USP standards to support the development of cell and gene therapy products

Ben Clarke, Ph.D.
Senior Scientist II, Biologics
USP

200 usp
The standard of trust

Outline

- USP Standards for Cell Therapies
- USP <1044> Cryopreservation
- USP <1043> Ancillary Material Standards
- New Chapters in PF
  - ⟨74⟩ Solid Phase Cytometry-Based Rapid Microbial Methods for the Detection of Contamination in Short Shelf-Life Products
  - ⟨77⟩ Mycoplasma Nucleic Acid Amplification Tests
  - ⟨1114⟩ Microbial Control Strategies for Cell Therapy Products

1

USP standards for cell therapy

USP public standards

- **Monographs**
  - Specifications for pharmaceutical articles in commerce (from release through product shelf life)
  - Tests, assays, and acceptance criteria needed to demonstrate the article meets required quality standards

- **General Chapters**
  - Procedural chapters less than 1000: validated methods
    - <90> *Fetal Bovine Serum—Quality Attributes and Functionality Tests*
  - Informational chapters 1000 to 1999: best practices and considerations
    - <1043> *Ancillary Materials for Cell, Gene and Tissue-Engineered Products*

- **Physical Reference Materials**
  - Provide traceable standards to demonstrate broad-based acceptability of procedures
  - Often associated with a procedural chapter

Existing USP CGT standards

Informational General Chapters

- *<1043> Ancillary Materials for Cell, Gene and Tissue-Engineered Products*
- *<1044> Cryopreservation of Cells*
- *<1046> Cell-Based Advanced Therapies and Tissue-Based Products*
- *<1047> Gene Therapy Products*
  - *Currently undergoing major revision*
- *<1024> Bovine Serum*
- *<1027> Flow Cytometry*

Procedural Chapters

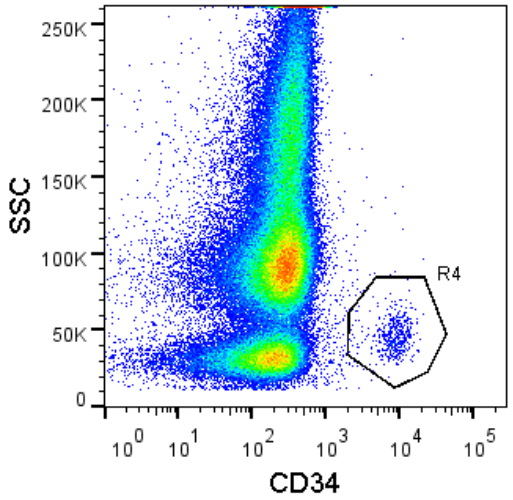
- *<127> Flow Cytometric Enumeration of CD34+ Cells*
- *<90> FBS Quality Attributes and Functionality Tests*
- *<89> Enzymes used as Ancillary Materials in Pharmaceutical Manufacturing (Trypsin)*
- *<89.1> Collagenase I, <89.2> Collagenase II*
- *<92> Growth Factors and Cytokines used in Cell Therapy Manufacturing (rhIL-4)*

Ancillary materials

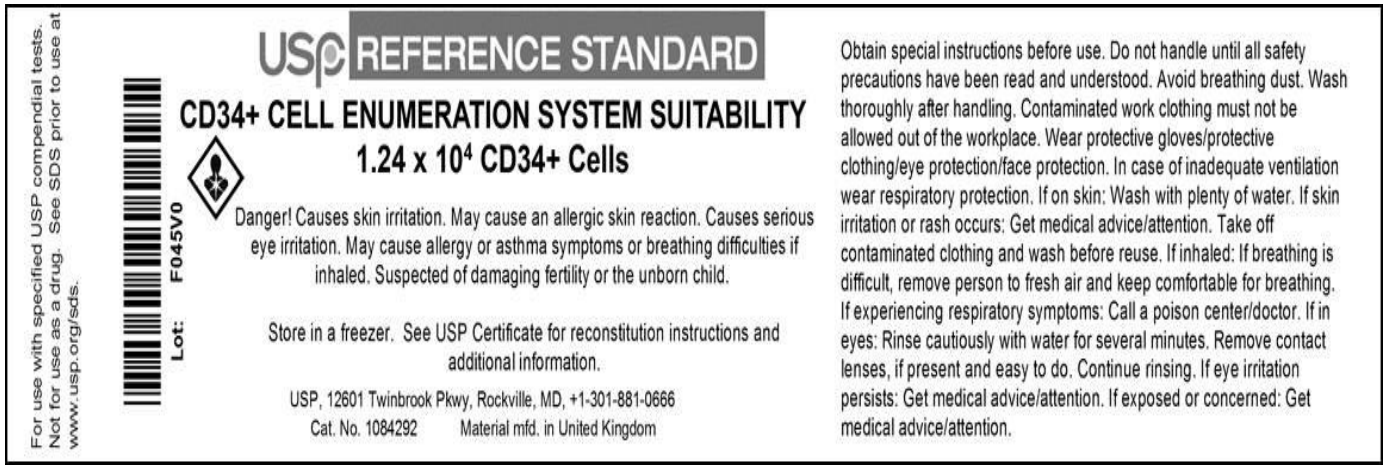
Reference Standards

- CD34+ Cell Enumeration System Suitability (1.24 x 10^4 CD34+ Cells)
- Physical RS associated with ancillary material monographs (FBS, Trypsin, rhIL-4, Collagenase)

Existing standards for method performance—USP <127> Enumeration of CD34+ Stem Cells



USP CD34+ Cell Enumeration System Suitability Reference Standard is used to calibrate instruments, assess reagents and ensure correct gating for data acquisition and analysis



CD34+ CELL ENUMERATION SYSTEM SUITABILITY

USP Catalog No.:	1084292
USP Lot No.:	F045V0

Additional Information:

USP CD34+ Cell Enumeration System Suitability Reference Standard is made from mobilized peripheral blood collected by apheresis of a G-CSF mobilized donor. The reference standard contains human leukocytes, erythrocytes and CD34+ cells that have been fixed and lyophilized.

Store USP CD34+ Cell Enumeration System Suitability Reference Standard in a freezer. Allow the vial to warm up to room temperature. Reconstitute the entire contents of the vial with 500 µL of water, use immediately as a system suitability standard as described in <127> Flow Cytometric Enumeration of CD34+ Cells. After reconstitution in 500 µL of water, the concentration range is 16-34 CD34+ cells/µL.

<1044> *Cryopreservation of Cells*

<1044> *Cryopreservation of Cells*

Principles of cryopreservation

- Colligative action, vitrification
- Membrane permeability, tonicity, and mechanisms of toxicity

Prefreezing

- Characterizing cell banks, cell status, growth rate
- Documentation and traceability
- Cell concentration range, media, washing, aggregation
- Donor and adventitious agent testing

<1044> *Cryopreservation of Cells*

Reagents and containers

- Freezing vessels: bags, cryotubes, straws
- Cryoprotectants
- Labels and ink

Addition of cryoprotectant and freezing

- Minimizing cryoprotectant toxicity during addition of cryoprotectant
- Freezing temperature, rate, and methodology

Storage and transport

- Appropriateness and hazards of different containers
- Temperatures and monitoring

<1044> *Cryopreservation of Cells*

Thaw

- Equipment, temperature, rate, considerations for staff

Post-thaw

- Mitigation of cryoprotectant toxicity by dilution, washing, and centrifugation
- Methods and considerations for testing of viability
- Adventitious agent testing and documentation

Specific considerations for cell-types

- Primary cells: lymphoid cells and hematopoietic, mesenchymal and pluripotent stem cells
- Cell substrates for Biologics: Mammalian and insect cell lines, bacterial and yeast strains

3

<1043> *Ancillary Materials for Cell, Gene and Tissue-Engineered Products*

Risk-based approach in USP <1043>

Level of Risk	Criteria that define the level of risk
Tier 1: Materials Intended for Use as Approved Biologics, Drugs, or Medical Devices	Intended for use as licensed drugs, biologics or medical devices. Suitability for use as a manufacturing component is required because the formulation, stability profile, and other quality aspects of these materials may change once the material has been introduced in the manufacturing process.
Tier 2: Well Characterized Materials with Intended use as AM	Intended to be used as ancillary materials. These materials are well-characterized and produced under quality systems well-suited for biological manufacturing, but the material is not a licensed medical product. Many are produced specifically for the manufacture of biological products.

Tier 2: Human Interleukin 4 (recombinant)

<92> *Growth factors and cytokines used in cell therapy manufacturing*

Identification

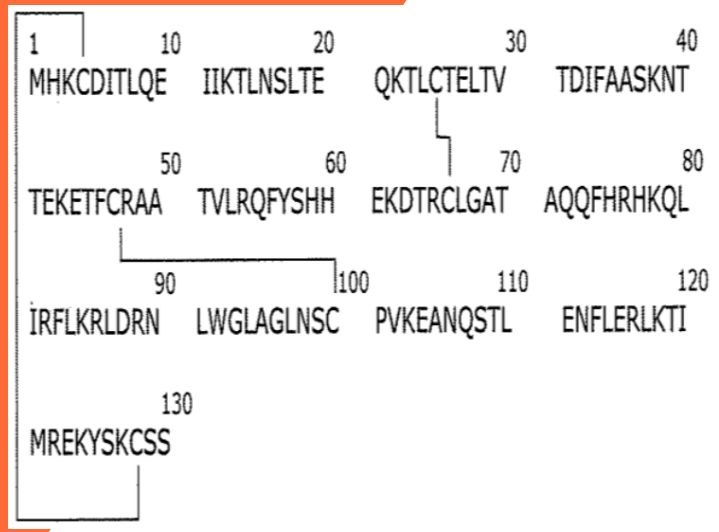
- Amino-terminal sequence analysis of at least eight amino acids
- Western Blot analysis to detect IL4 protein

Assay

- Purity, SDS-PAGE with silver staining
- Protein Content, Concentration of IL4 determined by A280 measurement

Specific tests

- Cell-based assay to determine identity and units/mg with acceptance criteria (NLT 0.5 × 10^7 USP Units of IL4/mg)
- Sterility Tests <71>
- Bacterial Endotoxin Test <85>



Molecular Formula

$C_{658}H_{1065}N_{193}O_{197}S_8$

Molecular Weight

15,088 daltons

CAS Number

n/f

Risk-based approach in USP <1043>

Level of Risk	Criteria that define the level of risk
Tier 1: Materials Intended for Use as Approved Biologics, Drugs, or Medical Devices	Intended for use as licensed drugs, biologics or medical devices. Suitability for use as a manufacturing component is required because the formulation, stability profile, and other quality aspects of these materials may change once the material has been introduced in the manufacturing process.
Tier 2: Well Characterized Materials with Intended use as AM	Intended to be used as ancillary materials. These materials are well-characterized and produced under quality systems well-suited for biological manufacturing, but the material is not a licensed medical product. Many are produced specifically for the manufacture of biological products.
Tier 3: Moderate-Risk Materials Not Intended for Use as AMs	These are research-grade materials not intended for use in biological manufacturing; sometimes approved by regulatory agencies as part of an in vitro diagnostic device. Tier 3 requires more qualification than Tier 1 or Tier 2 materials.

Tier 3: Trypsin (recombinant porcine)

<89> Enzymes used as ancillary materials in pharmaceutical manufacturing

Identification

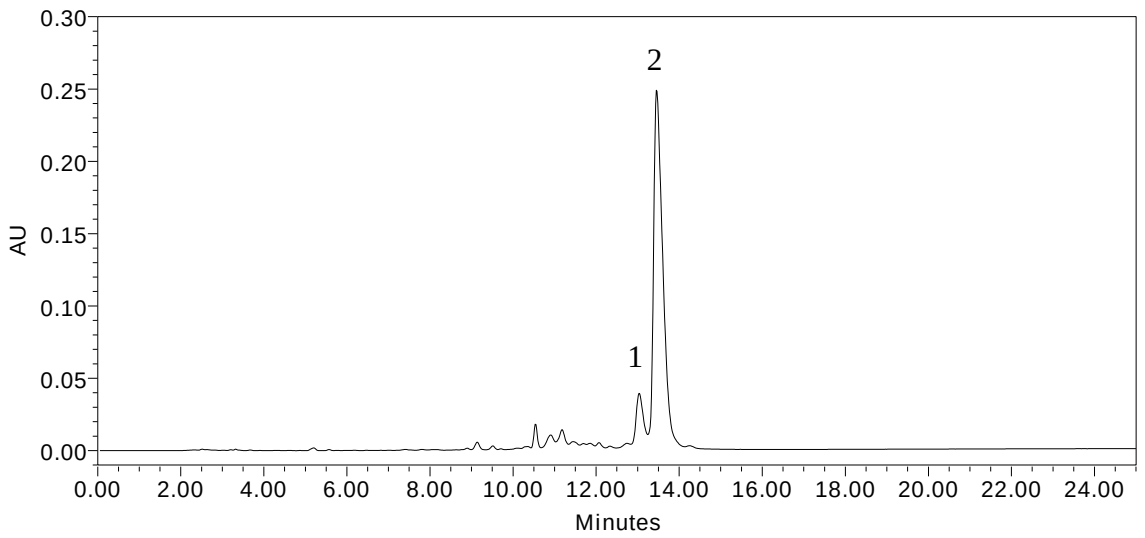
- Meets requirements under *Assay*
- Retention time corresponds to the *Standard solution* as described in *Purity*

Assay

- Ability to hydrolyze the peptide substrate Chromozym
- Trypsin Recombinant Porcine RS is used for system suitability (requirement: 90 - 110% of the labeled value)
- Acceptance criteria: At least 180 Units/mg of protein using Chromozym as the substrate or at least 3800 USP Units/mg of protein using BAEE substrate.

Purity by RP-HPLC

- Acceptance criteria: NLT 70% for the peak area of β-Trypsin and NMT 20% for the peak area of α-Trypsin.



Risk-based approach in USP <1043>

Level of Risk	Criteria that define the level of risk
Tier 1: Materials Intended for Use as Approved Biologics, Drugs, or Medical Devices	Intended for use as licensed drugs, biologics or medical devices. Suitability for use as a manufacturing component is required because the formulation, stability profile, and other quality aspects of these materials may change once the material has been introduced in the manufacturing process.
Tier 2: Well Characterized Materials with Intended use as AM	Intended to be used as ancillary materials. These materials are well-characterized and produced under quality systems well-suited for biological manufacturing, but the material is not a licensed medical product. Many are produced specifically for the manufacture of biological products.
Tier 3: Moderate-Risk Materials Not Intended for Use as AMs	These are research-grade materials not intended for use in biological manufacturing; sometimes approved by regulatory agencies as part of an in vitro diagnostic device. Tier 3 requires more qualification than Tier 1 or Tier 2 materials.
Tier 4: High risk Materials	These are materials produced as industrial or research-grade materials and may contain harmful impurities. They may also contain animal- or human-derived components with potential contaminants. This tier requires extensive qualification before use as a component in biological product manufacturing.

New microbial control chapters in PF 48(5)

⟨74⟩ Solid Phase Cytometry-Based Rapid Microbial Methods for the Detection of Contamination in Short Shelf-Life Products

Involves passing large volumes through a filter membrane followed by imaging and enumeration of fluorescently stained viable microbes

- Complement <72> for growth-based respiration method, <73> for ATP bioluminescence method, future PCR-based method and informational chapter ⟨1071⟩ Rapid Sterility Testing of Short Life Products - A Risk Based Approach
- Detection limit of 1 viable cell with test time of 2-3 hours
- Evaluation, Suitability, and Verification
  - Recommended method suitability microorganisms from national and international culture collections
- Test volume minimum: 1% for greater than 10 mL, 100 uL for less than 10 mL

⟨77⟩ Mycoplasma Nucleic Acid Amplification Tests

- Criteria for selecting a validated NAT-based test that is comparable to Method A and B of <63> Mycoplasma Tests
  - Mature method with validation data
  - Specificity – greater than 100 species of mollicutes, six species are most problematic
  - Limit of detection equivalent to <63>
  - Time to result less than a day (28 days for <63> Method A)
- Considerations for sample treatment, QC standards, suitability testing, interpretation of results and investigation of invalid results

⟨1114⟩ Microbial Control Strategies for Cell Therapy Products

- Considerations for aseptically manufactured products that build on <1211> Sterility Assurance
- Risk-based approach that focuses resources on common process weaknesses
- Risk assessment and considerations for
  - Manufacturing facilities – design, operation, cleaning, monitoring
  - Manufacturing operations – aseptic operations, in-process testing, adventitious agents
  - Materials – apheresis starting materials, media and buffers, raw materials

Call for Microbiology Expert Committee Members

We are looking for volunteers to join **USP's Microbiology Expert Committee** and help us shape standards in critical areas in quality, including sterility, bacterial endotoxins, rapid testing, and cell and gene therapy.

The Committee impacts pharmaceuticals, biologics, and dietary supplements and requires engagement across global regulators, industry, and other stakeholders.

You are welcome to **"apply"** by following the instructions through this link: **https://callforcandidates.usp.org/**

Summary

- The complexity and diversity of advanced therapies present challenges in standardization
  - Control of the quality of incoming and raw materials are essential for consistent manufacturing
  - Control of processes and analytical methods reduce the non-biological sources of variability

- USP is committed to working with stakeholders to streamline and expedite development of safe and effective therapies to patients

Thank You

Empowering a healthy tomorrow