Ancillary Materials for Cell and Tissue-Based Products

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USP Biologics and Biotechnology
Outline

• Introduction to USP
• Ancillary materials – definition, examples, and role in manufacturing of cell & tissue-based products
• How FDA regulates ancillary materials
• USP approach to CTG therapies and ancillary materials
The United States Pharmacopeia (USP)

- Oldest pharmacopeia in the world - founded 1820
- Official pharmacopeia in the US, published together with the National Formulary as the USP-NF
- USP Convention (also called USP) is an independent, science-based, nonprofit public health organization
- USP establishes written (compendial) and physical (reference) standards for medicines, dietary supplements, and food ingredients
USP and FDA

- History
  1906  First Federal Food and Drugs Act recognized USP as an official compendium
  1938  FD&C Act gave more prominent role for USP standards for identity, and strength, quality and purity

- By federal law, prescription and over-the-counter medicines available in the US must meet USP-NF public standards, where such standards exist

- USP standards are enforceable by FDA, but USP does not enforce

- USP-FDA Private-Public partnership
  USP’s mission complements FDA’s mission-- to ensure that patients have access to safe & effective medical products
Definition: Ancillary Materials

- Ancillary materials are materials that
  - Come into contact with the cell or tissue product during manufacturing, but
  - Are not intended to be part of the final product formulation

Terminology Notes:
- FDA/cGMP term **components** refers to a broader group of materials which includes ancillary materials as defined above
## Ancillary material vs other components

### Cell/tissue source
- Source of cells that become active ingredient in the final product

### Excipient
- Inactive ingredient intended to be in the final product
- *But note that some ancillary reagents may be considered excipients if intended to remain in final product*

### Device or other component of combination product
- Material normally classified as device that is intended to be part of final product
- *For example, structural component or delivery device*
Ancillary materials: common examples

Reagents
- Anticoagulants
- Buffered solutions & culture media
- Cryoprotectants
- Cytokines
- Antibodies & beads
- Enzymes
- Human or bovine serum

Containers/transfer devices
- Bags & tubing
- Culture flasks
- Plastic disposable sets for cell separation
- Pipettes, needles
Why are ancillary materials important?

- **Impact on safety and efficacy** of CTG products
  - Intended vs unintended effects
  - Variability: supplier-to-supplier, lot-to-lot

- **Availability** of high quality ancillary materials is a common problem in development & manufacturing of CTG products

- **Removal and residual testing**
  - Extensive measures to reduce AM residuals may impact quality of CTG products
  - Short shelf life of some cellular products may limit ability to test residuals before release
# How FDA regulates ancillary materials

## 361 HCT/Ps
- cGTP regs
  - Supplies & reagents
    - 21 CFR 1271.210
- No prospective reviews

## 351 HCT/Ps
- cGMP regs
  - Control of components, containers and closures
    - 21 CFR Part 211 Subpart E
  - Sliding scale approach to cGMP compliance, phase 1-4
  - Prospective review of INDs, BLAs
  - Guidance
    - Phase 1 cGMP
    - Somatic CMC
cGTPs vs cGMPs for ancillary materials

**In Common**

AM specifications
- Establish specifications & acceptance criteria
- Verify that AM meets acceptance criteria

Records
- Receipt (date, quantity, supplier name, lot number, exp date, storage conditions)
- Testing/Acceptance (local or vendor C of A)
- Manufacturing (record use & lot number)

**Differences**

**cGMPs much more detailed**
- Identity testing for all components
  - At least one test to verify identity
  - Use specific identity test if one exists
  - Must verify identity with in-house test

**CMC and Ph 1 cGMP guidances**
- Requirements for human- and animal-sourced AMs
USP Standards for B&B Product Classes

<xxxx> Overarching Guidance

<xxx> Common Product Class Quality Attributes

<xxx> Analytical Procedures

Monograph

Monograph

Monograph

<xxx> Ancillary Materials

Monograph

Monograph

Monograph
General Chapters

- Cell and Tissue Based Products
- Gene Therapy Products
- Ancillary Materials
- Flow Cytometry
- Bovine Serum
  - FBS Quality Attributes and Functionality Tests
  - Growth Factors & Cytokines Used in Cell Therapy Manufacturing
- Other Biologics & biotechnology related chapters

Monographs

- Monographs for Tissue-based products
- Monographs for Cell therapy products

Reference Standards

- Physical RS associated with Ancillary Material chapters
- Photomicrographs associated with tissue product histology tests
Ancillary Materials Standards: USP Approach

General Information Chapter (guidance)

Specific AM Chapters
- <1024> Bovine Serum
- <90> FBS Quality Attributes
- <92> Cytokines and Growth Factors Quality Attributes
- <123> Protein A Quality Attributes

Reference Standards:
- FBS
- Interleukin-4
- Protein A

Ancillary Material Requirements for Specific AMs

Ancillary Material Reference Standards

<1043> Ancillary Materials for Cell-, Gene-, and Tissue-Engineered Products
Chapter developed in 2005-2010 cycle; currently being updated/revised

Designed to address AM challenges for CTG products

Structure

- Introduction & definitions
- Qualification programs
- Risk classification (4 tiers)
- Performance testing
- Residual level assessment and removal
- Appendix (includes references for many FDA & ICH guidances)
Tier 1 – Low-Risk, Highly Qualified Materials with Intended Use as Therapeutic Drug or Biologic, Medical Device, or Implantable Material

Tier 2 – Low-Risk, Well Characterized Materials with Intended Use as AMs, Produced in Compliance with GMPs

Tier 3 – Moderate-Risk Materials Not Intended for Use as AMs (frequently produced for in vitro diagnostic use or reagent grade materials)

Tier 4 – High-Risk Materials, Materials not Produced in Compliance with cGMPs and materials not intended to be used in cell manufacturing
# Ancillary Material Risk Classification: Tier 1

Low-Risk, Highly Qualified Materials with Intended Use as Therapeutic Drug or Biologic, Medical Device, or Implantable Material

<table>
<thead>
<tr>
<th>Example</th>
<th>Typical Use in Cell, Gene, or Tissue-Engineered Product Manufacturing</th>
<th>Qualification or Risk Reduction Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant insulin for injection</td>
<td>Cell culture medium additive</td>
<td>- DMF cross reference (when possible or practical)</td>
</tr>
<tr>
<td>Organ preservation fluid</td>
<td>Process biological fluid employed in tissue transport or processing</td>
<td>- Certificate of analysis</td>
</tr>
<tr>
<td>Human serum albumin for injection</td>
<td>Cell culture medium</td>
<td>- Assess lot-to-lot effect on process performance</td>
</tr>
<tr>
<td>Sterile fluids for injection</td>
<td>Process biological fluid employed in tissue transport, cell processing, purification</td>
<td>- Assess removal from final product</td>
</tr>
<tr>
<td>Implantable biomaterials (formed collagen, silicone, polyurethane constructs intended for surgical implantation)</td>
<td>Scaffolds, matrices for immobilized cellular cultivation</td>
<td>- Stability assessment on AM as stored for use in manufacturing</td>
</tr>
<tr>
<td>Recombinant deoxyribonuclease for inhalation or injection</td>
<td>Process enzyme employed in viral vector manufacturing, stem cell processing</td>
<td></td>
</tr>
<tr>
<td>Antibiotics for injection³</td>
<td>Cell culture medium and biopsy transport fluid additive to reduce risk of bacterial contamination</td>
<td></td>
</tr>
<tr>
<td>Injectable monoclonal antibodies</td>
<td>Immunologically targeting specific cell populations for selection or removal</td>
<td></td>
</tr>
<tr>
<td>Injectable cytokines</td>
<td>Cell culture medium</td>
<td></td>
</tr>
<tr>
<td>Vitamins for injection; defined nutrients, chemicals, or excipients intended for injection</td>
<td>Cell culture medium additive employed in cell expansion, controlled cellular differentiation/activation step, or manufacture of a viral vector</td>
<td></td>
</tr>
<tr>
<td>IV bags, transfer sets and tubing, cryopreservation bags, syringes, needles</td>
<td>Storage vessels or container closure systems, closed aseptic transfer systems</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. AM Risk Tier 2
Low-Risk, Well Characterized Materials with Intended Use as AMs, Produced in Compliance with GMPs

<table>
<thead>
<tr>
<th>Example</th>
<th>Typical Use in Cell, Gene, or Tissue-Engineered Product Manufacturing</th>
<th>Qualification or Risk Reduction Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant growth factors, cytokines(^1)</td>
<td>Cell culture medium additive</td>
<td>• DMF cross reference (when possible or practical)</td>
</tr>
<tr>
<td>Immunomagnetic beads</td>
<td>Immunomagnetic separation of cells</td>
<td>• Certificate of analysis</td>
</tr>
<tr>
<td>Human AB serum</td>
<td>Cell culture medium additive</td>
<td>• Assess lot-to-lot effect on process performance(^2)</td>
</tr>
<tr>
<td>Progesterone, estrogen, vitamins, purified chemicals (USP-grade)</td>
<td>Cell culture medium additives, induction agents, buffer components</td>
<td>• Assess removal from final product</td>
</tr>
<tr>
<td>Sterile process buffers</td>
<td>Process biological fluid employed in tissue transport, cell processing, purification</td>
<td>• Stability assessment on AM as stored for use in manufacturing(^3)</td>
</tr>
<tr>
<td>Biocompatible polymers, scaffolds, hydrogels</td>
<td>Scaffolds, matrices for immobilized cellular cultivation</td>
<td>• When relevant, confirm certificate of analysis test results critical to product (could include functional assay)</td>
</tr>
<tr>
<td>Proteolytic enzymes</td>
<td>Process enzyme</td>
<td>• Vendor audit</td>
</tr>
<tr>
<td>Tissue culture media</td>
<td>Cell culture medium additive</td>
<td></td>
</tr>
<tr>
<td>Monoclonal antibodies</td>
<td>Immunologically targeting specific cell populations for selection or removal</td>
<td></td>
</tr>
<tr>
<td>Density gradient media</td>
<td>Cell separation via centrifugation</td>
<td></td>
</tr>
</tbody>
</table>
## Ancillary Material Risk Classification: Tier 3

**Table 3. AM Risk Tier 3**

Moderate-Risk Materials Not Intended for Use as AMs
(frequently produced for in vitro diagnostic use or reagent grade materials)

<table>
<thead>
<tr>
<th>Example</th>
<th>Typical Use in Cell, Gene, or Tissue-Engineered Product Manufacturing</th>
<th>Qualification or Risk Reduction Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant growth factors, cytokines</td>
<td>Cell culture medium additive</td>
<td>• DMF cross reference (when possible or practical)</td>
</tr>
<tr>
<td>Tissue culture media</td>
<td>Cell culture medium additive</td>
<td>• Certificate of analysis</td>
</tr>
<tr>
<td>Monoclonal antibodies (diagnostic-grade produced in cell culture)</td>
<td>Immunologically targeting specific cell populations for selection or removal</td>
<td>• Assess lot-to-lot effect on process performance$^1$</td>
</tr>
<tr>
<td>Process buffers</td>
<td>Process biological fluid employed in tissue transport, cell processing, purification</td>
<td>• Assess removal from final product</td>
</tr>
<tr>
<td>Novel polymers, scaffolds, hydrogels</td>
<td>Scaffolds, matrices for immobilized cellular cultivation</td>
<td>• Stability assessment on AM as stored for use in manufacturing$^2$</td>
</tr>
<tr>
<td>Proteolytic enzymes</td>
<td>Process enzyme</td>
<td>• When relevant, confirm certificate of analysis test results critical to product (could include functional assay)</td>
</tr>
<tr>
<td>Purified chemicals (reagent-grade)</td>
<td>Culture medium additives, induction agents, buffer components</td>
<td>• Vendor audit</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Upgrade manufacturing process for material to GMP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Develop stringent internal specifications</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Determine if lot-to-lot biocompatibility, cytotoxicity, or adventitious agent testing are needed</td>
</tr>
</tbody>
</table>
# Ancillary Material Risk Classification: Tier 4

## Table 4. AM Risk Tier 4

### High-Risk Materials

<table>
<thead>
<tr>
<th>Example</th>
<th>Typical Use in Cell, Gene, or Tissue-Engineered Product</th>
<th>Qualification or Risk Reduction Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS</td>
<td>Cell culture medium additive</td>
<td>- Same as in Table 3, plus</td>
</tr>
<tr>
<td>Animal-derived (including human) extracts</td>
<td>Cell culture medium additive</td>
<td>- Verify traceability to country of origin</td>
</tr>
<tr>
<td>Animal-derived polymers, scaffolds, hydrogels</td>
<td>Scaffolds, matrices for immobilized cellular cultivation</td>
<td>- Assure country of origin is qualified as safe with respect to source-relevant animal diseases, including TSE</td>
</tr>
<tr>
<td>Purified enzymes</td>
<td>Process enzyme</td>
<td>- Adventitious agent testing for animal source-relevant viruses</td>
</tr>
<tr>
<td>Ascites-derived antibodies or proteins</td>
<td>Immunologically targeting specific cell populations for selection or removal</td>
<td></td>
</tr>
<tr>
<td>Animal or human cells used as feeder layers</td>
<td>Cell culture substratum or source of medium components</td>
<td></td>
</tr>
<tr>
<td>Chemical entities with known toxicities (i.e. methotrexate, cholera toxin, <em>Staphylococcus aureus</em> pore-forming hemolysin, <em>Staphylococcus</em> enterotoxins A and B, toxic shock syndrome toxin)</td>
<td>Selection agents used in cell culture to improve or maintain transgene expression, enhance cellular proliferation, improve cell survival upon cryopreservation, superantigens for the activation of T cells</td>
<td></td>
</tr>
</tbody>
</table>
Chapters on specific ancillary materials
**USP <90> Fetal Bovine Serum (FBS)**

- **FBS Standards**
  - Osmolality: 280-360 mOsm/Kg
  - Total Protein: 30-45 mg/mL
  - pH: 7.00 - 8.00
  - Endotoxin: < 10 units/mL
  - Hemoglobin level < 30 mg/dL
  - Identification: Radial Immunodiffusion (RID): species ID, IgG levels
  - Functionality Assays (Growth Curve and Clonal Assay)

- **Associated Reference Standard (RS) in development**
  - Liquid frozen, 10 mL
  - Collaborative study will include several laboratories to test:
    - Identification (FBS sample positive for bovine IgG and content is < 500 mg/L)
    - Growth curve (doubling time in test sample is not less than 90% compared to RS)
Growth Factors and Cytokines Used in Cell Therapy Manufacturing

- Standards for specific GFs & cytokines
  - *Currently includes*
    - Recombinant human interleukin-4 (rhIL-4)
  - *In progress*
    - Recombinant human fibroblast growth factor-2 (rhFGF-2)
    - Others
Recombinant human interleukin-4 (rhIL-4)

- **rhIL-4 Standards**
  - Specific Activity: Not less than $0.5 \times 10^7$ Unit of IL-4/mg of total protein (determined using a TF-1 cell proliferation assay)
  - Purity: Not less than 97% (SDS-PAGE and silver stain)
  - Identity: N-term protein sequencing (10 residues) and Western Blot (comparable to Reference Standard)
  - Host-cell DNA: $\leq 1$ ng/mg

- **Associated Reference Standard**
  - Lyophilized powder
  - Mass per vial: $50 \, \mu g \pm 10\%$
  - Calibrated against International Standard (WHO)
  - Collaborative study to include several laboratories to test for:
    - SDS-PAGE/Western Blot using commercially available IL-4 antibody
    - Bioidentity test (Labeled potency of RS will be based on bioactivity using TF-1 cell line)
Figure 2. Example of MS/MS spectrum of identified peptide (R.GECWCVNPNTGK.L) from insulin-like growth factor-binding protein 2.

<table>
<thead>
<tr>
<th>approach</th>
<th>protein ID</th>
<th>protein reference</th>
<th>no. of unique peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (unfractionated sample)</td>
<td>TGF1</td>
<td>transforming growth factor β 1</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>GGF</td>
<td>glial growth factor</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>IGFBP2</td>
<td>insulin-like growth factor-binding protein 2</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>IGFII</td>
<td>insulin-like growth factor-II</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>TGF1</td>
<td>transforming growth factor β 1</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>GGF</td>
<td>glial growth factor</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>CAA33746.1</td>
<td>prepro-insulin-like growth factor 1</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>bFGF</td>
<td>basic fibroblast growth factor</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>IGFBP2</td>
<td>insulin-like growth factor-binding protein 2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>IGFBP4</td>
<td>insulin-like growth factor-binding protein 4</td>
<td>2</td>
</tr>
</tbody>
</table>

Summary & Conclusions

• USP AM standards are aimed to help industry develop safe & effective CTG products, and improve regulatory compliance

• Industry feedback has been mostly positive
  • Most want more guidance and specific AM standards
  • Concerns about how to consider cost in decision-making
  • Questions about how to do risk assessment
  • Many questions & concerns about animal-derived materials
Summary & Conclusions

- USP is seeking collaboration with academic & commercial parties to
  - Participate in USP collaborative studies (FBS, IL-4, etc.)
  - Propose new generation test methods for FBS (e.g., proteomics-based methods for identification)
  - Propose methods for residual testing (e.g., FBS)
  - Propose new Ancillary Material standards
  - Propose revisions of current chapters: <1043>, <92>, <90>
Summary & Conclusions

• Issues & plans for revision of <1043>
  – Update categories and examples of ancillary materials
  – “Animal-free”: definitions, and discuss current industry practices
  – Risk assessment: more detail & explanation, and alignment with ICH Q9 (quality risk management)
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