative assay to determine pyrogenicity in the section of the monograph on alternative methods, in case the tested product interferes with reagents used in other BET.
Response: Comment not incorporated. MAT is out of scope of <1085> as this chapter provides guidance for running the BET/rBET
Comment Summary #100: The commenter suggested not referencing the new General Chapter <86> Bacterial Endotoxins Test Using Recombinant Reagents in this section unless it appears in the USP-NF as “to be official” or “official.” The substance of the comment reflects a concern about the timing of referencing new chapters before they are officially recognized.
Response: Comment not incorporated. The chapter is published and is currently designated as "to be official," which supports its inclusion in the section.
Comment Summary #101: The commenter indicated that the statement regarding the validation and verification of alternative methods for detecting bacterial endotoxins is factually incorrect. They pointed out that while General Notices 6.30 requires validation, it does not mandate the use of <1225>. Additionally, they referenced USP's clarification that General Chapters numbered 1000 to 1999 are for informational purposes only and do not impose mandatory requirements. To address this, the commenter recommended modifying the language to clarify the references to <1225> and <1226>.
Response: Comment incorporated. The suggested changes have been accepted, and the text will be revised to read: "…. must be validated (see <1225>) and subsequently verified (see <1226>).”

CSE Calibration Gel Clot
Comment Summary #102: The commenter suggested adding a new sentence to clarify the use of full values of the labeled sensitivity when calculating the potency of the CSE, as the chapter does not currently discuss rounding.
Response: Comment not incorporated. The treatment of experimental data, including rounding rules, is covered in other general chapters, General Notices, and industry standard practices.

Additionally, all GMP facilities should have SOPs defining the treatment of test data. Defining rounding rules is beyond the scope of this chapter.

Table 1

Comment Summary #103: The commenter suggested that the example in Table 1 should include replicate determinations to align with the final bullet point, which states that replicate determinations for any given dilution should be considered individually rather than averaged.
Response: Comment not incorporated. The example is intended to illustrate the calculation method rather than prescribe specific experimental design requirements.

Table 3

Comment Summary #104: The commenter recommended updating the statement to clarify that recombinant reagents do not react with glucans, thereby eliminating glucan interference.
Response: Comment incorporated. The revised wording more accurately reflects the function of recombinant reagents in preventing glucan-related interference.
Comment Summary #105: The commenter recommended reinstating the statement about dispersing agents, emphasizing that they are still needed in some specific situations.
Response: Comment incorporated. The sentence has been restored to acknowledge the role of dispersing agents in mitigating interference during method development.
Comment Summary #106: The commenter recommended updating the wording to replace *lysate* with *reagent* to align with both USP <85> and <86>.
Response: Comment incorporated. The revised text ensures consistency with terminology used in both chapters while maintaining clarity on pH adjustment procedures.

Stability

Comment Summary #107: The commenter suggested that the stability BET sample is not an indicating test for stability and inquired about the frequency and criteria for BET sampling for stability studies.
Response: Comment not incorporated. The Expert Committee decided that this information is not needed at this time. However, they will consider adding this topic to the FAQ section in the future for further clarification.
Comment Summary #108: The commenter suggested that for stability samples, the testing frequency and number of samples need to be defined.
Response: Comment not incorporated. The Expert Committee decided that this information is not needed at this time, as endotoxin testing is not considered a stability-indicating method. Therefore, defining testing frequency and the number of samples for stability studies is not applicable in this context.

Laboratory Environmental Conditions

Comment Summary #109: The commenters suggested reinstating the *Laboratory Environmental Conditions* section, emphasizing its value in educating end users on best practices for laboratory conditions, aseptic techniques, and maintaining a controlled testing environment.
Response: Comment partially incorporated. Additional text was added to Equipment and Instrumentation Calibration and Qualification.
Plate readers, water baths, and dry heat blocks used for sample incubation should be on a laboratory bench away from heating, ventilation, and air conditioning (HVAC) ducts, significant vibration, and laboratory traffic that could affect the test results.
Comment Summary #110: The commenter suggested reinstating the *Laboratory Environmental Conditions* section, noting that it provided useful guidance on conducting BET in a controlled environment without requiring a hood, as well as recommendations on minimizing vibrations, foot traffic, and other potential interferences.

Response: Comment partially incorporated. A portion of the previous content has been reintroduced into Equipment and Instrumentation Calibration and Qualification, specifying that *plate readers, water baths, and dry heat blocks used for sample incubation should be placed on a laboratory bench away from HVAC ducts, significant vibration, and laboratory traffic that could affect test results.* However, the full section was not reinstated to maintain conciseness while preserving key environmental considerations.

Glossary
Comment Summary #111: The commenter requested to re-add the glossary that was removed.
Response: Comment not incorporated. The definitions are now located in Microbiological Chapters—Glossary ⟨1117.1⟩. Therefore, the glossary will not be re-added to this section. For definitions, please refer to the Microbiological Chapters—Glossary ⟨1117.1⟩.
Comment Summary #112: The commenter suggested re-adding the section on Activity Determination for a Liquid CSE.
Response: Comment not incorporated. Liquid CSE is covered in RSE: CSE Calibration.

General Chapter/Section(s):	<1251> WEIGHING ON AN ANALYTICAL BALANCE
Expert Committee:	General Chapters - Measurement and Data Quality
No. of Commenters:	8

Qualification
Comment Summary #1: The commenter recommended adding the instrument qualification section in GC<1251> to GC<41> as these are common requirements in industry.
Response: Comment not incorporated. The Expert Committee has made the decision not to include instrument qualification in GC<41> for PF50(5) draft as this is out the scope of GC<41>. Information related to instrument qualification can be located in GC<1058> or GC<1251> for balance.

Performance Qualification
Comment Summary #2: The commenter requested information on recommended tests for checking the balance environment (e.g., vibration and air currents).
Response: Comment not Incorporated. GC<1251> describes typical environmental effects that might affect the balance performance. It is out of the scope of this chapter to provide specific tests to check the balance environment due to the complexity and variety of environments. It is part of the education and training of the user to identify critical environments in the specific installation location.

Performance Qualification
Comment Summary #3: The commenter requested to clarify on the frequency of sensitivity test and repeatability test.
Response: Comment not incorporated. The frequency of the performance checks is defined by the user on a risk-based approach. GC<41> makes it evident that the most important property of accuracy is sensitivity which shall be periodically assessed (together with repeatability). Therefore the mandatory requirement of GC<41> is to periodically assess sensitivity and repeatability. It is at the discretion of the user to perform additional testing, e.g., according to Table 1 in GC<1251>. However, this is not a mandatory requirement.

Performance Qualification
Comment Summary #4: The commenter disagreed that "Performance Checks" is part of Performance Qualification. The commenter requested to define "Performance Checks" as the

intermediate checks performed between qualification activities and to address "Performance Check" as a part of routine operation of balances.
Response: Comment not incorporated. Routine tests as well as intermediate checks are part of performance qualification. This approach aligns with GC<1058>.

Performance Qualification
Comment Summary #5: The commenter requested clarification on "change in the displayed value" and "capacity of the balance".
Response: Comment not incorporated. Since "capacity" is a term with a very clear meaning in English, it should not affect the reader's understanding.

Performance Qualification
Comment Summary #6: The commenter requested to clarify the activities needed for daily use versus periodic calibration.
Response: Comment not incorporated. Both GC<41> and GC<1251>make it clear that calibration and performance checks are not the same activity. There are no requirements for daily performance checks. All performance checks are set up on a risk-based approach. It is stated clearly in GC<41> that the performance checks are done in-between the calibration intervals.

General Comment
Comment Summary #7: The commenter supported the revision that align closely with the European Pharmacopeia 2.1.7 Balance for Analytical Purposes.
Response: Comment acknowledged.

Performance Qualification
Comment Summary #8: The commenter inquired whether if a daily sensitivity test is sufficient and if the repeatability test needs to be performed at the same frequency as the sensitivity test.
Response: Comment not incorporated. The frequency of the performance checks are defined by the user on a risk-based approach. There is no requirement for a daily sensitivity or repeatability test. It is not a regulatory requirement that the frequencies of the individual performance checks must be the same.

Performance Qualification
Comment Summary #9: The commenter inquired if the daily checks are part of the performance qualification.
Response: Comment not incorporated. There is no requirement for daily checks. To emphasize this, the paragraph "Balance Checks" from the currently official GC<1251> was deleted. The performance checks are part of the performance qualification which is described in detail in the paragraphs on "Performance Qualification" in GC<1251>.

Performance Qualification
Comment Summary #10: The commenter requested to clarify the information in the parenthesis in Table 1 first bullet point under Acceptance Criteria. The commenter suggested change the sentence in parenthesis to the following: "with 1 being the sensitivity of a correctly adjusted balance, i.e., displayed value= load value), where…"
Response: Comment incorporated. Change was made per suggestion.

Performance Qualification
Comment Summary #11: The commenter requested clarity on triangular balances regarding eccentricity.

Response: Comment not incorporated. The current text states "... or at analogous locations for other balance pan...", which provides guidance for other platter shapes.

General Comment
Comment Summary #12: The commenter recommended to add blank space between numeric value and “%” to comply with ISO 80000 requirements.
Response: Comment not incorporated. The current format follows the USP Style Guide and is used consistently, regardless of ISO 80000 formatting requirements.

Performance Qualification
Comment Summary #13: The commenter requested clarity on which test weight needs to be used for sensitivity test.
Response: Comment not incorporated. For testing accuracy, test loads between 5% and 100% of capacity are permitted. Accuracy is influenced by sensitivity, eccentricity and non-linearity. If only sensitivity is assessed (which is the minimum requirement for assessing accuracy), the most efficient test load to test sensitivity is a test weight close to the capacity of the balance, however, any test load between 5% and 100% capacity fulfills the requirement.

Performance Qualification
Comment Summary #14: The commenter requested that the proposal be revised to state that the test weight should ideally be approximately 5% of the maximum capacity of the balance. In addition, the commenter requested to add a statement " Test weight below 100mg should not be used." The same comment was also made for GC<41>.
Response: Comment partially incorporated. For clarity, the sentence "Test weights below 100 mg shall not be used” was revised to read: "Test weights with a nominal mass below 100 mg shall not be used." The conventional mass may not be considered for the selection of the test load. The requirement of not using test loads below 100 mg is in alignment with Ph. Eur. and JP. "Nominal value" was added in Table 1 Repeatability.

Minimum Weight
Comment Summary #15: The commenter requested to add another nomenclature for minimum weight and safety factor.
Response: Comment not incorporated. It is sufficient to define minimum weight (balance performance) and smallest net weight (user requirement). Defining a name for an additional term (minimum weight under consideration of the safety factor) adds more confusion than clarity.

Performance Qualification
Comment Summary #16: The commenter requested clarity on whether the requirement for summing up the calibration uncertainties of weights is applicable for accuracy test.
Response: Comment not incorporated. The requirement for summing up the calibration uncertainties of weights is applicable for testing accuracy, i.e., when testing sensitivity, non-linearity or eccentricity. For testing eccentricity, the usage of non-calibrated weights is permitted. This is described already at the end of the paragraph on "performance qualification".

Performance Qualification
Comment Summary #17: The commenter requested clarity on pre-load requirements.
Response: Comment not incorporated. As stated in the footnotes to Table 1, in general, pre-loads can be taken for the assessment of repeatability to reflect more closely the actual weighing application. However, for multiple range devices, when the device is used during normal operation in the coarser ranges, repeatability also needs to be tested in the coarse ranges which is established by using a pre-load with a mass larger than the fine range capacity.

In contrast to multiple range devices, repeatability of multi-interval devices only needs to be tested in the fine range. As per the definition of multi-interval balances in the glossary, such a device will return into the fine range after applying a pre-load and taring the device. This information is made transparent in footnotes 1 to 3 underneath Table 1.

Receiver
Comment Summary #18: The commenter requested to clarify that the Receiver is the same as Tare Vessel, and add a definition in the Glossary section for "Receiver or Tare Vessel".
Response: Comment partially incorporated. In the "receiver" section, the text was revised to read as the following: "To ensure suitable performance in measuring the weight of a material, analysts should consider selection of a proper receiver **(tare vessel)** for the material."

Performance Qualification
Comment Summary #19: The commenter requested clarity on which tests need to be performed as performance check and which tests need to be performed as calibration.
Response: Comment not incorporated. Calibration requirements are included in GC<1251>.Calibration is an activity described in international standards, e.g. EURAMET cg-18 and ASTM E898. Calibration is a comprehensive activity comprising of a repeatability test, eccentricity test and error of indication test, and additionally requires the determination of the measurement uncertainty of the device.

Minimum Weight
Comment Summary #20: The commenter requested to change the weight tolerance in the calculation formula from 0.10% to 0.001.
Response: Comment not incorporated. 0.10% is a well-established value in GC<41> and GC<1251>. 0.001 would be a different requirement because of the significant figures.

Performance Qualification
Comment Summary #21: The commenter found that “ranges” and “intervals” are confusing in the chapter text. For example, it is stated that multi-interval balances repeatability should be performed in the finest “range” even if the balance does not have multiple ranges. The commenter requested clarity on the difference between large scale interval and small-scale interval. In addition, it is unclear about the maximum capacity. Specifically, the commenter requested clarity on whether it applies to all balance types or only for the single range balances in the statement “the maximum capacity refers to the capacity of the coarsest weighing range...”.
Response: Comment partially incorporated. The definitions of multi-interval and multiple range balances are listed in the glossary in GC<1251>. The definitions don't use the word “interval”, but “partial weighing range” with respect to multi-interval balances. The numerical value of a large-scale interval is larger than the numerical value of a small-scale interval. The sentence "Specifically, the maximum capacity..." refers to multi-interval and multiple range balances as this text is part of the paragraph that specifically addresses multiple range and multi-interval balances. A minor revision in Glossary, to clarify that the term “partial weighing range” is equivalent to “interval”.

Performance Qualification
Comment Summary #22: The commenter requested to revise the explanation of single-interval, multi-interval and multi-range balances in the Glossary section for clarity.
Response: Comment not incorporated. The definitions in Glossary align with the definitions used in OIML R76 and HB 44.

Minimum weight

Comment Summary #23: The commenter requested to correct the minimum weight calculation equation to $\mathbf{m}_{min}$=2000 x S.
Response: Comment incorporated.

Glossary
Comment Summary #24: The commenter requested to add scale interval d to the glossary section.
Response: Comment incorporated.

Performance Qualification
Comment Summary #25: The commenter suggested to use at least 5 points to calculate linearity instead of 4 points in the current proposal.
Response: Comment not incorporated. The recommended calibration guidelines EURAMET cg-18 and ASTM E898 suggest a minimum of four error-of-indication test points (plus the zero) for calibration, so there is no need to suggest more than four measurement points for linearity testing.

General Chapter/Section(s):	<1785> OSMOLALITY AND OSMOLARITY - PRACTICAL CONSIDERATIONS
Expert Committee:	General Chapters - Physical Analysis 2020
No. of Commenters:	1

Comment Summary #1: The commenter suggested under background, Calculations of Osmolality and Osmolality Coefficient, under the definition of the formula, revising the definition of some of the formula elements.
Response: Comment incorporated.
Comment Summary #2: The commenter suggested under Background, Calculations of Osmolality and Osmolarity Coefficient, first paragraph, to align the text with the last paragraph of this section.
Response: Comment incorporated.
Comment Summary #3: The commenter suggested under Recommended Procedure for Verification of Osmolality Measurement, item 3, revising the sentence related to the variance of the instrument to clarify the calculation.
Response: Comment incorporated.
Comment Summary #4: The commenter suggested under Osmolality and Tonicity, first paragraph, revising the text related to water movement to improve the accuracy of the statement.
Response: Comment partially incorporated.
Comment Summary #5: The commenter suggested under Considerations for Instrument Selection, Table 1 - Instrument Recommendations and Analytical Concerns According to the Sample Type, mitigation for nonaqueous formulations or water-in-oil emulsions, to add an explanation on the water activity determination.
Response: Comment incorporated.
Comment Summary #6: The commenter suggested under Determining Nonideal Behaviors in Mixtures of Solutes, second list, items 2 and 3, including an explanation on how to decide if solute-solute or solute-water interaction is the dominant factor of the deviation
Response: Comment Incorporated.
Comment Summary #7: The commenter suggested under Determining Nonideal Behaviors in Mixtures of Solutes, adding a definition of "MW".
Response: Comment incorporated.

Comment Summary #8: The commenter suggested under Background, Electrolyte Nonidealities, clarifying the definition of the equation elements.
Response: Comment incorporated.
Comment Summary #9: The commenter suggested under Osmolality and Tonicity, fourth paragraph, revising the text to align with the equations in the USP general chapter <785> Osmolality and Osmolarity.
Response: Comment incorporated.
Comment Summary #10: The commenter recommended under considerations for Instrument Selection, Table 1 - Instrument Recommendations and analytical Concerns According to Sample Type, mitigation for high-concentration polymer or proteins, adding more information about the instrument.
Response: Comment incorporated.
Comment Summary #11: The commenter suggested under Background, Zwitterions and Other Nonideal Solutes, adding an explanation of the equation elements.
Response: Comment incorporated.
Comment Summary #12: The commenter suggested under electrolyte nonidealites, paragraph above Figure 3, explaining the deviations at low and high concentrations and how to evaluate the deviations.
Response: Comment incorporated.
Comment Summary #13: The commenter suggested under Background, Calculations of osmolality and Osmolality Coefficients, last paragraph, first sentence, removing i ≠ ni" to improve the understanding of the statement.
Response: Comment incorporated.
Comment Summary #14: The commenter suggested under Considerations for Instrument Selection, Recommended Procedure for Verification of Osmolality Measurement, item 3, clarifying the text in the sentence on variance of instrument.
Response: Comment incorporated.
Comment Summary #15: The commenter recommended under Background, Determining Nonideal Behaviors in Mixtures of Solutes, second list, renumbering the second list.
Response: Comment incorporated.
Comment Summary #16: The commenter recommended under Considerations for Instrument Selection, Recommended Procedure for Comparison of Two Osmolality Measurements, Evaluation for Statistically Significant Differences, checking if the formulas were correct.
Response: Comment incorporated.
Comment Summary #17: The commenter suggested under Relevance of Osmolality to Pharmaceutical Development and Production, clarifying the text related to sample dilutions.
Response: Comment incorporated.
Comment Summary #18: The commenter suggested under Considerations for Instrument Selection, Recommended Procedure for Verification of Osmolality Measurements, item 3, clarifying the units.
Response: Comment incorporated.
Comment Summary #19: The commenter suggested under Considerations for Instrument Selection, Table 1 - Instrument Recommendations and analytical concerns According to Sample Type, clarifying the term "low volume instrument".
Response: Comment incorporated.
Comment Summary #20: Under Background, Determining Nonideal Behaviors in Mixtures of Solutes, legend under Figure 4, the commenter recommended checking the concentration of the solution to be aligned with the text.
Response: Comment incorporated.
Comment Summary #21: The commenter recommended under Relevance of Osmolality to Pharmaceutical Development and Production, the deletion of the sentence on accuracy and precision.

Response: Comment not incorporated. The sentence was rewritten for clarification.
Comment Summary #22: The commenter suggested under Osmolality and Tonicity, first paragraph eighth sentence, revising the sentence to improve the accuracy of the statement.
Response: Comment incorporated.
Comment Summary #23: The commenter recommended under Recommended Procedure for Verification of Osmolality Measurement, item 3, revising the sentence related to the variance for the instrument.
Response: Comment incorporated.

b. Nonelectrolyte Nonidealistic
Comment Summary #24: The commenter suggested under Nonelectrolyte nonidealistic section, second paragraph, last sentence, clarifying the unit for the sucrose concentration.
Response: Comment incorporated.

Monograph

Monograph/Section(s): ANHYDROUS LACTOSE / Multiple sections
Expert Committee(s): Simple Excipients
No. of Commenters: 9

Comment Summary #1: The commenters indicated that performance related properties (PRP) including their limits should be established in Quality by Design (QbD) studies performed by the excipient users. These properties were probably identified as potential PRPs on theoretical grounds and the testing of these mentioned PRPs does not guarantee “substitutability” between different lactose excipients for inhalation without robust QbD studies.
Response: Comment not incorporated. Although USP Simple Excipients Expert Committee (SE EC) agrees that robust QbD studies need to be performed, compendial standards can help ensure that quality excipients are used in inhalation products. The performance related tests proposed in the monographs provide necessary guidance and help excipient users to select suitable lactose excipients for their intended use for inhalation products. No acceptance criteria for such tests are included in the monograph to help the users select suitable lactose excipients for inhalation products.
Once the lactose quality necessary for a given product has been established, it is the responsibility of the excipient user (drug product manufacturer) to verify the capability of the excipient manufacturer to supply consistent excipient that meets the requirements for their application. If performance-related considerations cannot be implemented by the excipient manufacturer or supplier, then the user (drug product manufacturer) must process and control the excipient accordingly.
Comment Summary #2: The commenters recommended abolishing reference to the performance-related test of Morphology and Shape completely. They believe the testing of these physical characteristics adds little value because the results are difficult to quantify and correlate with drug performance, especially for very fine powders.
Response: Comment not incorporated. USP SE EC believes that the morphology and shape test should remain as a performance-related test in the Lactose monographs because particle morphology and shape are straightforward tests and provide useful information for Lactose used in inhalation product, consistent with the second Stimuli article published in PF 49(5), *USP Responses to Comments on Stimuli Article “Revisions to the USP–NF Lactose Monographs — Focusing on Inhalation and Injection Grades”.*

If the surface morphology consideration cannot be implemented by the excipient manufacturer or supplier, then the user (drug product manufacturer) must be able to process and control the excipient accordingly.

Comment Summary #3: The commenters proposed to omit the requirement of "span" in the performance-related test of Particle Size Distribution, as "span" is not defined in General Chapter <429> and in their opinion does not provide additional info as it can be calculated from d_{10}, d_{50}, and d_{90} by the user itself.

Response: Comment not incorporated. USP SE EC agreed to keep the requirement of "span" as span indicates the overall distribution of the particles. Since d_{10}, d_{50}, and d_{90} have been determined already, span can be calculated easily.

Comment Summary #4: The commenters proposed to omit the term "surface energetics" under the performance-related test of Amorphous Content because surface energetics and amorphous content are not identical.

Response: Comment incorporated. USP SE EC agreed to delete the sentence "Surface energetics should be characterized by a suitable technique."

Comment Summary #5: The commenters requested to provide justification why amorphous content is a PRP due to two challenges: a) the relevance of measuring amorphous content is limited, given its unstable character and variation over time. This means that data on the amorphous content at release provided by excipient supplier can provide some indications, but do not absolve the excipient user from measurements of the amorphous content and establishing appropriate in-process controls when this is shown to be a critical factor; and b) difficulty for excipient suppliers to create QbD samples with the amorphous content as an independent variable.

Response: Comment not incorporated. Justifications were provided in the second *Stimuli* article published in PF 49(5), *USP Responses to Comments on Stimuli Article "Revisions to the USP–NF Lactose Monographs — Focusing on Inhalation and Injection Grades"*. Despite the challenges, the amorphous content needs to be considered when Lactose is used for inhalation products. Therefore, the Amorphous test in the Lactose monographs cross-reference the general chapters for determining the relevance of amorphous content in performance of inhalation products, such as <891>, <696>, <1241>, and <941>, etc.

Comment Summary #6: The commenters proposed to omit the Content of Alpha and Beta Anomers method from the Anhydrous Lactose monograph and leave the determination of the method to the drug product manufacturer and excipient supplier. The agreed method should be validated with relevant test method validation criteria.

Response: Comment not incorporated. Consistent with the second Stimuli article published in PF 49(5), *USP Responses to Comments on Stimuli Article "Revisions to the USP–NF Lactose Monographs — Focusing on Inhalation and Injection Grades*", USP SE EC agreed to retain the existing Content of Alpha and Beta Anomers test in the Anhydrous Lactose monograph to ensure reproducible and/or consistent manufacturing process and quality of the material.

Comment Summary #7: The commenters proposed to include a statement in the labeling section that the parameters determined by the QbD studies of the drug product manufacturers [Critical Material Attributes (CMA)] will direct whether Performance related Properties (PRPs) have to be determined and subsequently will be labelled for example on certificates of analysis. The labeling requirement can easily be misunderstood that it is mandatory to provide data on all mentioned PRPs (amorphous content, morphology and shape, anomeric purity). Instead, performance related properties including their limits should be established by QbD studies performed by the excipient users. This is exactly the approach that is described in General Chapter <1059> on Excipient Performance.

Response: Comment partially incorporated. The sentence - "*This section provides information on excipient properties that are recognized as being relevant control parameters to achieve the desired performance (See Excipient Performance ⟨1059⟩)*" will be included to refer to chapter

<1059>, however, the performance related tests will still be included in the “Labeling” section under “Other requirements” without acceptance criteria.
According to <7> Labeling, “*The term ‘labeling’ includes all labels and other written, printed, or graphic matter on an article’s immediate container or on, or in, any package or wrapper in which it is enclosed, except any outer shipping container. The term ‘label’ is that part of the labeling on the immediate container*.” Certificate of Analysis (CoA) is also part of the Labeling, so these performance-related tests can be listed on the CoA.
USP *General Notices, 10.20 Labeling* states that “*All articles in USP or NF are subject to the labeling requirements specified in <7> Labeling, unless different requirements are provided in an individual monograph,*” so the performance-related tests under the Labeling section should be considered when lactose is intended for use in inhalation dosage forms.
Comment Summary #8: The commenters recommended using the sentence: “the following properties may be relevant…” to replace the sentence “the following specifications must be met…”.
They agreed on the framework for applying QbD principles to excipient performance provided in General Chapter <1059> on Excipient Performance, however, only if excipient property is determined to be a CMA, then both the method and limits need to be agreed upon between user and supplier.
Response: Comment partially incorporated. USP SE EC agreed to modify the sentence to read as follows: “the following attributes should be evaluated.“
Comment Summary #9: The commenters indicated that, in their view, the current draft monograph implies that the same approach should be used in other monographs e.g. other excipients used for inhalation. They wonder whether this is the intention of the USP-NF and whether the impact of this is overseen by the USP Expert Committee.
Response: Comment noted. It will be discussed further by the USP SE EC regarding whether to apply the same approach (placing performance-related tests under *Labeling – Other Requirements)* to other excipients intended for inhalation. At this time, this will be decided on a case-by-case basis.
Comment Summary #10: The commenters recommended some verbiage changes to the “*Labeling*” and “*Other Requirements*” section. See below.
Response: Comment partially incorporated.
USP SE EC accepted the following modified edits under Labeling:
“- Where Lactose Monohydrate is intended for use in the manufacture of inhalation or injectable dosage forms, the labelling indicates that it complies with compendial requirements for inhalation or injectable use.”

“- For modified Lactose Monohydrate, the labeling also indicates the method of modification.”

However, USP SE EC does not agree to remove the “span” from the test of Particle Size Distribution, as mentioned above in Comment Summary #3.

Comment Summary #11: The commenters suggested some edits to the tests of Particle Size Distribution and Amorphous Content, and recommended deleting the test of Particle Shape and Morphology. These comments are similar to the aforementioned Comments #2, #3, #5, and #10.
Response: Comments not incorporated. See responses in Comment Summaries #2, #3, #5 and #10.
Comment Summary #12: The commenter requested clarification on why “tight container”, not “well-closed container”, is required in the Packaging and Storage section.
Response: Comments not incorporated. Based on the definitions of “tight container” and “well-closed container” in the general chapter *<659> Packaging and storage requirements,* USP SE

EC believes that the current packaging and storage requirement – “tight container” is more appropriate as Lactose is hygroscopic at ambient conditions.

Comment Summary #13: The commenters recommended separating the microbial limits requirements for inhalation and injection grades.

The commenters noted that the concern for Burkholderia cepacia complex (BCC) is first and foremost related to the water used for the product. In case the final product subsequently is not subject to sterilization, presence of BCC may pose a risk to patients. That could be the case for inhalation grades but not injection grades. The FDA Guidance for Industry "Microbiological Quality Considerations in Non-sterile Drug Manufacturing" addresses this topic for non-steriles.

Test for (BCC): Even though the PDG has agreed to include the requirement for BCC, the test for BCC is not available in Ph. Eur. or JP. As this is a prerequisite in terms of harmonization, actual harmonization is not likely to happen within a reasonable period of time.

Response: Comments incorporated. The following changes, which are local requirements, will be made to the revised Lactose monographs:

- ⧫For inhalation grades: The total aerobic microbial count is NMT 1×10^1 cfu/g and the total combined molds and yeasts count is NMT 1×10^1 cfu/g. It meets the requirements of the tests for the absence of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella* species, and *Burkholderia cepacia*.⧫
- ⧫For injection grades: The total aerobic microbial count is NMT 1×10^1 cfu/g and the total combined molds and yeasts count is NMT 1×10^1 cfu/g. It meets the requirements of the tests for the absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella* species.⧫

Comment Summary #14: The commenters indicated that there is revision required in General Chapter <1111> for Inhalation products and substances to include B.cepacia.

Response: Comments noted. This comment has been passed to the USP Microbiology Expert Committee. The current chapter *<1111> MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: ACCEPTANCE CRITERIA FOR PHARMACEUTICAL PREPARATIONS AND SUBSTANCES FOR PHARMACEUTICAL USE* does not have *Burkholderia cepacia complex (BCC)* requirement. However, the USP Microbiology Expert Committee may consider revising the chapter <1111> to include BCC test.

Comment Summary #15: The commenters noted that they have no objection to adding the Microbial Limits and Bacterial Endotoxin tests to the USP local text, but if USP plans to add these tests to the PDG harmonization text, they would appreciate it if USP could provide a proposal to the PDG.

Response: Comments noted. Regarding PDG harmonization of Lactose monographs, USP, as the coordinating pharmacopeia of these monographs, will submit Stage 1 proposals to PDG so that each PDG pharmacopoeia can discuss the feasibility and necessity to specify these tests.

Comment Summary #16: The commenters recommended USP to consider a wider Bacteria Endotoxin test (BET) specification for lactose as 5 EU/g isn't always necessary. They indicated that this specification shouldn't be mandatory but more on a case-by-case basis depending on lactose concentration and final formulation.

Response: Comments not incorporated. Based on the information provided by major Lactose manufacturers globally and the FDA’s input, the BET limit of NMT 5 EU/g will remain unchanged.

Comment Summary #17: The commenter recommended removing "VS" after 0.1N sodium hydroxide in the Acidity or Alkalinity test, unless the intent is to require this solution to be standardized prior to use.

Response: Comments not incorporated. 0.1N sodium hydroxide solution needs to be standardized before testing for acidity or alkalinity, so "VS" should be retained.

Comment Summary #18: The commenters noted that they appreciate that USP-NF incorporated aspects of the feedback they provided in 2020. Accordingly, they are pleased to

note that that the thin layer test for identification will be removed, and that optical rotation will – for the time being - remain as the assay method for lactose in all routes of application.
Response: Comments acknowledged.
Comment Summary #19: The commenters noted that they appreciate USP's efforts on improving and harmonizing Lactose monographs. They strongly suggest that there is no need for HPLC tests for Lactose in light of many decades of safe use and experience therefore proposed related substances test only be applied to lactose intended for injectable or inhalation use if needed.
Response: Comments noted. Since the HPLC test method for Assay and Impurity analyses will be part of the Phase 2 monograph update and not within the scope of this revision, USP SE EC will consider the comment at that time.

Monograph/Section(s):	DICLOFENAC SODIUM COMPOUNDED TOPICAL FOAM
Expert Committee:	Compounding
No. of Commenters:	1

Comment Summary #1: The commenter noted that the monograph for Diclofenac Sodium Compounded Topical Foam, 30 mg/mL may produce a drug product that is essentially a copy of an FDA-approved product. Please see the final guidance for industry Compounded Drug Products That Are Essentially Copies of a Commercially Available Drug Product Under Section 503A of the Federal Food, Drug, and Cosmetic Act (January 2018). FDA recommends using only FDA-approved drug products unless the patient has a specific medical need that cannot be met by the approved drug products. Because compounded drug products do not go through the drug approval process, they should only be used when an FDA-approved product is not available to meet the medical needs of an individual patient. As stated in the final guidance for industry Compounded Drug Products That Are Essentially Copies of a Commercially Available Drug Product Under Section 503A of the Federal Food, Drug, and Cosmetic Act (January 2018), under section 503A they do not consider a drug product to be commercially available if the drug product appears on the FDA Drug Shortage list, however, diclofenac sodium is not currently listed on the FDA Drug Shortage list.
Response: Comment not incorporated. This monograph is being published to provide compounders with a preparation to compound when the drug product is unavailable, such as when it is on shortage. USP Compounded Preparation Monographs are also used outside of the United States, where it may be the case that the product is unavailable.
Comment Summary #2: The commenter has noted concerns about developing monographs for preparations that may present particular safety risks (e.g., Diclofenac Sodium Compounded Topical Foam) given the limited labeling that generally accompanies compounded drug products. FDA-approved diclofenac sodium products are labeled with a boxed warning regarding the risk of serious cardiovascular and gastrointestinal events. Because of the serious side effects that can occur with the use of this drug, an approved package insert should be available to inform both practitioner and patient of the serious side effects and provide recommendations for appropriate follow up. Such package inserts are not required and will not be available for this compounded drug.
Response: Comment not incorporated. Compounded preparations from 503A and other state-licensed compounders are exempt from FDA Product Labeling requirements.
Comment Summary #3: The commenter noted that the monograph states to use FoamaDerm from RS Therapeutics Inc., Edmonton, Alberta as the base. However, according to the RS Therapeutics Inc. website, FoamaDerm is only available in Canada. It is unclear whether compounders in the US will be able to obtain this required product to create the topical foam drug product as described in the monograph.

Response: Comment not incorporated. USP Compounded Preparation Monographs are used by compounders outside the United States. The component may be available in additional countries in the future.
Comment Summary #4: A commenter has noted concerns with using proprietary excipients when there is no information about the identity of the excipient provided in the monograph. They recommend that the ingredients in the proprietary excipients be provided so that the identity of the excipients is understood by the public. It is important that the public has the information that will help them understand the risks and benefits associated with the use of the drug product, including any excipients in the drug product.
Response: Comment not incorporated. This information is obtained from the manufacturer.
Comment Summary #5: The commenter noted that the assay acceptance criteria range is given as 90.0%-110.0%. FDA liaisons to the Compounding Expert Committee note that this is inconsistent with observations of the raw data. They recommend tightening this range to be consistent with the raw data.
Response: Comment not incorporated. The Expert Committee bases its acceptance criteria on existing USP monographs and expert opinion, not solely on laboratory testing criteria.
Comment Summary #6: The commenter recommends revising the Appearance section for additional clarity, as follows: “Translucent Yellowish clear solution”.
Response: Comment not incorporated. Appearance in the monograph matches data from the stability study.
Comment Summary #7: The commenter recommends including *USP* <61> *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* and <62> *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms* in the Specific Tests section.
Response: Comment not incorporated. Requiring compliance with *USP* <60>, *USP* <61>, and *USP* <62> would not be consistent with the requirements in *USP* <795> *Pharmaceutical Compounding – Nonsterile Preparations*.
Comment Summary #8: A commenter notes that the Packaging and Storage section differs from the observed study. They recommend revising the section to be consistent with the study, which states to pack in a light-resistant hermetic container, sachet, under nitrogen gas.
Response: Comment not incorporated. The storage conditions mentioned in the comment are for the stock solution, not the final preparation.
Expert Committee-initiated Change #1: The original stock solution ingredients and instructions were merged into the final preparation ingredients and instructions for clarity.

Monograph/Section(s): DIETHYLENE GLYCOL MONOETHYL ETHER / Additional Requirements: Labeling
Expert Committee(s): Simple Excipients
No. of Commenters: 1

Comment Summary #1: The commenter requested retaining the statement in the Labeling section: “Label it to indicate that it is intended for topical or transdermal use only and it is stored under an atmosphere of an inert gas. The material is not to be used for parenterals,” because removing this statement would allow this substance to be used in all dosage forms without appropriate toxicological assessment. This impacts all legally marketed, unapproved drug products, including compounded products and over-the-counter products.
Response: Comment not incorporated. Because of evidence of Diethylene Glycol Monoethyl Ether’s low toxicity and its history of safe global use, the Simple Excipients Expert Committee (SE EC), in consultation with the Nomenclature Labeling Expert Committee (NL EC), opted not to incorporate the comment. Importantly, any use of Diethylene Glycol Monoethyl Ether or any other excipient in legally marketed, over-the-counter (OTC) or unapproved drug products

remains subject to FDA oversight, including toxicological evaluation when required by the intended use, dosage form, or route of administration.

Monograph: FONDAPARINUX SODIUM INJECTION
Expert Committee(s): Biologics Monographs 3 - Complex Biologics and Vaccines
No. of Commenters: 1

Specific Tests, Bacterial Endotoxin Test <85>
Comment Summary #1: The commenter questioned the rationale for setting up a bacterial endotoxin specification when the maximum human dosage is determined by the clinician.
Response: Comment not incorporated. The comment is out of scope for the proposed revision.

Specific Tests, Sterility Test <71>
Comment Summary #2: The commenter suggested that the sterility test requirement should not be dependent on product labeling.
Response: Comment not incorporated. The comment is out of scope for the proposed revision.

Specific Tests, pH <791>
Comment Summary #3: The commenter suggested that the specified pH range is too broad to serve as a critical quality attribute.
Response: Comment not incorporated. The comment is out of scope for the proposed revision.

Monograph/Section(s): LACTOSE MONOHYDRATE / Multiple sections
Expert Committee(s): Simple Excipients
No. of Commenters: 9

Comment Summaries and Responses: See Anhydrous Lactose.

Monograph/Section(s): POMEGRANATE FRUIT DRY EXTRACT
Expert Committee: Botanical Dietary Supplements and Herbal Medicines
No. of Commenters: 1

Molecular formula Punicalin
Expert Committee initiated-change: Change the molecular formula for Punicalin from $C_{16}H_{18}O_9$ to $C_{34}H_{22}O_{22}$

Monograph/Section(s): PROPYL GALLATE /Mutliple Sections
Expert Committee: Simple Excipients
No. of Commenters: 1

Comment Summary #1: The commenter indicated that USP's method is an unnecessary burden because no new impurities are detected or controlled in the proposed Related Substances test in the NF monograph for Propyl Gallate, and worldwide suppliers already adhere to the corresponding Ph.Eur. monograph.
Response: Comment not incorporated. The proposed USP method provides superior resolution of the gallic acid peak from the void volume/solvent peak. Avoiding the use of methanol in the preparation of standard and sample solutions prevents a transesterification reaction and formation of methyl gallate and perhaps some other impurities that are not present in Propyl

Gallate. Additionally, the mobile phase in the USP method is phosphate-free, making it compatible with mass spectrometry for further impurity identification if necessary.
Comment Summary #2: The commenter indicated that trace level impurities were observed in the propyl gallate sample after the propyl gallate peak when using the Ph. Eur. monograph method. However, due to the extraneous peaks observed with the USP monograph method, these trace level impurities in the same sample would likely go undetected.
Response: Comment not incorporated. The trace level impurities (less than 0.05%) in the Propyl Gallate sample after the propyl gallate peak were also observed using the USP proposed method. All peaks generated by forced degradation were fully separated from the propyl gallate peak and the extraneous peaks appearing after 20 min.
Comment Summary #3: The commenter indicated that a separate standard solution of gallic acid is unnecessary because the peak counts of the gallic acid and propyl gallate peaks from 0.1% solutions do not show a significant response factor difference.
Response: Comment not incorporated. While the response factors for gallic acid and propyl gallate are similar, using a separate standard for gallic acid supports the correct identification and quantification of this specified impurity.
Comment Summary #4: During feasibility testing of the proposed USP Assay and Related Substance method, the commenter observed extraneous peaks after 20 min, where the gradient is set for column cleanout and re-equilibration. The commenter requested clarification on whether these peaks should be monitored and asked for the integration stop time to be specified in the official monograph to address this issue.
Response: Comment incorporated. The method sponsor stopped integrating peaks after 20 minutes because it marked the start of the column cleanout stage, and the peaks resembled those in the injection of Diluent. The phrases "Calculate the percentage of any impurity in the portion of Propyl Gallate taken:" has been modified as "Calculate the percentage of any impurity, disregarding any peaks corresponding to those of the Diluent, in the portion of Propyl Gallate taken" to clarify the integration.

Harmonization
Comment Summary #5: The commenter requested that USP consider harmonizing the Assay and Related Substance HPLC method with the analytical method in the Ph. Eur. monograph for Propyl Gallate. The Ph. Eur. monograph's HPLC method for Assay and Related Substance can detect and separate all known impurities (gallic acid, methyl gallate, and ethyl gallate) listed in the briefing of the revised USP monograph.
Response: Comment not Incorporated. The USP procedure demonstrated great separation of solvent front and the gallic acid peak; USP procedure does not use methanol in the sample prep, which can contribute to transesterification and creating impurities that do not exist in propyl gallate; USP's mobile phase is phosphate-free, making it compatible with mass spectrometry for further substance identification.
