Risk Assessment Approach to Microbiological Controls of Cell Therapies

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TECHNOLOGY/APPLICATION

Risk Assessment Approach to Microbiological Controls of Cell Therapies

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ABSTRACT: This technology review, written by a small group of pharmaceutical microbiologists experienced in cell therapies, discussed a risk-based approach to microbiological contamination detection and control during gene and cell therapy production. Topics discussed include a brief overview of cell therapies, a risk analysis related to donor selection, cell collection and infectious agent testing, cell transformation and expansion, packaging, storage, and administration, and cell therapy microbial contamination testing and release.

KEYWORDS: Risk-based approach, Microbial contamination, Cell therapies, Donor selection, Cell collection, Cell transformation and expansion, Bacterial endotoxin, Sterility, Mycoplasma, Adventitious viruses, Biological safety cabinets, Restrictive access barrier systems, Isolator systems, In-process sterility testing, Rapid microbial detection testing.

Introduction

The intent of this article was to promote a holistic, risk-based approach to microbial contamination monitoring and control during cell therapy production. The authors believe it is important to identify and mitigate risks associated with contamination by bacteria, fungi, and mycoplasma and institute monitoring programs that reflect these levels of risks. Adventitious viruses, although discussed in the raw materials section, were outside the scope of this paper. As cell therapy production involves continuous, stepwise, aseptic processing over multiple days, the emphasis should be placed on microbial contamination prevention and detection and not on sterility testing. As many cell therapy products do not have a label claim of “sterile”, firms should consider calling the final product test a microbial contamination test instead of a sterility test. This is consistent with other cell-based growth manufacturing processes (e.g., nonsterile monoclonal antibody drug substance production) where the drug substance and/or process was not intended to be sterile; however, the expectation was that no microbial contamination was introduced.

As expected, the longer the bioprocessing takes and the greater the number of aseptic manipulations, especially when using systems (i.e., open systems) with a higher potential for microbial ingress, the greater the risk of microbial contamination during processing. Whenever practical, rapid microbial detection methods should be employed instead of growth-based microbial methods (e.g., sterility tests). The authors caution that approaches widely used in classical biopharmaceutical manufacturing may not be applicable to bioprocessing for cell-based therapies.

The 2019 Regenerative Medicine Advanced Therapy (RMAT) guidance clarifies the U.S. Food and Drug Association’s (FDA’s) interpretation of regenerative medicine therapies as defined in Section 506(g)(8) of the Federal Food, Drug and Cosmetic Act (“FDC Act”) (1). Section 506(g)(8) defines regenerative advanced therapy as “including cell therapies, therapeutic tissue engineering products, human cell and tissue products, and combination products using any such therapies or products, except for those regulated solely under Section 361 of the Public Health Service Act (“PHS Act”) (42 U.S.C. 264) and [21 C.F.R. Part 1271]”(2). The first update to the interpretation of this provision is with regards to cell therapies. The FDA clarified that it interprets “cell therapies” to include both allogeneic and autologous cell therapies, as well as xenogeneic cell products.
Particular attention will be given to human cord blood, bone marrow, peripheral blood, and adipose tissue mesenchymal stem cells because of their wide use in cellular therapy. Xenogeneic cell products are outside of the scope of this article.

Brief Overview of Cell Therapies

Cell therapies are at the forefront of scientific innovation, medical sciences, and rapid technology changes in the field of immunotherapy. They often target a need for treatment where no traditional or alternative therapies exist, including treatment for rare diseases or for cancer where other treatments have failed. Cell therapy products may be autologous, allogeneic, or xenogeneic cells that have been subjected to substantial manipulation outside the body, altering their biological characteristics, physiological functions, or structural properties so that they can be administered to humans for the purpose of prevention, cure, treatment, diagnosis, or mitigation of disease or injuries (3). Cell therapies work through the pharmacological, immunological, or metabolic action of these modified cells.

The manufacture of cell therapies involves a process referred to as adoptive cell transfer (ACT). Steps in this process may include cell selection, insertion of replication-limited viral vectors and/or genetic constructs, propagation, and expansion. The final product is composed of genetically modified cells that target specific cell types in the recipient’s body. Such cells may have a different functional capability in the recipient than they had in the donor (4).

One of the emerging ACT cell therapies is chimeric antigen receptors-T cells (CAR-T) technology. In 2017, two such CAR-T cell therapies were approved by the FDA, one for childhood acute lymphocytic leukemia (ALL) and the other for diffuse large B cell lymphoma (DLBCL). CAR-T cells are the equivalent of “giving patients a living drug” according to Reiner Brentjens, MD, PhD, a pioneer in CAR-T technology at Memorial Sloan Kettering Cancer Center in New York City. T cells are the backbone of CAR-T technology. After apheresis of the patient or a donor, the T cells are selected and genetically engineered against the disease using a nonreplicating virus to produce antibodies, called chimeric antigen receptors (CARs). These T cells are then expanded in the manufacturing area. The final step is the infusion of the CAR-T cells into the patient where they continue to replicate and identify and destroy the cancer cells, inactivating the cancer cell antigens using the targeted receptors on the CAR-T cell surfaces.

Cell therapy products fall under the regulatory definition of biological products. Regulations for cell therapies include the EudraLex guidance specifically for Advanced Therapeutic Medicinal Products (ATMPs) (5) and the European Medicines Agency (EMA) Guideline on Human Cell-Based Medicinal Products (6). ATMPs have mostly been developed in academic or hospital settings and now need to be transferred for commercial development to a suitable manufacturing environment with appropriate quality systems. This poses challenges in that cell therapy products do not follow the conventional pharmaceutical manufacturing route and clinicians lack experience with current good manufacturing practices (cGMPs). Consider the following:

1. The risk of microbial contamination: cell therapy products cannot be sterilized using current industry practices. The manufacturing process involves many manual aseptic manipulations, which are operator dependent.

2. The entire process is manipulation of the product.

3. The drug substance in some cases can be the drug product (i.e., a continuous process).

4. There is no viral removal/inactivation step.

5. Antibiotics may be used for specific parts of the process to suppress microbial growth.

6. The product may be manufactured and applied during the same surgical procedure.

7. One lot is reserved for a single patient in an autologous therapy.

There are testing challenges:

1. Cell viability and microbial contamination in-process checks are operator dependent, unless automated.

2. A number of quality control tests are destructive and thus require extra material to be prepared or consumed, which can be a significant loss in cell-based therapies.

3. Sampling for testing materials may inadvertently introduce contamination.

4. The short time to get the product to the patient makes growth-based compendial contamination tests less suitable.
New technologies used in the research setting may not be designed to meet the rigor of cGMPs and may have to undergo modifications from development to commercial production. Cell therapies may have a short shelf life because of the clinical advantage of using fresh cells or because of urgent medical need; therefore, conventional release testing may not be possible. Because of these unique issues, controls for raw materials and consumables must be clearly defined.

**Example of a Cell Therapy Manufacturing Process**

The following diagram (Figure 1) provides an example of the manufacturing steps for a cell therapy product, using a CAR-T manufacturing process as a model.

**Overview of Contamination Control**

Recently published pharmaceutical good manufacturing practices (GMPs), including the revisions to the EU Good Manufacturing Practices Annex 1, emphasized that processes, equipment, facilities, and manufacturing activities should be managed in accordance with quality risk management principles that provide a proactive means of identifying, scientifically evaluating, and controlling potential risks to product quality.

In view of these expectations, Friends of Cancer Research (7) have stated:

There can be varying interpretations of FDA guidance regarding phase-appropriate current GMP requirements for manufacturing products for use in the early investigational setting. In consequence, some institutions have imposed very strict cGMP requirements that are more applicable for later stage clinical development ... significantly increasing the cost and time to manufacture early investigational cell products ... Implicit in any approach for manufacturing ... is a focus on patient safety ... with an emphasis on risk assessments and analytical testing to determine and manage potential impact to patient safety.

It is the opinion of the authors of this article that risk assessments should be used to justify using alternative
approaches for cell therapy manufacture versus using those specified in classical pharmaceutical GMPs.

Quality assurance is particularly important in the manufacture of sterile products like gene and cell therapies and must strictly follow carefully established and validated methods of manufacture and their controls. A contamination control strategy should be implemented across the cell therapy facility to assess the effectiveness of all the control and monitoring measures employed. This strategy should consider all aspects of contamination control and its life cycle with ongoing and periodic reviews and assessments to update the strategy as appropriate. This assessment should lead to corrective and preventative actions being taken as necessary to reduce the risk of microbial contamination.

At a high level, a typical cell therapy manufacturing process involves the following areas where a strategy should be employed (Figure 2).

How prevalent is microbial contamination of cell therapy products? Reports in the literature summarized by a recent review article differed by cell type, national origin, and time period so it was difficult to establish the prevalence of microbial contamination (8). The reviewers reported that the most common contaminants were mycoplasma, bacteria, fungi, and viruses. The most prominent bacteria found in contaminated cell cultures were Gram-positive spore-forming rods like Bacillus cereus, Bacillus coagulans, and Bacillus brevis; Gram-positive cocci like Enterococcus malodoratus, E. casseliflavus, and Staphylococcus epidermidis; and Gram-negative bacteria such as Escherichia coli. Based on the identity of these contaminants, it was most likely that they originated from either the cell collection, the processing facility, or the technicians responsible for the manipulation of the cell culture, although the exact origin of the contaminants was uncertain. Furthermore, this example may not be representative of other cell therapy findings.

Microbial Contamination Control Strategy

Most cell therapy manufacturing processes employ steps that are time-sensitive; therefore, it is imperative that a risk-based contamination control/prevention approach that does not compromise the quality, safety, efficacy, and identity of the product is used. A risk-based approach must also be utilized to ensure a successful and timely treatment of the patient.

It is also important for cell therapy manufacturers to note that terminology such as “open” and “closed” (as used in aseptic processing for sterile manufacturing) may not directly translate to aseptic manipulations that are performed during cell therapy manufacturing. Considerations must be given to the types of manipulations as well as the duration of such manipulations when developing a contamination control/prevention strategy.

This is in alignment with the Guidelines on Good Manufacturing Practices specific to ATMPs (5) which states,

These Guidelines do not intend to place any restrain on the development of new concepts of new technologies. While this document describes the standard expectation, manufacturers may implement alternative approaches, if it is demonstrated that the alternative approach is capable of meeting the same objective. Any adaptation applied must be compatible with the need to ensure the quality, safety, ecacy, and traceability of the product.
A control strategy should, at a minimum, consist of the considerations shown in Table I.

The cell therapy manufacturing wherever possible will be performed in closed systems (low risk of microbial ingress) using sterile single-use disposable materials with advanced aseptic manipulation occurring in biosafety cabinets, restrictive access barrier systems (RABS), or isolator systems.

### Input Materials

Input materials may represent the highest risk for the introduction of adventitious contamination for a cell therapy process; therefore, it is important that a well-defined strategy is employed to mitigate these risks.

After the cells are sourced from a donor or a cell bank, they are isolated, transduced, selected, and expanded by cell culture to obtain sufficient cells for therapeutic use, harvested from the culture medium, formulated, and packaged as a therapeutic dose for delivery to the recipient. To mitigate the risk of microbial contamination, the inputs to the production of cell therapies must be selected, controlled, and tested. The recent 2019 Parenteral Drug Association Technical Report No. 81: Cell-Based Therapy Control Strategy (9) provides a comprehensive control strategy that mitigates all risks to product quality. The authors concentrated in this article on what the technical report identified as critical microbial safety concerns, that is, the presence of mycoplasma, bacteria, fungi, and bacterial endotoxin (see Table II).

### Overall Risk Analysis

A number of risk analysis tools, including quality risk management, may be used when assessing risk factors.

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**TABLE I**

Control Strategy Points to Consider for Cell Therapy Production

<table>
<thead>
<tr>
<th>Input Materials</th>
<th>Manufacturing Facilities</th>
<th>Manufacturing Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>● Cell harvesting, for example, leukapheresis</td>
<td>● Design</td>
<td>● Personnel qualification and training</td>
</tr>
<tr>
<td>● Raw materials control, including in-process</td>
<td>● Equipment</td>
<td>● Design</td>
</tr>
<tr>
<td>● Product containers and closures</td>
<td>● Utilities</td>
<td>● Process risk assessment</td>
</tr>
<tr>
<td>● Vendor approval (key component suppliers, sterilization of components and single-use systems, and services)</td>
<td>● Preventative maintenance—maintaining equipment and premises (planned and unplanned maintenance) to a standard that will not add significant risk of contamination</td>
<td>● Process simulation</td>
</tr>
<tr>
<td>● For outsourced services, such as sterilization, sufficient evidence should be provided to the contracting organization to ensure that the process is operating correctly</td>
<td>● Cleaning and disinfection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>● Monitoring systems—including an assessment of the feasibility of the introduction of scientifically sound, modern methods that optimize the detection of environmental contamination</td>
<td></td>
</tr>
<tr>
<td></td>
<td>● Prevention—trending, investigations, corrective and preventive actions (CAPA), root cause determination, and the need for more robust investigational tools</td>
<td></td>
</tr>
<tr>
<td></td>
<td>● Continuous improvement based on information from the above systems</td>
<td></td>
</tr>
</tbody>
</table>
Tables III–V show an example of a hazard analysis and critical control points program approach (originating in the food industry), which summarizes the common input materials, identifies the various risks to the recipient of the cell therapy with ratings from low to high, and suggests common risk mitigations or critical process controls.

The following section discusses in more detail the risk mitigation strategies of these inputs. Testing strategies will be summarized later in the document.

**Donor Qualification**

**Autologous Cells (Patient Specific):** According to 21 CFR 1271.90(a)(1) (10), a donor eligibility determination or donor screening for infectious agents is not required on autologous cells. However, as pathogens may be present in the donor, it should be determined if bioprocessing will increase the risk to the recipient owing to further propagation of a pathogenic agent present in the donor that on administration could cause infection. For example, donation would not be scheduled immediately after a dental procedure or minor surgery. The presence of pathogens could also influence the manufacturing site control and the safety of the manufacturing operators. All biological materials must be handled as potentially infectious materials.

**Allogeneic Cells (Universal):** For allogeneic cells or tissues, manufacturers must perform donor screening and testing, as required in 21 CFR Part 1271 (10). Donors of all types of cells and tissues must be screened for risk factors and clinical evidence of relevant communicable disease agents and diseases, including: human immunodeficiency virus, hepatitis B virus, hepatitis C

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Critical Assessment for Microbial Safety Concerns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attributes</td>
<td>Severity</td>
</tr>
<tr>
<td>Bacterial endotoxins</td>
<td>High</td>
</tr>
<tr>
<td>Sterility</td>
<td>High</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>Moderate–High</td>
</tr>
<tr>
<td>Adventitious viruses</td>
<td>High</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE III</th>
<th>Risk Analysis with Cell Therapy Products Related to Input Materials—Donor Selection and Cell Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input Materials</td>
<td>Risk Identification</td>
</tr>
<tr>
<td>Donor qualification</td>
<td>Autologous donation</td>
</tr>
<tr>
<td></td>
<td>Allogeneic donation</td>
</tr>
<tr>
<td>Source of the cells</td>
<td>Peripheral blood</td>
</tr>
<tr>
<td></td>
<td>Adipose tissue</td>
</tr>
<tr>
<td></td>
<td>Bone marrow</td>
</tr>
<tr>
<td></td>
<td>Cord blood</td>
</tr>
<tr>
<td>Collection process</td>
<td>Systems with a high probability of microbial ingress</td>
</tr>
<tr>
<td></td>
<td>Systems with a low probability of microbial ingress</td>
</tr>
</tbody>
</table>

“May be high if the patient has a preexisting microbial infection.
<table>
<thead>
<tr>
<th>Input Materials</th>
<th>Risk Identification</th>
<th>Risk Rating</th>
<th>Risk Mitigation/Critical Process Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-use consumables</td>
<td>Open (e.g., transfer bags, roller bags, cassettes, etc.)</td>
<td>High</td>
<td>Sterile, disposable consumables</td>
</tr>
<tr>
<td></td>
<td>Closed</td>
<td>Low</td>
<td>Sterility, mycoplasma and infectious agent screening</td>
</tr>
<tr>
<td>Cell banks</td>
<td>Contamination</td>
<td>Moderate</td>
<td>Infectious agent screening; Sterile filling/sterility testing</td>
</tr>
<tr>
<td>Transduction</td>
<td>Viral and bacterial vectors</td>
<td>Low to Moderate</td>
<td>BSC, RABS and isolator systems; Bioburden Monitoring</td>
</tr>
<tr>
<td>Cell expansion</td>
<td>Multiple aseptic manipulations</td>
<td>Low to Moderate</td>
<td>Source certification; Adventitious agent screening; Terminal sterilization (pasteurization, chemical treatment or irradiation)</td>
</tr>
<tr>
<td>Culture media</td>
<td>Fetal bovine serum and other components</td>
<td>Low to Moderate</td>
<td>GMP and compendial compliance; Microbial monitoring, Endotoxin monitoring</td>
</tr>
<tr>
<td>Microcarrier</td>
<td>Immunomagnetic beads</td>
<td>Low</td>
<td>Terminal sterilization</td>
</tr>
<tr>
<td>Utilities</td>
<td>Pharmaceutical-grade water</td>
<td>Moderate</td>
<td>Sterile filtration at Point of use</td>
</tr>
<tr>
<td>Compressed gases</td>
<td></td>
<td>Moderate</td>
<td>Sterile filtration at Point of use</td>
</tr>
<tr>
<td>Facility</td>
<td>Containment</td>
<td>Low to Moderate</td>
<td>BSC, isolators, RABS, etc. Labelling, consideration for dedicated work areas for high-risk contamination steps, appropriate disinfection between steps, process flows, and HVAC cascades</td>
</tr>
<tr>
<td></td>
<td>Segregation of process steps</td>
<td>Low to Moderate</td>
<td>Clearly defined and physically separate or procedurally segregated entry and exit. Transfer disinfection procedures for materials, adequate protection of sterile materials for transfer</td>
</tr>
<tr>
<td></td>
<td>Personnel and materials flow</td>
<td>Low to Moderate</td>
<td>Terminal sterilization; Use of non-animal-derived materials</td>
</tr>
<tr>
<td>Cell harvesting</td>
<td>Digestive enzymes (e.g., trypsin, collagenase, DNase/ RNase, restriction endonucleases), growth factors, cytokines, and monoclonal antibodies</td>
<td>Low to Moderate</td>
<td>Terminal sterilization; Use of non-animal-derived materials</td>
</tr>
<tr>
<td>Cell storage solution</td>
<td>Buffers; saline; surfactants; cryo-protectants</td>
<td>Low</td>
<td>Sterile filtration</td>
</tr>
</tbody>
</table>
virus, human transmissible spongiform encephalopathies, including Creutzfeldt–Jakob disease, and Treponema pallidum (syphilis) (21 CFR 1271.75) (10). If cord blood or other maternally-derived tissue is used, screening and testing on the birth mothers, as described in 21 CFR Part 1271.80(a) (8), must be performed.

For donor eligibility testing, facilities must use appropriate FDA-licensed, approved, or cleared tests (21 CFR 1271.80(c)) (8).

**Cell Source Risk:** The relative microbial contamination rate for different sources of stem cells may be bone marrow > adipose tissue > cord blood > peripheral blood. This hierarchy of risk is related to the invasiveness of the cell collection method, the environmental controls, and the number of manipulations required.

**Collection Process**

Closed systems for cell collection, processing, and delivery are preferred to open systems in terms of both microbial contamination and personnel safety. For example, the risk of microbial contamination is reduced in leukapheresis using closed systems for blood collection, cell separation and washing and I.V. bag preparation.

**Testing Based on Types of Donation:** Autologous and allogeneic cell cultures are tested for only bacterial endotoxin, sterility, and mycoplasma. In addition to these tests, allogeneic cell cultures and some autologous cell cultures are screened for blood-borne infectious agents.

**Cell Culture Media**

Animal-derived or human-derived cell culture media ingredients, for example, fetal bovine serum or human AB serum, have potentially higher risks of microbial contamination; therefore, a strong trend exists toward the use of synthetic cell culture media, media ingredients certified as low-risk materials, and sterilization techniques (e.g., gamma irradiation) to inactivate microbial contaminants in the medium before its use in cell culture.

**Microcarriers for Cell Adhesion**

These are beads used for stimulation of cells. There is a low potential for microbial contamination if the carrier can be terminally sterilized by irradiation or steam sterilization.

**Utilities**

**Pharmaceutical-Grade Water**

If the water is processed on-site, the water systems must be well-designed, maintained, and operated in a GMP-compliant manner. Periodic monitoring for microbial counts and endotoxin is indicated.

**Compressed Gases**

Air, oxygen, nitrogen, and carbon dioxide are sterile filtered in-line and/or at the point-of-use.

**Single-Use Culture Equipment**

The use of traditional flat-bottom tissue culture flasks, which are disposable, may not provide the capacity or

<table>
<thead>
<tr>
<th>Input Materials</th>
<th>Risk Identification</th>
<th>Risk Rating</th>
<th>Risk Mitigation/Critical Process Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packaging</td>
<td>I.V. bags</td>
<td>Low</td>
<td>Irradiation sterilization; aseptic transfer Container-closure integrity</td>
</tr>
<tr>
<td>Storage</td>
<td>Ambient temperature; refrigeration temperature; frozen/cryopreserved</td>
<td>Low</td>
<td>Frozen storage should include liquid nitrogen vapor not liquid nitrogen</td>
</tr>
<tr>
<td>Shipping</td>
<td>Refrigerated or frozen</td>
<td>Low</td>
<td>Shipping frozen in liquid nitrogen or controlled refrigerated transport</td>
</tr>
<tr>
<td>Donor/Recipient</td>
<td>Allogeneic only</td>
<td>Moderate-High</td>
<td>Vaccination; pre- and post-antibiotic treatment</td>
</tr>
</tbody>
</table>

**TABLE V**

**Risk Analysis with Cell Therapy Products Related to Input Materials—Packaging, Storage and Administration**

<table>
<thead>
<tr>
<th>Input Materials</th>
<th>Risk Identification</th>
<th>Risk Rating</th>
<th>Risk Mitigation/Critical Process Controls</th>
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</tbody>
</table>
growth conditions needed for cell therapy products. The use of single-use stirred cell bioreactors, wave platforms, and perfusion cells with advanced aseptic connections will provide flexibility, improve scheduling, produce larger yields, and promote higher sterility assurance levels.

Cell Storage Solutions

Buffers, saline, human serum albumin, dimethyl sulfoxide (DMSO), cryoprotectants, and so forth, may be a source of microbial contamination.

Final Packaging Material

Sterile, pyrogen-free stoppered vials, syringes, or I.V. bags are commonly used.

Product Storage Conditions

Cell therapies may be stored at ambient temperature, or they may be refrigerated, frozen, or cryopreserved to improve their stability, extend their shelf life, or mitigate the proliferation of microbial contaminants.

If liquid nitrogen is used for cryopreservation, vapor-phase liquid nitrogen is preferred as microorganisms can survive in the liquid form and potentially contaminate the product.

Route of Administration Risk

The route of administration, that is, intramuscular, intravenous, or intrathecal administration, increases the invasiveness of the therapy. Another related risk factor, often overlooked, is the volume of the administration (Table VI).

Manufacturing Facilities

The manufacturing facilities of cell therapy products should take into consideration a design to protect the operators from the potential hazards associated with the donor cells, because often either disease screening is limited or very little information is available about the donor cells, and the facility must be configured so that starting cells can be managed. Also, the design should take into account the protection from cross-contamination of the donor cells and of the production process. A separation strategy is required to prevent cross-contamination between patients and product types.

<table>
<thead>
<tr>
<th>Route of Administration</th>
<th>Typical Volume (mL) Administered</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intradermal</td>
<td>0.1</td>
<td>Very Low</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>1</td>
<td>Low</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>3</td>
<td>Moderate</td>
</tr>
<tr>
<td>Intrathecal</td>
<td>1–10</td>
<td>High</td>
</tr>
<tr>
<td>Intravenous Push</td>
<td>1–60</td>
<td>Moderate-High</td>
</tr>
<tr>
<td>Intravenous Piggyback</td>
<td>25–250</td>
<td>High</td>
</tr>
<tr>
<td>Intravenous</td>
<td>25–250</td>
<td>High</td>
</tr>
</tbody>
</table>

Flow of Materials

In cell therapy operations, the expectation is that there are documented and defined material flow routes. Material flow procedures should be designed so that contamination risks are eliminated, and the path of donor cells into the manufacturing space is expedited. An optimized route will include segregated paths for incoming starting material and completed finished material. Physical segregation utilizing separate incoming and outgoing material paths is preferred.

A material disinfection program for materials entering the manufacturing space should appropriately decontaminate the materials to a level commensurate with the processing environment. A typical flow has materials unpacked from shipping containers and transferred either to interim storage or directly to transport containers to move into the manufacturing space. A segregated space is used to perform the unpacking and transfer to transport containers. Personnel are able to perform incoming inspection for appropriate documentation and visible defects on the incoming materials at this stage. Operator safety at this stage should consider protective apparel to safeguard them from any potential exposure risk including liquid spills, aerosols, and sharps.

A material kitting program that is able to maintain lot accountability for each processing batch can keep materials organized per each processing step. Material kitting can be placed into secondary containment containers for ease of movement into the manufacturing space.
Secondary containment systems can also be used to aid in transport into the manufacturing space. Secondary containment can be single-use or multiple-use containers. For multiple-use transport containers, a program of sanitization after each use should be employed.

Airlocks or pass-throughs employed to maintain environmental control of the manufacturing space are used as a location for disinfection of the outer packaging of the materials. During development of the disinfection program, the sample container should be shown to be impervious to the disinfectant to ensure that cell viability is not adversely impacted.

Types and Use of Water

Water used for cell therapy applications must be suitable for the purpose. Water for injection or sterile cell culture grade water should be used for in-process applications. Water for disinfectant preparation should be appropriate for the classification of the area being cleaned. If the facility has an in-house water system, appropriate monitoring of the water quality should be performed at regular intervals.

Modular Clean Rooms and Isolators

Modular style clean rooms offer the advantage of scalability. When there is increased need for manufacturing space, modular clean rooms can be quickly placed into use. Standard layouts can be used to justify reduced validation activities. Another advantage of modular clean room design is the physical separation offered by the modular style rooms.

Barrier systems, such as an isolator or restricted access barrier (RAB), are alternative improvements to contamination prevention. Isolators offer the advantage of providing high-quality aseptic environments and protection for the operators and the product. Alternatively, the use of isolators in cell therapy product manufacturing may not be preferred for the following reasons:

1. Preparation and start time to use an isolator is longer than the time required to perform the cell culture step.

2. Operator capability to perform activities through glove ports requires training and adequate fine motor skills.

3. Long decontamination cycles are an obstacle to quick transfers.

4. Decontamination chemical ingress into the cell culture materials can inhibit cell growth or otherwise affect the materials unless they have external packaging not permeable to decontaminant gas.

5. Potential cell death caused by the long periods when the cells are not maintained at the correct temperature and environment.

6. An isolator system is expensive, and there are added complications and costs to reconfigure the isolator layout if process changes are required. Also, the inflexibility of the configuration for product or system changes can add to the validation cost.

Biological Safety Cabinets

Biological safety cabinets (BSCs) are used because they protect both the product and the personnel. BSCs are easily cleaned and require minimal operator training. BSCs facilitate a varied selection of gowning that can be easily donned by the operator, is available worldwide, and is readily accepted by regulatory agencies. BSCs have an integrated high-efficiency particulate air filtration system that isolates the workspace from the room heating, ventilation, and air conditioning (HVAC) system. There are other parameters to consider, such as: (1) the use of a viral retentive filter depending on product risk, (2) airflow visualization studies to demonstrate that open operations can be performed with minimal risk, (3) training of operators to correctly perform the operations and at the correct speed that is demonstrated in the hood qualification, (4) a cleaning program for the BSC with consideration of cross-contamination risks for hoods used for multiple applications, (5) microbiological monitoring of BSCs used to perform aseptic operations should be performed, and (6) any containment choice must be compatible with the cell process, and any risks associated with containment and personnel protection must be addressed.

Personnel Gowning and Gloving

1. Personnel should be appropriately gowned for the manufacturing environment.

2. Aseptic gowning and qualification are required when working in aseptic environments.
3. A personnel monitoring program should be employed depending on the working environment.

4. Universal blood-borne pathogen precautions should be followed.

5. If reused and laundered gowning is utilized, work with the supplier to have an appropriate decontamination program based on the contamination risk.

General Training Considerations

As cell therapies advance toward commercialization from earlier phases (i.e., investigational new drug), it is important to have a higher level of controls to prevent microbial contamination events, particularly for manual process steps. Operators and laboratory personnel who have worked in lesser controlled (academic or clinical) laboratory settings will need to be assessed and trained to work within a cGMP space appropriate for cell therapies. Some considerations will include:

1. operation of equipment and disposables, including operation of the BSC, specific cell therapy instruments, and single-use technologies;

2. microbiology fundamentals that include organism types and sources of contamination; and

3. aseptic behaviors and techniques, as applicable, for open processing steps and aseptic process qualifications.

Facility Design and Environmental Controls

In 21 CFR 1271.190, it is stated that, “the facility is to be designed to provide the appropriate construction and size to prevent contamination and mix-ups. The facility must be maintained clean and designed to prevent spread of disease” (10). Increasingly specialized equipment is available that can perform all cell processes in a closed environment. Depending on the specific product requirements, the cell therapy product should be manufactured in an environment suitable to achieve aseptic conditions and operator safety. For aseptic manipulations where a higher level of control is needed, ISO 5 BSCs are utilized. Based on a microbiological risk assessment, it is common to operate ISO 5 BSCs within an ISO 7 background environment (or ISO 6 at rest). As the design of processes are moving toward the use of closed systems, where applicable, a risk assessment should be performed. ISO 7 background should be acceptable for incorporating closed systems. The environmental monitoring (EM) program should have a risk-justified sampling plan. The EM program should be reviewed and trended, updated as appropriate, and should include sampling sites that represent the actual room conditions during manufacturing. The current expectation is to have a contamination control strategy appropriate for the identified risks of the cell therapy operations. Specifically included in the strategy (and complementing points in Table I) are:

1. room classifications/pressures, personnel and equipment flow;

2. in-process monitoring;

3. cleaning and disinfection program;

4. qualifications of equipment and cycles;

5. process simulation;

6. air visualization studies;

7. trending;

8. environmental monitoring;

9. gowning;

10. training;

11. supervision; and

12. material controls.

All systems working together and trending for signals will enable maintenance of effective facility control.

Manufacturing Process

Raw Material Program

As per the EudraLex guideline for Advanced Therapy Medicinal Products (ATMP) (5), “the level of supervision and further testing by the ATMP manufacturer should be proportionate to the risks posed by the individual materials”.

A well-defined raw materials program is integral for cell therapy manufacturing.
As patient cells cannot be sterilized, it is critical that all raw materials and consumables used in a cell therapy manufacturing process are sterile and free of contaminants such as endotoxin, virus, and mycoplasma.

At a minimum, a raw materials program should take into consideration:

1. critical quality attributes;
2. global regulatory or compendial testing requirements;
3. the supplier and their manufacturing/supply chain capabilities;
4. quality agreement;
5. material sourcing;
6. use of authorized medicinal products; and
7. extractable/leachable substances from packaging materials.

**Consumables (Presterilized, Single-Use Devices)**

Because there are no sterilization steps in a cell therapy manufacturing process, all consumables used should be sterile, pyrogen-free, and single-use. Consumables are typically purchased as sterilized (via gamma irradiation or ethylene oxide). It is incumbent on the end user to ensure that the manufacturer of consumables has a robust sterilization validation process, because many of the consumables come in direct contact with the patient’s cells.

Another key aspect of consumables is that they are typically customized for the end user, thus considerations must be given to sourcing. Some customized consumables are sourced from a single supplier. This could create potential issues with supply and demand, which eventually could impact the ability to manufacture. This is extremely critical in autologous cell therapy manufacturing because each lot of the product represents a single patient.

Because most steps in cell therapy manufacturing are time critical, care should also be given to the ability of the consumable to fit the needs of the manufacturing process. Consumables should allow easy performance of advanced aseptic manipulations via tube welding, and so forth, and provide minimal risk of the introduction of microbial contamination.

**Process—Manual Steps**

Prior to commencing manufacturing activities, a microbiology risk assessment (failure modes and effects analysis or another appropriate tool) should be performed. The risk assessment should cover the entire manufacturing process from receipt of the incoming leukapheresis to final product formulation and filling. Consideration must also be given to the design of the facility and the personnel and material flows. Manual steps are high-risk areas for microbial contamination; they should be identified and, where possible, the appropriate mitigating actions implemented. These identified areas should be considered for inclusion in aseptic process simulation (e.g., validation).

During the risk assessment, rather than using the terms “open” and “closed” for the manual aseptic manipulations that are performed, use of terminology such as a high, medium, and low risk is recommended.

Mitigating actions should include both engineering (e.g., BSCs, controlled environments) and procedural controls (e.g., cleaning, gowning, training, and so forth). Because of advanced aseptic connection techniques, such as sterile welding, and the improved design of the product contact consumables, most aseptic manipulations present a minimal risk of microbial contamination ingress. An example of better design is the use of cassettes for manual filling during the electroporation process. Additionally, operators are typically required to demonstrate the use of appropriate aseptic techniques through an aseptic operator qualification program.

The microbiology risk assessment should be completed as early as possible as the outcome of the risk assessment should, at a minimum, direct the level of control for gowning, cleaning, and environment needed at each phase of the manufacturing process.

Appropriate gowning is an important aspect to consider when performing manual aseptic manipulations. Consideration must be given to the task being performed by the manufacturing operators. Because most manipulations require hand to eye coordination as well as enough hand dexterity, operators should wear gowning that is sufficient to prevent contamination but that does not impede the operator’s visibility or the dexterity of...
the operator’s gloved hands. As most of the cell therapy manufacturing process is of a manual nature, the highest risk of microbial contamination would typically stem from the potentially contaminated gloved hands of personnel.

Process—Automated Steps

Cell therapy manufacturing is still developing. Most of the manufacturing process involves manual aseptic manipulations. As more companies develop cell therapy manufacturing, vendors and current cell therapy manufacturers are exploring options to automate certain aspects of the manufacturing process. Robotic systems are compatible with gloveless isolator systems.

Cell Culture/Cell Harvesting

The cell culture process for cell therapy products involves a growth in culture dishes or biofermentors (e.g., vessels or bags) with an incubation period over several days. The culture process within a biofermentor or using a robotic system for culture dishes typically involves minimum handling by the manufacturing operators and thus presents a lower risk of the introduction of microbial contamination. The cell harvesting process (e.g., media separation after the growth phase) may typically last for a few hours and should be handled similarly to reduce the risk of introduction of microbial contamination.

Compressed Gas

CO₂-enriched air is typically used during the cell expansion phase of the process. A testing strategy must be defined for receipt and release of the CO₂ and any other compressed gas used, along with filter integrity testing. A risk-based filter integrity testing frequency should be used in place of routine microbiological testing of compressed gas to minimize potential impact to the manufacturing process and to ensure the quality of the compressed gases.

Environmental Monitoring

The facility and manufacturing process are designed to incorporate engineering controls to ensure the intended operations minimize potential contamination. As people are one of the largest sources of contamination, well-designed procedures and practices for aseptic operators can reduce potential contamination sources.

Microbiological monitoring of the manufacturing process, facility, and operators is expected to provide ongoing measurement of the capabilities for control of microbiological contamination. The selection of sampling sites, frequency of sampling, target acceptance levels, and methods for sampling should be relevant and appropriate for the particular cell therapy operations. The EM program can be developed with a risk-assessment approach, and use of a detailed map indicating locations of samples is a useful tool for both current application and periodic revisits to make improvements. As the level of containment increases and operators are excluded, the importance of EM decreases, but a robust EM program is still needed.

Recommendations for Monitoring Practices

Table VII provides risk-based recommendations for EM, aseptic process simulations, and microbial testing for different manufacturing environments (11).

Cell Therapy Product Testing and Release

Real-Time Release—Autologous and Allogeneic Cell Lines

Release test parameters include cell identity, viability, count, and potency that lie outside of the scope of this white paper. Screening for sterility (microbial contamination), mycoplasma, and bacterial endotoxin may be conducted on the finished product, raw materials, or in-process materials, as justified by risk analysis.

Sterility/Microbial Contamination Testing

The absence of microbial contamination of gene and cell therapies is a critical quality attribute, so the ability to detect contamination is important to patient safety. Classical sterile drugs are tested before release (except for those parametrically released) for sterility, accounting for the allowance of sufficient test time to results. Sterile pharmaceutical drug products have large batch sizes and expiration dating as long as three years, and the release cycle allows for the completion of a sterility test. As cell viability declines rapidly with time, cell therapies are short-lived products manufactured in a smaller batch size or are formulated with cryoprotectants and stored frozen, and then thawed before administration.
This testing may include in-process microbial contamination or final product sterility testing. The growth-based sterility test, as described in USP <71> Sterility Tests (12), requires at least a 14-day incubation and is often not suitable as a release test for gene and cell therapies as they may have a short shelf life and be administered prior to freezing with cryoprotectants for longer storage.

The positions taken by regulatory agencies and standard-setting organizations are summarized as follows:

The FDA states in their July 2018 Draft Guidance for Industry, Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) (13) that the compendial sterility test may not be suitable for all products. As they mentioned in “Analytical Procedures” (section 3.2.S.4.2 of this guidance), rapid sterility tests may be needed for ex vivo genetically modified cells administered fresh or with limited hold times before final formulation, packaging, and patient administration.

The FDA recommended that if ex vivo genetically modified cells are administered immediately after manufacturing, samples should be taken 48–72 hours prior to final harvest for product release for in-process sterility testing. For such products, aside from an in-process sterility test, the FDA also recommended that cell therapy manufacturers perform a rapid microbial detection test, such as a Gram stain, on the final formulated product and a sterility test, compliant with 21 CFR 610.12, on the final formulated product. The authors of this article question the use of a 48- to 72-hour window when nongrowth-based methods can be completed in close to real time, so that screening for microbial contamination can be conducted immediately prior to release. Furthermore, a Gram stain that has a limit of detection of around 10^2 to 10^3 colony-forming units (CFUs) per milliliter will only detect gross levels of microbial contamination and therefore is unsuitable for release testing.

Using the above-mentioned approach, the FDA believes that the release criteria for sterility would be based on a negative result of the Gram stain and a no-growth result from the 48–72 hour in-process sterility test. The FDA conveys that although the results of the sterility culture tests may not be available for release testing, performed on the final product will not be available for

<table>
<thead>
<tr>
<th>Manufacturing Environment</th>
<th>Air Cleanliness Standards</th>
<th>Environmental Monitoring Frequency</th>
<th>Environmental Monitoring Action Levels (CFU)</th>
<th>Aseptic Process Simulation Requirements</th>
<th>Microbial Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSC in a classified area</td>
<td>ISO 5/ISO 7</td>
<td>Each shift /Each operator</td>
<td>&lt;1/&lt;5</td>
<td>Initial 3 batches/semi-annual</td>
<td>In-process and final product testing</td>
</tr>
<tr>
<td>Barrier system in a classified area</td>
<td>ISO 5/ISO 7</td>
<td>Each shift</td>
<td>&lt;1/&lt;5</td>
<td>Initial 3 batches/semi-annual</td>
<td>In-process and final product testing</td>
</tr>
<tr>
<td>Open isolator system</td>
<td>ISO 5/ISO 7</td>
<td>Each shift</td>
<td>&lt;1/&lt;5</td>
<td>Initial 3 batches/semi-annual</td>
<td>In-process and final product testing</td>
</tr>
<tr>
<td>Closed isolator system</td>
<td>ISO 5/ISO 8</td>
<td>Periodic</td>
<td>&lt;1/&lt;50</td>
<td>Initial 3 batches/annual</td>
<td>Final product testing</td>
</tr>
<tr>
<td>Gloveless, robotic, isolator system</td>
<td>ISO 5/ISO 8</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Each manufacturer should perform a risk assessment to determine the type of environmental monitoring program and to determine the product testing strategy.
product release, this testing will provide useful data. A negative result will provide assurance that aseptic technique was maintained during cell culture. A positive result should lead to identification of the microbial isolate and an antibiotic susceptibility profile that will provide information for the medical staff responsible for the patient being treated so that they can intervene. The contamination event will also trigger an investigation of the cause of the sterility failure. The sterility culture on the final formulated product should be continued for the full duration (usually the qualified end point) to obtain the final sterility test result, even after the product has been administered to the patient.

The original 21 CFR 610.12 stated that the sterility of each lot of a biological product would be determined by the performance of bulk and final container sterility testing using fluid thioglycollate medium (F) and soybean casein digest medium (SCDM) as described in USP <71> Sterility Tests (10).


The amendments to the sterility test requirements provided cell therapy manufacturers the flexibility to use state-of-the-art test methods. The changes are summarized as follows:

1. Elimination of specified sterility test methods, culture media, and test requirements.

2. Elimination of specified sterility requirements for most bulk materials.

3. Provision for a single repeat sterility test when the original test was invalidated because of error or the use of faulty materials.

4. Replacement of the prescribed sample size or amount requirement with a general requirement that the sample be appropriate to the material being tested.

5. Replacement of the “Interpretation of Test Result” paragraph in 610.12 with a requirement that manufacturers establish, implement, and follow written procedures for their chosen sterility test that, at minimum, describe the test method used, the method of sampling, and the written specification for the acceptance or rejection of each lot of product tested.

Note: 21 CFR 610.12 contains exemptions from the sterility testing requirements for a range of human blood cell and plasma derivatives. It is possible that an exemption for no sterility testing may be extended to include cell therapy products collected in closed systems with no manual manipulation during processing on a risk-based case-by-case basis.

The EU Guide to the Quality and Safety of Tissues and Cells for Human Application as Applied to Cell Therapy Products, which they termed Advanced Therapy Medicinal Products (ATMPs), addressed the issue of sterility testing. The regulations stated that the application of the sterility test to the finished product in accordance with the European Pharmacopoeia (Ph. Eur. 2.6.1) and USP <71> Sterility Tests (10) may not always be possible owing to the scarcity of available materials, or it may not be possible to wait for the final result of the test before the product is released because of the short shelf life or medical need. As pointed out by the regulators, in these cases, the strategy regarding sterility assurance has to be adapted. For example, the use of alternative methods for preliminary results combined with sterility testing of media or intermediate product at subsequent (relevant) time points could be considered.

The EU regulators have mentioned the use of validated alternative rapid microbiological methods to consider. For example, sole reliance on alternative microbiological methods according to Ph. Eur. 2.6.27 Microbiological Control of Cellular Products may be acceptable when this is justified with regard to the specific characteristics of the product and the related risks, and provided that the suitability of the method for the specific product has been demonstrated.

The Australian Therapeutic Goods Authority (TGA) takes a flexible approach to microbiological testing. The Australian Code of Good Manufacturing Practice for Human Blood and Blood Components states, “In order to ensure both the reliability of the manufacturing process and the quality of the final product there should be routine microbial contamination testing. Where contamination is demonstrated, records should show the corrective action taken.”

The USP recently published General Chapter <1071> Rapid Sterility/Microbial Contamination Testing of Short-Life Products: A Risk-Based Approach (14). The chapter states that the current growth-based sterility tests with an incubation period of at least 14 days are
not suitable for short-life products or for products prepared for immediate use, which are usually infused into patients before the completion of the test. The discussion also says that patient safety is best served through the completion of a test that detects microbial contamination before use. Short-life products including compounded sterile preparations, positron emission tomographic products, and cell and gene therapies, may require a new generation of risk-based rapid microbial tests.

The microbial tests would be risk-based so the stakeholder can select the preferred technology for their intended use and balance competing user requirement specifications, including time to result, specificity, limit of detection, sample size, and product attributes.

What approach do the authors of this article recommend? Microbial contamination detection options include:

1. After completion of a batch record step that includes aseptic manipulations, for example, manual replenishment of the cell culture medium, a sample should be taken to screen for microbial contamination (i.e., in-process testing).

2. A sample should be taken in advance of the final step with the timing reflecting the screening method employed, that is, 48-72 hours prior to release for a growth-based contamination check or a shorter time for a more rapid microbial test (i.e., surrogate finished product testing).

3. The final product should be subjected to a compendial or growth-based microbial test and released before the completion of the test with the clinician responsible for the treatment of the patient being notified if the test becomes positive (i.e., traditional finished product testing).

4. The final product should be tested for microbial contamination using a rapid microbial test and released at the successful completion of the test (i.e., real-time finished product testing).

Table VIII describes these different release-testing strategies with the detection options 1 through 4 as described above.

Options deployed by the manufacturer should be determined by a risk assessment and will depend on the source of the cells, extent of processing, excipients used, and extent of containment in the manufacturing facility (Table IX).

**Mycoplasma Testing**

Growth-based mycoplasma screening tests are described in USP <63> Mycoplasma Tests (15) and the FDA Points to Consider document. However, these methods require at least a 28 day incubation so they are not suitable as an in-process or release test for gene and cell therapies.

Mycoplasma contamination may be detected by routine testing using one or more specific techniques, including direct growth on broth/agar, specific deoxyribonucleic acid (DNA) staining, polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), ribonucleic acid (RNA) labeling, and enzymatic procedures. Next generation mycoplasma detection methods employing nucleic acid-based methods that obtain same-day results are available.

These rapid methods will enable cell therapy facilities to monitor cell culture media, working cell lines, in-process samples, and final product in close to real time.

**Bacterial Endotoxin Testing**

Bacterial endotoxins testing described in USP <85> Bacterial Endotoxins Test (16) can be completed within 1–2 hours so the current tests are suitable as an in-process or release test for gene and cell therapies. Recombinant reagent assays are an alternative approach. Also, hand-held or benchtop cartridge test units are available for in-process testing, and robotic systems are available for large-volume endotoxin testing.

**Summary**

In summary, the following points should be considered. Based on the point to consider, recommendations are also included that may be used for implementing a contamination control/prevention and testing strategy for a cell therapy manufacturing process.

**Collection Process**

Points to consider for a contamination control/prevention strategy.
The initial patient material may present an increased risk to the patient and the manufacturing process. Issues include:

1. Inability to obtain cells from the patient because of the patient’s declining health.
2. Death of the patient’s cells during cell expansion.
3. Introduction of non-facility-related microorganisms into the manufacturing facility.
4. Lapse of several days before a definitive root cause of microbial contamination is determined.

Recommendations for testing

### TABLE VIII
In-Process and Release Testing Strategies Based on Technology Employed

<table>
<thead>
<tr>
<th>Technology</th>
<th>Basis of the Test</th>
<th>Time to Result</th>
<th>Detection Option</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram Stain</td>
<td>Differential staining of bacterial cells</td>
<td>Around 30 minutes</td>
<td>Final product testing (3)</td>
</tr>
<tr>
<td>USP &lt;71&gt; Sterility Tests</td>
<td>Growth in soybean-casein digest and fluid thioglycollate medium</td>
<td>At least 14 days incubation</td>
<td>Final product testing (3)</td>
</tr>
<tr>
<td>Respiration</td>
<td>CO₂ production in proprietary aerobic and anaerobic broth</td>
<td>48–72 hours</td>
<td>In-process monitoring; timed pre-release testing; final product testing (1, 2, or 3)</td>
</tr>
<tr>
<td>RT-PCR method</td>
<td>PCR amplification</td>
<td>2–3 hours</td>
<td>Rapid release testing of final product (4)</td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>Vital staining</td>
<td>1–2 hours</td>
<td>Rapid release testing of final product (4)</td>
</tr>
<tr>
<td>Solid phase cytometry</td>
<td>Vital staining</td>
<td>2–3 hours</td>
<td>Rapid release testing of final product (4)</td>
</tr>
<tr>
<td>ATP bioluminescence</td>
<td>ATP production in soybean casein digest and fluid thioglycollate medium</td>
<td>48–72 hours</td>
<td>In-process monitoring; timed pre-release testing; final product testing (1, 2, or 3)</td>
</tr>
</tbody>
</table>

*Number represents microbial contamination detection option.

### TABLE IX
Recommendations for Risk-Based Microbial Testing of Cell Therapies Based on Type of Containment

<table>
<thead>
<tr>
<th>Manufacturing Environment</th>
<th>Containment System</th>
<th>In-Process Monitoring</th>
<th>Final Product Microbial Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological safety cabinet in a</td>
<td>Open</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>classified area</td>
<td>Closed</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Barrier system in a classified</td>
<td>Open</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>area</td>
<td>Closed</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>pen isolator system</td>
<td>Open</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Closed</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Closed isolator system</td>
<td>Open</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Closed</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Gloveless, robotic, isolator system</td>
<td>Open&quot;</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Closed</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*Open systems would not be used in this environment.
1. Microbial contamination testing at the collection center and/or upon receipt at the manufacturing facility (See Table III).

**Raw Materials/Consumables**

Points to Consider for a contamination control/prevention strategy.

1. Product contact materials are sterile and single-use.
2. Certificate of Analysis has been received from qualified suppliers confirming sterility of input materials.
3. Incoming and release program are established.
4. Potential microbial contamination would be detected upstream via established testing program.
5. Effective disinfection programs for transferring materials are established.

**Recommendations for Testing**

1. Confirmation of vendor supplied testing results for lower risk materials stated on certificate of analysis, via testing a minimum of three (3) lots.
2. Use of only qualified suppliers.
3. Performance of microbial contamination testing for high-risk materials prior to release (See Table IV).

**Media**

Points to consider for a contamination control/prevention strategy.

1. Critical aseptic manipulations are performed in an ISO 5 area.
2. Only sterile media supplements are used.
3. Aseptic manipulations are typically of short duration.
4. Subsequent sterile filtration is employed, where possible.
5. Risk-based environmental and personnel monitoring program is used.

**Recommendations for testing**

1. Microbial testing is risk-based (See Table IV).

**Manufacturing Process**

Points to consider for a contamination control/prevention strategy.

1. High-risk aseptic manipulations are performed in an ISO 5 area, using RABs or isolators where possible.
2. Low-risk aseptic manipulations are performed in classified areas.
3. Manufacturing is automated, when possible, to reduce manual manipulations.
4. Aseptic manipulations are typically of short duration.
5. Area-specific personnel gownsing is used.
6. Sterile single-use consumables are used.
7. Advanced aseptic connection systems are used.

**Recommendations for testing**

1. Microbial testing is risk-based (See Table IV).

**Final Product**

Points to consider for a contamination control/prevention strategy.

1. Low-risk aseptic manipulations are performed in classified areas.
2. Aseptic manipulations are typically of short duration.
3. Area-specific personnel gownsing is used.
4. Sterile single-use consumables are used.
5. Sterile cell protectant is used.
6. Liquid nitrogen storage is used.

**Recommendations for testing**

1. Risk-based strategy (see Tables IV and V)
2. Sufficient in-process testing could reduce risk and/or need for final product testing

3. Rapid endotoxin testing, based on risk

4. Rapid mycoplasma testing, based on risk

5. Rapid microbial contamination testing (see Table VI) or

6. Growth-based absence of contamination testing, based on risk

7. Evaluate aseptic manipulations to reduce risk

Conclusions

The following conclusions have been reached:

1. Cell therapy manufacturing must utilize a different contamination control/prevention strategy than that of traditional pharmaceutical manufacturing. It is important to remember that each product lot represents an individual patient and that cell therapy treatments may be the last treatment option for the patient population receiving such treatments.

2. A risk-based strategy must be employed that will provide a contamination/prevention program consistent with regulatory requirements while providing practical procedures that will not impede the manufacturing process nor cause delay in return of the product back to the patient.

3. Cell therapy manufacturers must refrain from trying to directly correlate and implement the requirements of a traditional pharmaceutical manufacturing process, as they are not always comparable (i.e., like for like). This is especially true when it comes to the execution of the process validation (or aseptic process simulation). For example, in cell collection and cell therapy manufacturing, the process may consist of both manual and automated aseptic operations being performed by qualified aseptic operators using the same equipment with sterile single-use consumables. So, in contrast to traditional pharmaceutical manufacturing, only high-risk operations (e.g., manipulations made in ISO 5 areas) must be included in the design of aseptic process simulation. Additionally, cell therapy manufacturing processes employ a cell growth phase using cellular growth medium during the manufacturing process, which makes this phase of the process “self-contamination” checking. Any adventitious contamination at this phase could, and most often does, cause the death of the patient’s cells and thus termination of the process. Traditional pharmaceutical processes do not employ such a growth phase and hence the need to rely on the traditional media fill simulations using SCDM (or tryptic soy broth) or another suitable medium.

4. The collection process may present a slightly increased risk of microbial contamination and not the manufacturing process itself, because contamination introduced at this stage of the process could cause both manufacturing delays as well as delayed return of the product back to the patient. Controls and testing must be implemented for this stage to ensure both prevention and detection are adequate.

5. Where possible, manufacturers should consider either a just-in-time release process or a reduced-time release process (see Table VI). Patient safety should be based on a comprehensive contamination control/prevention and testing strategy that can consistently demonstrate that the process remains in a state of contamination control based on all the factors that have been discussed throughout this document. The release strategy must take into consideration a risk-based approach to evaluating the entire process from collection to the final product.

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Conflict of Interest Declaration

The authors have no conflicts of interest related to this publication.

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References


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