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Standard Guide for Cell Culture Growth Assessment of Single-Use Material[1]

This standard is issued under the fixed designation E3231; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide outlines best practices to consider when setting up a representative leachable test method to detect if a material is compatible with cell culture media or manufacturing processes. This guide does not replace or supersede cell growth tests like USP <87>, USP <88> (plastic/elastomeric materials), or ISO 10993 (medical device materials), that are used in assessing biological reactivity in humans. Polymeric materials that have passed these tests have been found to leach compounds under normal process conditions that can inhibit cell culture growth for some cell lines. See Refs (**1-5**).[2] Test methods that are representative of the manufacturing conditions will help identify materials that are appropriate for use during manufacturing.

1.2 This guide may be relevant to biopharmaceutical manufacturing, cell-based therapeutics, vaccines, cell-based diagnostics, and other areas.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *USP Documents:*[3]
USP <87> Biological Reactivity Tests, In Vitro
USP <88> Biological Reactivity Tests, In Vivo

2.2 *ISO Documents:*[4]
ISO 10993 Biological Evaluation of Medical Devices

3. Terminology

3.1 *Definitions:*

3.1.1 *bis(2,4-di-tert-butylphenyl)-phosphate (bDtBPP), n*—leachable compound known to inhibit cell growth (CAS # 69284-93-1).

3.1.2 *dimethyl sulfoxide (DMSO), n*—(CH3)2SO (CAS # 67-68-5).

3.1.3 *test articles, n*—the material being tested.

3.1.4 *test media, n*—cell culture media that has been used to extract potential leachables from the test articles.

3.1.5 *the gray (Gy), n*—the SI unit for absorbed radiation dose, and defined as the absorption of one joule of energy, in the form of ionizing radiation, per kilogram of matter, that is one gray = 1 J/kg.

4. Summary of Guide

4.1 This guide outlines best practices to assess compatibility of polymeric materials with animal cell cultures used in the manufacture and processing of vaccine, gene, cell and protein therapies reliant on cell-based processes. The best practices may be used to reveal compatibility issues in a cell culture system that includes the cell culture medium, the cell line and the polymeric test articles that are under evaluation.

4.2 The guide starts with an overview of typical cell culture compatibility studies and then outlines best practices for each aspect of the study.

[1] This guide is under the jurisdiction of ASTM Committee E55 on Manufacture of Pharmaceutical and Biopharmaceutical Products and is the direct responsibility of Subcommittee E55.07 on Single Use Systems.

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[2] The boldface numbers in parentheses refer to a list of references at the end of this standard.

[3] Available from U.S. Pharmacopeial Convention (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.

[4] Available from International Organization for Standardization (ISO), ISO Central Secretariat, BIBC II, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, http://www.iso.org.

5. Significance and Use

5.1 A risk-based approach must be used to determine the cell lines, test articles, and materials used for testing. An evaluation of relevant factors should be made to determine if a test article is representative of the intended use.

5.2 Cell culture compatibility should be assessed if the material is in direct contact with cell culture medium regardless of duration of contact. Test articles can be of a single material or assembled from a multitude of materials.

5.3 Two perspectives to single-use material cell culture compatibility assessments are the supplier and the end user perspectives. It is understood that the supplier may have better access to single-use materials and material manufacturing processes, while having limited access to representative cell lines. Supplier assessment of materials are best tested using cell lines available that have shown known material sensitivity. The end users may have more limited access to materials but access to more representative cell lines and processes. Therefore assessment of compatibility of material with a specific cell line in a process is best evaluated by the end user.

5.4 This guide outlines best practices to establish test procedures. Appendix X1 outlines an example test procedure for a commercially available CHO cell line.

6. Cell Culture Compatibility Testing Overview

6.1 Fig. 1 highlights the main stages of organizing cell culture compatibility testing. Key aspects are highlighted in the figure and detailed best practices for each stage are covered in the subsequent sections. Appendix X1 shows an example of what a test could look like using an industrially available media and cell line.

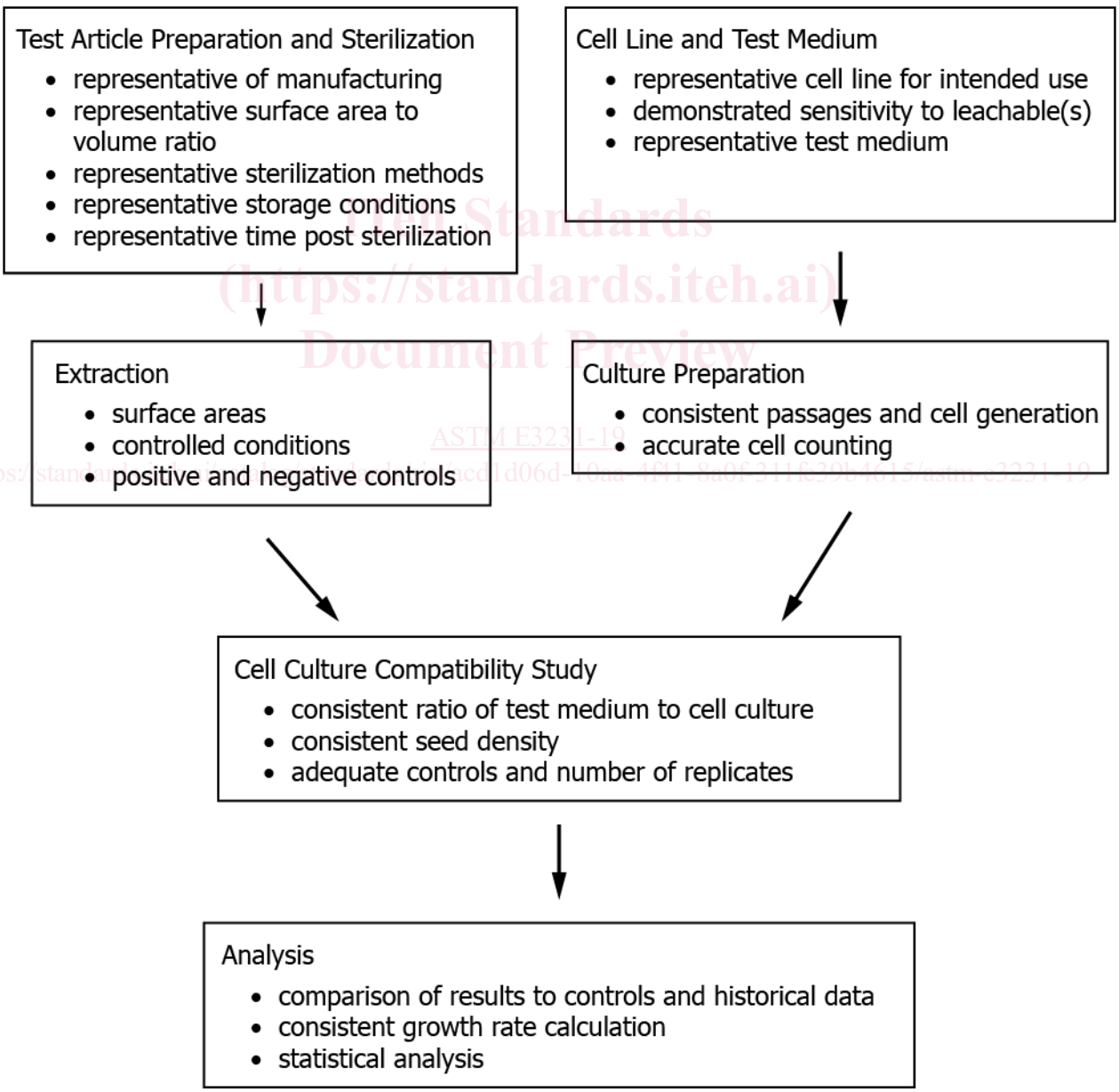

FIG. 1 Cell Culture Compatibility Testing Design: Key Considerations for Success

7. General Study Design and Controls

7.1 Fig. 2 shows a typical process flow for cell culture compatibility testing. The test article is first prepared to a size that is scaled to be representative of the contacted surface area during use. Then the test article is sterilized in a representative way. Following sterilization the test article is extracted in the cell culture medium (test medium) in parallel to control conditions. Then these test medium are inoculated with cell culture and cell growth is monitored. The growth rate is used to analyze the material's effect on cell growth.

7.2 *Controls:*

7.2.1 Several replicates of negative and positive controls should be used during each test. These flasks filled with media must be incubated under the same conditions as the extraction containers of the test articles. The number of replicates should be chosen based on expected cell growth variability. Typically a minimum of three replicates are recommended.

7.2.2 Multiple positive and negative controls could be used. For example, each test could include multiple standard growth inhibitors to demonstrate growth inhibition. Multiple vessel types could be used to demonstrate representative cell growth (glass and plastic flasks).

7.3 *Negative Controls (Growth Benchmark):*

7.3.1 The media from the negative controls should exhibit normal growth performance following the extraction period. This controls for any problem with the extraction process affecting cell growth. This extraction container could have no test article or a material known to not inhibit cell growth in the media during the extraction duration.

7.4 *Positive Control (Extracted or Spiked with Growth Inhibitor or Growth Promoter):*

7.4.1 The media from the positive controls should demonstrate reduced or enhanced growth performance following extraction. The positive controls show that the assay was executed properly to demonstrate sensitivity to a known leachable that has proven to effect the cell line used. It does not indicate that if the test article media also demonstrates an effect that the same leachable compound is present. For this reason, positive controls are not an absolute requirement.

7.4.2 One example of a positive control would be to spike the media from the flask with bDtBPP or similar growth inhibitor of interest.

7.4.3 The sensitivity of the cell line to the positive control should be demonstrated with a dose-response curve test.

8. Cell Line and Test Medium

8.1 *Cell Lines:*

8.1.1 The cell lines should be representative of the final application. In order to detect leachables better it is recommended to use a cell line that demonstrates a known sensitivity to known leachables that effect cell growth.

8.2 *Extraction Solution (Test Medium): Cell Culture Medium:*

8.2.1 Media that is representative of the intended process should be used. When possible serum-free, chemically defined cell culture medium will help ensure test reproducibility. Serum-containing media are known to give false negative test results and should not be used if possible. See Ref (**4**). If your intended process and the representative test cell line requires serum containing medium, then serum containing medium can be used to best represent the final intended use case.

8.2.2 The medium must be temperature stable for the duration of the testing.

9. Material Preparation and Sterilization

9.1 A representative test article should be used. The material should be manufactured using methods as similar as possible to the envisioned manufacturing process of the component. It is important to use representative chemical grades, multiple production lots, anticipated process (machining or molding), type of irradiation process, use of cleaning agents or process aids, etc.

9.2 Select the test article size based on the worst case use condition and contact area during extraction. Using consistent surface areas during extraction helps when comparing materials. It is recommended, as a worst case test condition, to test a surface area of 3 cm^2 of material per ml of extraction medium for materials >0.5-mm thick (ISO 10993). Smaller test articles can be used as multiples to achieve a surface area of 3 cm^2 per millilitre of extraction medium. If the material floats, this should be accounted for in the surface area calculation. For some materials with a low surface area, an extraction ratio of 3 cm^2/mL cannot be achieved and a lower ratio is required.

9.3 If cutting is required to achieve the target sample size, cutting tools need to be clean to prevent introduction of foreign matter. Test articles should be dusted to remove small particles that could be generated during cutting, sawing, or similar procedures. Avoid cleaning fluids, if possible, as they can extract leachables and reduce reactivity prior to the test. Consider that cutting the material might expose material layers that would normally not contact the cell culture.

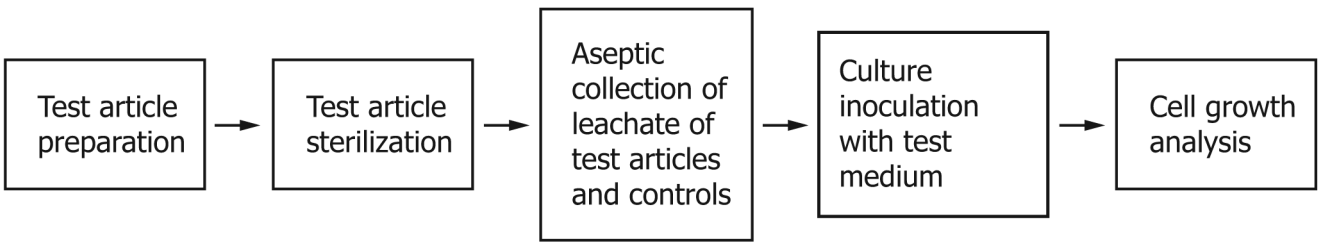

FIG. 2 Cell Culture Compatibility Study Process Flow