⟨1043⟩ ANCILLARY MATERIALS FOR CELL, GENE, AND TISSUE-ENGINEERED PRODUCTS

Change to read:

INTRODUCTION

A wide variety of reagents and materials, many of which are unique or complex, are ▲used in▲ (USP 1-May-2020) the manufacture of cell, gene, and tissue-engineered ▲(CGT)▲ (USP 1-May-2020) products.

▲The purpose of this chapter is to provide guidance on the development of appropriate material qualification programs for CGT products. Because CGT products are not usually amenable to extensive purification, filtration, and terminal sterilization procedures, reagents and material qualification are critically important to ensuring CGT product quality.

The term "ancillary materials" (AMs) refers to materials that come into contact with the cellular starting material, CGT product intermediates, or final CGT product during manufacturing, but are not intended to be present in the final CGT product. AMs are therefore distinct from the cellular starting material, the intermediates, and final CGT products. Excipients, which are intended to be present in the final product, are therefore not AMs. Processing devices such as vessels and transfer tubing sets may be considered AMs if they have surfaces and materials that contact the cells during production. Processing devices and containers that are considered AMs typically incorporate natural or synthetic biomaterials. Scaffolds or delivery devices that are intended to be part of the final product are not AMs. Cell banks and virus banks are generally not considered AMs; feeder layer cells that are not intended to be incorporated in the final product may be considered an AM or an impurity. Guidance documents (i.e., ICH Q5D) describe specific requirements for cell and virus bank certification and regulatory approval. However, "helper" viruses and "helper" plasmids may be considered AMs if they are not intended to be part of the final product.

Reagents that fall under the AM classification include well-characterized chemicals, complex compounds (antibiotics, anticoagulants, density gradients, toxins), multi-component mixtures (buffers, culture media), and complex biological compounds or mixtures (enzymes; blood-, plasma-, or serum-derived products; biological extracts; cytokines; antibodies; and conditioned media from cultured cells).▲ (USP 1-May-2020)

Add the following:

▲REGULATORY CONSIDERATIONS

Regulatory agencies have referred to AMs (as defined within this chapter) as ancillary products, ancillary reagents, processing materials, processing aids, and process reagents. The US FDA first introduced the AM concept with the term "ancillary product" in a *Federal Register* notice, *Application of Current Statutory Authorities to Human Somatic Cell Therapy Products and Gene Therapy Products* [*Federal Register* 58(197), October 14, 1993, pp. 53248–53251], which established the FDA's authority to regulate human somatic cell therapy products and gene therapy products. In a subsequent guidance, the FDA defined the term "ancillary reagent" as a material "used in the manufacture or production of a biological product that may or may not end up as part of the final product," but the definition was not formalized or used consistently in other FDA guidance (see *Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications*, February 2010). The term "AM" is also synonymous with "processing material", as defined in 21 CFR 1271, *Current Good Tissue Practice for Manufacturers of Human Cellular and Tissue-Based Products; Inspection and Enforcement; Proposed Rule* [*Federal Register* 66(5), January 8, 2001, pp. 1508–1559]. The US FDA also provides information to cell and tissue product manufacturers in 21 CFR 876.5885, *Tissue Culture Media for Human Ex Vivo Tissue and Cell Culture Processing Applications*.

Various labeling terminology has been used by AM manufacturers. The labeling terms "Research Use Only" (RUO) and "Investigational Use Only" (IUO) originated in the medical device labeling regulations and apply to materials intended for in vitro diagnostic use (see *Distribution of In Vitro Diagnostic Products Labeled for Research Use Only or Investigational Use Only: Guidance for Industry and FDA Staff*, November 2013). Because a given AM's label may or may not be relevant to its intended use in CGT product manufacturing, it is ultimately the responsibility of the CGT product manufacturer to define and qualify the suitability of a given AM in the CGT product manufacturing process.

Global and regional differences in AM labeling and documentation may dictate CGT product regulatory submission requirements. In the US, the CGT product sponsor can sometimes cross-reference an AM manufacturer's Drug Master File (DMF), which allows proprietary AM information to be reviewed by regulators, but not necessarily by the CGT product manufacturer. Other countries and regulatory authorities manage the submission of AM information differently, and it is critically important for the CGT product manufacturer to be aware of these differences if the same CGT product will be clinically tested or marketed in more than one regulatory jurisdiction. In any case, the CGT product manufacturer is responsible for ascertaining the AM's labeling, essential features, quality characteristics, and suitability for use.▲ (USP 1-May-2020)

Add the following:

▲IMPACT OF ANCILLARY MATERIAL QUALITY ON PRODUCT QUALITY

AM quality can pose risks to the stability, safety, potency, and purity of any product. These risks are often heightened with CGT products, whether due to their short shelf lives, limited ability to conduct extensive in-process and release testing, or the fact that many CGT products expand in vivo after their administration to the patient. To minimize the risks associated with

these commonly occurring scenarios, rigorous AM qualification and prudent application of manufacturing process controls are necessary.

Sourcing of Ancillary Materials

AMs used in the manufacture of CGT products are often selected for their unique functional or biological effects, and not necessarily on their quality attributes. Many AMs are only intended for research use but may not be readily available in a grade comparable to that of well-characterized licensed therapeutic products manufactured by controlled and documented methods. For this reason, many CGT product manufacturers source AMs that are approved or licensed therapeutic products. More recently, many AM suppliers have committed to providing higher grade AMs, using high-quality manufacturing and testing approaches with extensive documentation. Although the terms “clinical grade” and “good manufacturing practices” (GMP) are often applied to these higher quality AMs, their documentation may not be fully compliant with regulatory requirements for finished pharmaceutical products and excipients. Regardless of the AM labeling claims or descriptions, CGT product manufacturers are ultimately responsible for ensuring that the AM meets the necessary functional, quality, and documentation requirements demanded by the relevant regulatory authorities.

Specific safeguards are necessary in order to minimize or eliminate the risk of transmitting adventitious agents (viruses, bacteria, mycoplasma, protozoa, fungi, prions, etc.) when sourcing AMs using human- or animal-derived components such as sera, antibodies, or growth factors. In general, manufacturers of human- or animal-derived components and AMs should practice the following procedures to ensure CGT product safety:

1. Screening and qualifying (e.g., through bacterial or viral testing) and documenting (e.g., through herd certification and traceability) the sources of human- or animal-derived components as being free of suspected adventitious agents
2. Validating the inactivation (e.g., through chemical, thermal, or irradiation treatments) and removal (e.g., through chromatographic or filtration methods) of suspected adventitious agents during the processing of human- or animal-derived AM components
3. Testing (e.g., through polymerase chain reaction or virally-responsive cell lines) the initial raw material and final purified human- or animal-derived AM components for the presence of suspected adventitious agents

Industry and regulators have not yet established standard terminology to fully describe the presence or absence of human- or animal-derived components in AMs. Nevertheless, CGT product manufacturers should strive to eliminate all possible risk of adventitious agents in their final CGT products. The most direct way to achieve this goal is to eliminate AMs containing materials of human or animal origin. If AMs containing human- or animal-derived components are used, CGT product manufacturers should require AM suppliers to verify and document the absence of adventitious agents through the proper sourcing, processing, and testing of their human- or animal-derived components.

Regardless of the stated grade of the AM, the CGT product manufacturer is responsible for developing comprehensive and scientifically sound qualification plans to ensure the traceability, consistency, suitability, purity, and safety of the AM. Developers would be well served by referring to the International Council for Harmonisation (ICH)Q8(R2) guideline on *Pharmaceutical Development*, which provides internationally harmonized methodology for incorporating a quality-by-design (QbD) approach into development processes. This guidance allows systematic consideration of the critical quality attributes of a manufacturing process, including materials, resulting in the design of a high quality product.▲ (USP 1-May-2020)

Change to read:

QUALIFICATION OF ANCILLARY MATERIALS

▲AM qualification is the process of acquiring and evaluating data to establish the source, identity, purity, safety, and overall suitability of a specific AM used in the manufacturing process. CGT product developers and manufacturers are responsible for establishing and periodically reevaluating rational and scientifically sound AM qualification programs. These programs should include the characterization and quantification of each AM throughout the manufacturing process (i.e., measuring its tendency to be removed, degraded, or taken up by cells during manufacturing). Although the heterogeneity of many CGT products and AMs makes it difficult to recommend specific tests or protocols for a qualification program, the following provides a practical approach applicable to most AMs.

Thorough documentation is the cornerstone of any qualification program. A well-designed and well-documented AM qualification program becomes more comprehensive as product development progresses. Development of progressive specifications for AMs typically runs in parallel to the development of progressive specifications for the CGT product, including analytical testing. In early stages of CGT product development, safety is the primary focus; a complete description of AM characteristics, and precise quantitation of residual AM in the CGT product, may be limited by a lack of validated assays. In later CGT product stages, AM quality and residuals specifications, along with analytical test methods, should be more comprehensively elaborated to support eventual licensure of the CGT product.

In cases where AMs are products licensed for therapeutic use and manufactured under current good manufacturing practices (cGMP), the extent of qualification is typically less than that for a material intended for research purposes. While use of licensed therapeutic products as AMs may ensure a higher level of quality and safety, it is outside the scope of the licensed product’s intended use and labeling; therefore, the AM’s suitability for use in the CGT product manufacturing process still requires qualification. Modification of the licensed product, such as dilution, reformulation, mixture with another agent, aliquotting, or repackaging, could trigger additional qualification. Modification of the licensed product’s container–closure system may also be necessary if it will be used as an AM.

In many situations, complex or unique substances essential for CGT product process control or production will not be available in pharmaceutical grade. Even if an AM manufactured in compliance with cGMP is used, the CGT product manufacturer must develop a scientifically sound strategy for AM qualification based on assessments of criticality and principles of risk management. This assessment takes into account major risks to the CGT product, AM or CGT product failure modes,

and impacts to the product or patient in the event of an AM or CGT product failure. A qualification program for AMs used in CGT product manufacturing should address each of the following areas: 1) identification and sourcing, 2) selection and suitability for use in manufacturing, 3) characterization, 4) vendor qualification, and 5) quality assurance and control.▲ (USP 1-May-2020)

Identification ▲and Sourcing▲ (USP 1-May-2020)

The first step in any qualification program is the listing of all of the AMs used in a given ▲CGT▲ (USP 1-May-2020) product manufacturing ▲process, including details on▲ (USP 1-May-2020) where in the manufacturing process they are ▲▲ (USP 1-May-2020) employed. The source and intended use for each material should be established, and the necessary quantity or concentration of each material should be determined. Alternate sources for each material should be identified ▲and qualified.▲ (USP 1-May-2020)

Selection and Suitability for Use

Developers of CGT products should establish and document selection criteria for AMs and qualification criteria for each vendor early in the design phase of ▲CGT▲ (USP 1-May-2020) product development. Selection criteria should include assessments of microbiological and chemical purity, identity, and biological activity pertinent to the specific ▲CGT product▲ (USP 1-May-2020) manufacturing process. It is important to address these issues early in product development because certain AMs that are initially considered necessary may be impossible or prohibitively expensive to qualify, thereby justifying the investigation of alternatives or replacements. Examples include some animal- or human-derived materials that ▲may▲ (USP 1-May-2020) have alternate (i.e., plant or chemically synthesized) sources.

AMs ▲containing components▲ (USP 1-May-2020) of animal or human origin ▲require careful selection because of the▲ (USP 1-May-2020) potential ▲risk of▲ (USP 1-May-2020) infectious or zoonotic disease, ▲and the associated regulatory scrutiny. Vendors should provide documentation on the country of origin for animal-derived AMs to address concerns regarding transmissible spongiform encephalopathies and other diseases of agricultural concern (e.g., tuberculosis, brucellosis, etc.).▲ (USP 1-May-2020) In many cases, the chain of custody for animal-derived AMs (i.e., abattoir → intermediate processing center → final processing center) ▲must▲ (USP 1-May-2020) be documented. Vendors of human-derived AMs should ▲▲ (USP 1-May-2020) supply documentation regarding material traceability. For instance, human plasma-derived AMs should be sourced from licensed facilities that control the donor pool and appropriately screen the individual donors for relevant human infectious diseases. ▲▲ (USP 1-May-2020) Vendors of animal- and human-derived AMs ▲may supply different grades of materials, some more suitable for use in CGT product manufacturing than others.▲ (USP 1-May-2020) Many animal- and human plasma-derived components are subjected to ▲validated▲ (USP 1-May-2020) chemical (detergent or solvent treatment) or physical (heat, ▲irradiation, or filtration)▲ (USP 1-May-2020) treatments that have been shown ▲▲ (USP 1-May-2020) to significantly reduce the risk of ▲contamination by▲ (USP 1-May-2020) adventitious microbial or viral ▲agents.▲ (USP 1-May-2020)

Characterization

▲Quality control and characterization tests for each AM need to be developed (or adopted) and implemented. AM testing should assess a variety of quality attributes, including identity, purity, functionality, and freedom from microbial or viral contamination. The appropriate level of testing for each AM is derived from its risk assessment profile and the knowledge gained during CGT product development. AM test specifications should be established and justified to ensure consistency and performance in the CGT product manufacturing process. Acceptance criteria can be based on data from lots used in preclinical and early clinical studies, lots used for demonstration of manufacturing consistency, and relevant development data, such as those arising from analytical method development and stability studies. AM testing data should be tracked over time for consistency and retained samples should be archived for later analysis, if necessary.▲ (USP 1-May-2020)

Some AMs that are biological in nature may be difficult to fully characterize. Because these materials exert their effects through complex biological activities, and ▲because physicochemical▲ (USP 1-May-2020) testing may not be predictive of the AM's process performance, ▲it is frequently necessary to include▲ (USP 1-May-2020) functional or performance testing. ▲▲ (USP 1-May-2020) Performance variability of ▲AMs▲ (USP 1-May-2020) may have a detrimental impact on the potency and consistency of the ▲CGT▲ (USP 1-May-2020) product. Examples of complex ▲functional▲ (USP 1-May-2020) testing for AMs include growth promotion testing ▲▲ (USP 1-May-2020) of fetal bovine serum (FBS) ▲or human plasma/serum supplements,▲ (USP 1-May-2020) performance testing of digestive enzyme preparations, and in vitro tissue culture cytotoxicity assays (see *Performance Testing*).

Vendor Qualification

Vendors ▲▲ (USP 1-May-2020) of AM should be qualified at the earliest opportunity. Auditing the vendor's manufacturing facility, including their GMP ▲capabilities▲ (USP 1-May-2020) and AM testing program, are basic elements of a vendor qualification program. A review of the vendor's processing procedures and documentation program is essential in establishing confidence in the vendor as a reliable supplier. Vendors certified through an ISO inspection program or audited by ▲▲ (USP 1-May-2020) governmental agencies ▲typically▲ (USP 1-May-2020) have robust quality systems in place. Reports of past audits of US suppliers obtained through the Freedom of Information (FOI) Act may augment the qualification process.

▲The CGT product developer should establish a written quality agreement with each AM vendor in order to clearly define the roles and responsibilities, including communication channels, change control, audit procedure, and dispute resolution.▲ (USP 1-May-2020) It is important to develop a good working relationship with vendors. In some cases, the vendor may provide higher manufacturing standards, custom formulation services, or replacement of substandard components upon request, with or without additional costs. ▲▲ (USP 1-May-2020) Vendors should be familiar with the principles of validation, especially

cleaning, viral inactivation, and sterilization validation. It is also critical to ensure that the ▲AM▲ (USP 1-May-2020) vendor takes appropriate steps to prevent cross contamination between ▲different AMs▲ (USP 1-May-2020) during manufacture. Finally, ▲AM vendors should▲ (USP 1-May-2020) supply written certification of processing or sourcing changes to customers, well in advance of the implementation of the changes so that customers can evaluate the potential impact of such changes.

Quality Control and Quality Assurance

Because the components of the ▲AM▲ (USP 1-May-2020) qualification program are multifaceted and need to be in compliance with cGMP, they should be monitored by a quality assurance/quality control unit (QAU). Typical QAU activities include the following systems or programs: 1) incoming receipt, segregation, inspection, and release of materials prior to use in manufacturing, 2) vendor auditing and certification, 3) certificate of analysis verification testing, 4) formal procedures and policies for out-of-specification materials, 5) stability testing, and 6) archival sample storage.

Change to read:

RISK ▲MANAGEMENT

The evaluation of AM risk to CGT product quality should be based on scientific knowledge, with the ultimate goal of patient protection. ICH Q9 (step 4) "Quality Risk Management" provides a useful approach to risk management principles, process, methods, and definitions. Risk management processes include identifying, analyzing, and evaluating risks, followed by controlling potential risks. Tools or methods, such as a risk evaluation matrix (REM), can quantify risk and facilitate appropriate decision-making and risk acceptance.

Each AM is potentially subject to the risk management process, from selection and qualification to storage and distribution, and use in manufacturing. For reasons described earlier in this chapter, and especially because of the inability to thoroughly remove some AM residuals from the final CGT product, AM selection should incorporate a risk-based approach. Before conducting an AM risk assessment the CGT product manufacturing process must be understood, including identification of the AM's relationship to the CGT product's critical quality attributes (CQAs); these considerations are described in ICH Q11 (step 4) "Development and Manufacture of Drug Substances". Assessment tools can then be used to quantitate each critical parameter, resulting in an assessment of overall risk. For example, if an AM had a suitable safety profile, was used in minimal amounts in upstream steps of the CGT product manufacturing process, and was thoroughly removed from the final CGT product, it would earn a low risk category since both the likelihood of failure (toxicity) and the occurrence severity (consequence to the patient) are low. Conversely, an AM with known toxicity which is introduced downstream would have higher potential to appear as a toxic residual in the final product, and would therefore be assigned to a medium or high risk category. Regardless of the risk category, assessment of AM removal should be completed during CGT product manufacturing process characterization and validation.

As part of the risk assessment process a rational and scientific qualification program should be designed for each AM, taking into account sources and manufacturing processes. Whenever available, AMs that are approved or licensed therapeutic products are preferable because they are well-characterized, possess an established toxicological profile, and are manufactured according to controlled and documented procedures. Incorporating licensed or approved biologics, small molecule drugs, and medical devices or implantable materials into CGT product manufacturing processes presents a more favorable patient safety profile, compared to unapproved versions. Qualification programs for these AMs should reflect the extensive scrutiny to which they were subjected during development and manufacture. Consequently, greater emphasis may then be placed on the impact of inherent AM variability on final CGT product function. For example, if a manufacturer selects approved human serum albumin (HSA) as a cell culture medium supplement for CGT product manufacturing, it may be prudent to assess the impact of the HSA's lot-to-lot variability on cell growth rate and differentiation, its stability during manufacturing, or its interaction with other processing components. Such qualification approaches focus on the AM's variability as it influences final product potency and safety. AM qualification programs should be comprehensive to minimize risks to the consumer and the CGT product.

To aid CGT manufacturers and developers in the design of their AM risk management and qualification programs, potential risk categories are presented as Tiers 1–4 in *Table 1*, *Table 2*, *Table 3*, and *Table 4*, respectively; these are provided as a guide to create an REM. The REM should also consider the amount of the AM used, the stage at which the AM is used in the manufacturing process, and the residual AM remaining in the final CGT product.▲ (USP 1-May-2020)

Tier 1

▲The AM is a highly qualified material that is well-suited for use in manufacturing of CGT products, such as a licensed biologic, drug, or medical device. Tier 1 AMs generally come with sterile packaging systems or dosage forms labeled with their intended use, and the CGT product developer clearly defines and documents their use within the manufacturing process. Changes to the packaging, dosage form, formulation, or storage conditions must be qualified and documented; these changes may also trigger further AM release testing and stability studies.▲ (USP 1-May-2020)

Tier 2

▲The AM is a well-characterized material produced under an established quality system well-suited for CGT product manufacturing, but the AM is not a licensed or approved medical product. Many Tier 2 AMs are produced specifically for the manufacture of CGT products. Most animal-derived materials are excluded from this category.▲ (USP 1-May-2020)

Tier 3

▲The AM is either intended for research use, locally produced under laboratory conditions, or not intended for use in CGT product manufacturing; it may be approved by regulatory agencies as part of an in vitro diagnostic device. Tier 3 AMs require more qualification than Tier 1 or Tier 2 materials. Using a risk-based approach, an AM manufacturing process upgrade may be necessary to fully qualify the AM for CGT product manufacturing. For example, robust viral inactivation and removal steps may need to be added to the production process for a diagnostic-grade monoclonal antibody in order to produce a reagent suitable for CGT product manufacturing. The manufacturing process for an AM produced in an academic or pilot-stage manufacturing facility will likely need to be upgraded for late-stage clinical trials and eventual commercialization of the CGT product.▲ (USP 1-May-2020)

Tier 4

▲The AM is produced for industrial or research use and may contain harmful impurities, and/or may contain animal- or human-derived components harboring adventitious agents. High-risk Tier 4 AMs require extensive qualification before they may be used in CGT product manufacturing. These materials may require one or more of the following measures:

1. An upgrade of the AM manufacturing process
2. Selection, screening, and testing of the animal or human sources
3. Testing of each AM lot to ensure that it is free of adventitious agents or specific contaminants
4. Treatment of the AM to inactivate or remove adventitious agents or specific contaminants
5. Validation of the CGT product manufacturing process to assess consistency of removal of a known toxic substance, and/or lot-release testing to demonstrate safe residual levels
6. Validation of the CGT product manufacturing process to demonstrate removal or inactivation of adventitious agents or specific contaminants

For Tier 4 AMs deemed critical to the CGT product manufacturing process, CGT product developers should explore alternative AMs or sources as early as possible in development.▲ (USP 1-May-2020)

Table 1. AM Risk Tier 1 ▲Materials Intended for Use as Approved Biologics, Drugs, or Medical Devices▲ (USP 1-May-2020)

Example	Typical Use in CGT Product Manufacturing	Qualification or Risk Reduction ▲▲ (USP 1-May-2020)
Recombinant insulin for injection	▲Chemically defined basal cell culture medium▲ (USP 1-May-2020)	• DMF cross reference (when possible or practical) • Certificate of analysis • Assess lot-to-lot effect on process performance[b] • Assess removal from final product • ▲Assess AM stability as stored and used in CGT product manufacturing[c] • Visual inspections • Assess particulates and extractables▲ (USP 1-May-2020)
Organ ▲transport/▲ (USP 1-May-2020) preservation fluid	Process biological fluid employed in tissue transport or processing	
Human serum albumin for injection	Cell culture ▲additive▲ (USP 1-May-2020)	
Sterile fluids for injection	Process biological fluid employed in tissue transport, cell processing, purification	
Implantable ▲materials▲ (USP 1-May-2020) (collagen, silicone, polyurethane constructs intended for surgical implantation)	Scaffolds, matrices for immobilized cellular cultivation	
Recombinant deoxyribonuclease for inhalation or injection	Process enzyme employed in viral vector manufacturing, stem cell processing	
Injectable antibiotics[a]	▲▲ (USP 1-May-2020) Biopsy transport fluid additive to reduce risk of bacterial contamination	
Injectable monoclonal antibodies	Immunologically targeting specific cell populations for selection or removal	
Injectable cytokines, ▲vitamins, chemicals, nutrients▲ (USP 1-May-2020)	Cell culture ▲additive▲ (USP 1-May-2020)	
▲▲ (USP 1-May-2020)	▲▲ (USP 1-May-2020)	
IV bags, transfer sets and tubing, cryopreservation bags, syringes, needles	Storage vessels or container–closure systems, closed aseptic transfer systems	

[a] Beta lactam antibiotics should not be used as AMs due to the risk of patient hypersensitivity.

[b] Beta lactam antibiotics should not be used as AMs due to the risk of patient hypersensitivity. See *Performance Testing*.

[c] Often AMs are aliquoted or stored at different concentrations, in different buffers, or under conditions that are different from those stated on the label or previously validated. Data should be generated that demonstrate the stability and preservation of activity of the AM under the conditions that are specific to the manufacturing application.

Table 2. AM Risk Tier 2 ▲Well-Characterized Materials with Intended Use as AMs▲ (USP 1-May-2020)

Example	Typical Use in CGT Product Manufacturing	Qualification or Risk Reduction ▲▲ (USP 1-May-2020)
Recombinant growth factors, cytokines[a]	Cell culture ▲▲ (USP 1-May-2020) additive	▲All of the qualification and risk mitigation activities in Tier 1, plus the following:▲ (USP 1-May-2020) • DMF cross reference (when possible or practical) • ▲Confirm certificate of analysis test results that are critical to CGT product identity, purity, or potency • Verify AMs containing animal- or human-derived materials have been purified, tested, and certified to be free of adventitious agents▲ (USP 1-May-2020) • Vendor audit
Immunomagnetic beads	▲Cell selection▲ (USP 1-May-2020)	
Human AB serum	Cell culture ▲▲ (USP 1-May-2020) additive	
Progesterone, estrogen, vitamins, purified chemicals (USP-grade)	Cell culture ▲▲ (USP 1-May-2020) additives, induction agents, buffer components	
Sterile process buffers	Process biological fluid employed in tissue transport, cell processing, purification	
Biocompatible polymers, scaffolds, hydrogels	Scaffolds, matrices for immobilized cellular cultivation	
Proteolytic enzymes	▲Digest tissue or protein▲ (USP 1-May-2020)	
Tissue culture media	Cell culture ▲▲ (USP 1-May-2020) additive	
▲▲ (USP 1-May-2020)	▲▲ (USP 1-May-2020)	
Density gradient media	Cell separation via centrifugation	

[a] ▲AMs▲ (USP 1-May-2020) produced from nonmammalian, recombinant sources (i.e., microbially grown in the absence of animal-derived growth medium components).
▲ ▲ (USP 1-May-2020)

Table 3. AM Risk Tier 3 Moderate-Risk Materials Not Intended for Use as AMs▲▲ (USP 1-May-2020)

Example	Typical Use in CGT Product Manufacturing	Qualification or Risk Reduction ▲▲ (USP 1-May-2020)
Recombinant growth factors, cytokines	Cell culture ▲▲ (USP 1-May-2020) additive	▲All of the qualification and risk mitigation activities in Tier 2, plus the following:▲ (USP 1-May-2020) • DMF cross reference (when possible or practical) • ▲Confirm certificate of analysis test results▲ (USP 1-May-2020) • Upgrade manufacturing process ▲and/or testing▲ (USP 1-May-2020) for material to ▲be suitable for therapeutic use▲ (USP 1-May-2020)
Tissue culture media	Cell culture ▲▲ (USP 1-May-2020) additive	
Monoclonal antibodies ▲(for diagnostic use▲ (USP 1-May-2020) produced in cell culture)	▲Targeting cells for selection, activation, or removal▲ (USP 1-May-2020)	
Process buffers	Process biological fluid employed in tissue transport, cell processing, purification	
Novel polymers, scaffolds, hydrogels	Scaffolds, matrices for immobilized cellular cultivation	
Proteolytic enzymes	▲Digest tissue or protein▲ (USP 1-May-2020)	
Purified chemicals (reagent-grade)	Culture medium additives, induction agents, buffer components	

▲▲ (USP 1-May-2020)

Table 4. AM Risk Tier 4 High-Risk Materials

Example	Typical Use in CGT Product Manufacturing	Qualification or Risk Reduction
▲Animal- and human-derived materials▲ (USP 1-May-2020)	Cell culture ▲▲ (USP 1-May-2020) additives; ▲targeting moieties for cell selection, activation, or removal▲ (USP 1-May-2020)	▲All of the qualification and risk mitigation activities in Tier 3, plus the following: • Safety testing for residuals in CGT product • Recombination-competent retroviral testing for relevant gene therapy AMs▲ (USP 1-May-2020) • Adventitious agent testing for animal source-relevant viruses
▲▲ (USP 1-May-2020)	▲▲ (USP 1-May-2020)	
Animal-derived polymers, scaffolds, hydrogels	Scaffolds, matrices for immobilized cellular cultivation	
Purified enzymes	Process ▲additives such as trypsin or collagenase for digesting protein or tissue▲ (USP 1-May-2020)	
▲▲ (USP 1-May-2020)	▲▲ (USP 1-May-2020)	
Animal or human cells used as feeder layers	Cell culture substratum or ▲conditioned medium▲ (USP 1-May-2020)	
▲Toxic entities (i.e., methotrexate, bacterial toxins, etc.)	Cell culture additives to maintain transgene expression, enhance proliferation, or improve cell survival upon cryopreservation▲ (USP 1-May-2020)	

Change to read:

PERFORMANCE TESTING

▲In cases where an AM is chosen for its effect on a particular biological function of an intermediate or final CGT product, performance testing is an essential element of its overall qualification. This is especially true when the AM plays a critical role in modulating a complex biochemical effect or has a large impact on yield, purity, and/or final product potency. Performance testing is particularly critical for AMs that are complex substances, mixtures, or biologically sourced; these AMs are more likely to show significant lot-to-lot variability, lack a simple identity test, and be difficult to characterize. The development of well-defined performance assays for complex AMs will not only ensure process reproducibility and final product quality, but in many cases will satisfy regulatory requirements for identity testing.

Often, the initial qualification of an AM for use in CGT product manufacturing investigates the dose-response effect of the AM on yield, purity, or potency of the therapeutic product. The optimal amount of AM used in CGT product manufacturing should consistently yield the desired effect, while minimizing residuals. Performance testing frequently utilizes a scaled-down or simulated manufacturing process.

Some examples include:

- For a cellular proliferation AM added to culture medium, the assay could demonstrate that each lot of AM produces the expected rate and degree of cellular proliferation
- For a monoclonal antibody used to purify a particular cell type, the assay could demonstrate that each new lot of monoclonal antibody purifies a standard cell population with the expected recovery and purity
- For a density gradient material used to purify a vector or cell, the assay could demonstrate that each new lot of material purifies the vector or cell with the expected recovery and purity
- For a helper viral vector used to produce a gene therapy vector, the assay could demonstrate that new lots of the helper vector produce the expected amounts of active gene therapy vector

Functional assays may evolve as CGT product manufacturing processes develop, and the critical relationships between AM and final products are better understood.

When performance tests yield relative results, it is helpful to compare each new lot of AM side by side with a reference standard AM, if available. This simultaneous comparison reduces the variability due to different lots of cells or vectors used in testing.▲ (USP 1-May-2020)

Change to read:

▲RESIDUAL▲ (USP 1-MAY-2020) ASSESSMENT AND REMOVAL

▲Though AMs are not intended to be present in the final CGT product, it is often challenging to completely remove AMs without impairing the CGT product yield or quality. To establish appropriate specifications for AM residuals, developers should address both AM removal procedures and AM analytical methods early in development. Developing appropriate residuals specifications and detection methods ensures CGT product and process consistency, allows characterization of the final CGT product, and reduces negative effects on product quality, clinical efficacy, and safety.

Thresholds for AM residuals are established, and may evolve, throughout product development. In early development, the fold reduction in AM concentration is often estimated for each manufacturing unit operation, and AM removal is typically expressed as a dilution factor or log reduction factor. In late development, direct residuals measurements utilize validated assays. However, all AM residuals measurements must be interpreted with some caution, because intracellular and extracellular AM concentrations may differ.

As a part of CGT product manufacturing process validation, spiking experiments with high AM concentrations may be required to mimic a worst case scenario and demonstrate manufacturing process robustness. Validation studies should incorporate the following considerations:

1. Residuals assay should accurately quantitate the AM in each sample matrix
2. If a scaled-down validation is conducted, the comparability of the small-scale and full-scale processes must be demonstrated
3. In spiking studies, the higher AM levels must not interfere with the purification process

Specifications for residual AM in the final CGT product are typically based on the residual AM in the CGT product lots used in toxicological or clinical studies, or on known toxicological data.▲ (USP 1-May-2020)

Change to read:

CONCLUSION

▲AM quality is critical to CGT product quality. AMs must be carefully selected, characterized, and qualified, so that they perform consistently and as intended in the CGT product manufacturing process. AM qualification programs should focus on developing appropriate AM specifications, robust CGT product manufacturing processes, and AM risk assessments and mitigations, in order to prevent negative impacts on CGT product quality, clinical efficacy, and patient safety.▲ (USP 1-May-2020)

Change to read:

APPENDIX

Lists of Relevant *USP* Chapters and Regulatory References

AMs used in CGT products will be regulated in the context of the manufacturing process of the CGT products. ▲▲ (USP 1-May-2020) The following lists of documents include relevant regulatory ▲and technical guidances on▲ (USP 1-May-2020) product and process development, manufacturing, quality control, and quality assurance.

***USP* Chapters**

- *Biological Reactivity Tests, In Vitro* ⟨87⟩
- *Biological Reactivity Tests, In Vivo* ⟨88⟩
- ▲*Cell-based Advanced Therapies and Tissue-based Products* ⟨1046⟩▲ (CN 1-May-2020)
- ▲*Gene Therapy Products* ⟨1047⟩▲ (USP 1-May-2020)
- *Biotechnology-Derived Articles—Amino Acid Analysis* ⟨1052⟩
- *Capillary Electrophoresis* ⟨1053⟩
- *Biotechnology-Derived Articles—Isoelectric Focusing* ⟨1054⟩
- *Biotechnology-Derived Articles—Peptide Mapping* ⟨1055⟩
- *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩
- *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩

Code of Federal Regulations (CFR)

▲Regulations	Title
21 CFR 211 Subpart E, 211.80 through 211.94 and 211.101	Control of Components and Drug Product Containers and Closures
21 CFR 312	Investigational New Drug Application
21 CFR 314	Applications for FDA Approval to Market a New Drug
21 CFR 801.109(b)(1)	Labeling—Prescription Devices
21 CFR 807 Subpart E, 807.81 through 807.97	Premarket Notification Procedures
21 CFR 812	Investigational Device Exemptions
21 CFR 814	Premarket Approval of Medical Devices
21 CFR 876.5885	Tissue Culture Media for Human Ex Vivo Tissue and Cell Culture Processing Applications▲ (USP 1-May-2020)

FDA Guidance Documents

- ▲▲ (USP 1-May-2020)*Guidance for Industry: Monoclonal Antibodies Used as Reagents in Drug Manufacturing*, ▲March 2001. https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm080417.pdf.▲ (USP 1-May-2020)
- *Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals*, ▲May 1993. https://www.fda.gov/downloads/biologicsbloodvaccines/safetyavailability/ucm162863.pdf.▲ (USP 1-May-2020)
- *Class II Special Controls Guidance Document: Tissue Culture Media for Human ex vivo Tissue and Cell Culture Processing Applications—Final Guidance for Industry and FDA Reviewers*, May 16, 2001. ▲https://www.fda.gov/RegulatoryInformation/Guidances/ucm073567.htm.
- *Guidance for Industry: Contract Manufacturing Arrangements for Drugs: Quality Agreements*, November 2016. https://www.fda.gov/downloads/drugs/guidances/ucm353925.pdf.
- *Guidance for Industry and Food and Drug Administration Staff: Use of International Standard ISO 10993-1, "Biological evaluation of medical devices—Part 1: Evaluation and testing within a risk management process"*, June 16, 2016. https://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm348890.pdf.▲ (USP 1-May-2020)

National and International Regulatory Documents

- ICH Q5A: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human and Animal Origin. ▲Available at: http://www.ich.org.▲ (USP 1-May-2020)
- ICH Q5D: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products. ▲Available at: http://www.ich.org.
- ICH Q8(R2): Pharmaceutical Development. Available at: http://www.ich.org.
- ICH Q9: Quality Risk Management. Available at: http://www.ich.org.▲ (USP 1-May-2020)
- Public Health Service Guideline on Infectious Diseases Issues in Xenotransplantation. ▲Available at: https://www.cdc.gov/.
- Health Canada "Guidance Document: Preparation of Clinical Trial Applications for use of Cell Therapy Products in Humans". Available at: https://www.canada.ca/en/health-canada.html.▲ (USP 1-May-2020)