CBER Standards Recognition Program for Regenerative Medicine Therapies
Standards Recognition Summary (SRS)

Recognition Number: 060 **Date of Recognition:** 12/8/2025

SDO Name/Designation: ASTM F3716 **Year of Publication:** 2025

Title: Standard Test Method for Cumulative Population Doubling Analysis of the Proliferation of Vertebrate Tissue Cell Preparations

Scope: 1.1 This test method, within the limitations defined, describes procedures for performing serial *in vitro* cell cultures of vertebrate tissue cells whose quantified total cell numbers over culture time are used to derive cumulative population doubling (CPD) data.

1.2 This test method describes how to derive CPD data from total cell count data from serial tissue cell cultures.

1.3 This test method describes how CPD data can be used to perform scientifically valid quantitative comparisons of the mathematical form (that is, linear versus hyperbolic), rate (that is, slope), and extent of cell proliferation of vertebrate tissue cell populations with known differences in their organ or tissue source, their cell culture media (for example, supplements and test agents), their cell culture conditions, or their intrinsic cellular properties (for example, normal versus tumorigenic).

1.4 This test method describes how CPD data from long-term serial cell culture studies provide more information about the history, rate, and extent of cell proliferation by tissue cell preparations than short-term cell proliferation assays.

1.5 This test method is applied in an *in vitro* cell culture laboratory setting.

1.6 This test method does not recommend use of a specific cell counting method. Many different types of cell counting methods exist that may be suitable for this test method. Suitable cell counting methods may include the following: trypan blue hemocytometer counting; trypan blue automated cell counter; electrical zone sensing cell counting (Coulter counter) (Test Method F2149); or acridine orange-propidium iodide automated fluorescence cell counter. See Annex A of ISO 20391-1 for a summary of cell counting methods.

1.7 Although live cell and dead cell counting may be performed for this test method, neither is required. Only total cell counts are necessary.

1.8 Although in most cases counts of individual cells are used for this test method, it can be performed with surrogate measures of changes in cell number (for example, light absorbance by cells, light scatter by cells, cell mass).

1.9 This test method can be broadly applied to isolated cell preparations from any vertebrate animal. The following statements are illustrative and non-exclusive:

1.9.1 The test method can be applied to mammalian cells isolated from human organs and tissues for regenerative medicine applications, pharmaceutical drug development applications, and toxicological analysis applications.

1.9.2 The test method can be applied to mammalian cells isolated from the organs and tissues of animals used in research (for example, mice, rats, dogs, monkeys, pigs, goats, sheep, etc.), used for pharmaceutical and toxicological evaluations, and treated in veterinary medicine.

1.9.3 The test method can be applied to freshly isolated uncultured tissue cells (that is, primary cells) or to cell populations after varying degrees of culture, after specific processing (for example, cell fractionation) or after other varied manipulations (for example, genetic modification, neo-plastic transformation, subcloning).

1.9.4 The test method can be applied to cells isolated from normal, diseased, or injured organs and tissues.

1.10 The test method can be applied to cells in any culture format that permits serial cell culture and quantification of the total number of cells in the culture system at each transfer of cells to a next culture vessel (that is, at each serial cell culture passage).

1.10.1 Applicable cell culture formats include adherent cell culture, suspension cell culture, and microcarrier cell culture.

1.10.2 Applicable cell culture formats include other cell culture formats (for example, three-dimensional matrix formats) that permit complete cell harvest and uniform sampling and quantification of cells at each serial cell culture cell transfer (see, for example, Guide F2739).

1.10.3 During serial cell culture incubation periods, cells may exist as single cells, cell clusters, or even in solid states, as long as they can be completely harvested, uniformly sampled, counted, and allow a portion of the harvested cells, of known number or fraction, to be transferred into the next culture vessel.

1.11 This test method may be performed with a variety of serial cell culture schedules. However, for comparative analyses of the rate and extent of the cell proliferation of different cell populations, it is crucial that the same serial cell culture schedule is used for the compared cell populations.

1.11.1 The serial cell culture can be performed by transferring either a constant number or a constant fraction of the cells harvested from the existing cell culture at each cell culture passage.

1.11.2 Serial cell culture based on transferring either a constant number of cells or a constant fraction of cells can be performed with either regular time periods of incubation between transfers or irregular time periods of incubation between transfers.

1.11.3 Serial cell culture based on transferring either a constant number of cells or a constant fraction of cells can be performed with transfers occurring when cell cultures have reached a designated quantity of cells. With adherent cell culture formats, the designation can be the

cultures' degree of confluency (for example, when 100 % coverage of the culture surface has been achieved). However, other independent measures of the quantity of cells may be applied (for example, absorbance level).

1.11.4 This test method can be performed with any variegating combination of serial cell culture schedules, as long as the schedules are well documented for subsequent CPD determinations. However, for comparative analyses of the mathematical form, rate, or extent of the cell proliferation of different cell populations, it is crucial that the same serial cell culture schedule and culture format are used for the compared cell populations.

1.12 This test method has been evaluated (herein) for its ability to provide accurate and precise comparisons of the mathematical form, rate, and extent of proliferation by human primary cell preparations in both intralaboratory and interlaboratory evaluations.

1.13 Limitations are described as follows:

1.13.1 The quality of this test method depends on accurate and precise cell counting, whether performed manually with cell counting slides or with well-calibrated automated electronic cell counters.

1.13.2 The quality of this test method depends on well-maintained, well-calibrated, and properly operated cell culture equipment, culture vessels, and culture media.

1.13.3 The quality of this test method depends on technically proficient cell culture personnel who are well trained in tissue cell culture maintenance and cell counting procedures.

1.13.4 The quality of this test method depends on consistent technical procedures throughout, including maintaining standard cell culture passaging and counting techniques, methods, and equipment performance.

1.13.5 In the case of adherent cell cultures, dead cells that detach during the cell culture interval will be lost to the accounting. This loss is acceptable for the standard test method, as it is concerned with quantifying the number of 6population doublings by cells that remain attached in adherent cell cultures.

1.14 Though developed primarily based on experience with vertebrate tissue cell culture, the standard test method described herein may also be applied to analyses of invertebrate tissue cells and plant cells.

1.15 *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.*

1.16 *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.

Extent of Recognition: Complete Recognition

Rational for Recognition: The standard is applicable to cell - based therapies. there are no conflicts with FDA regulations or policies, and the standard is scientifically sound.

Standards Development Organization: www.ASTM.org

Please note that this standard may also be recognized under the Center for Devices and Radiological Health's (CDRH) Recognized Consensus Standards Database for Medical Device, found here: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm .