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Standard Test Method for Automated Analyses of Cells—the Electrical Sensing Zone Method of Enumerating and Sizing Single Cell Suspensions[1]

This standard is issued under the fixed designation F2149; 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 test method, provided the limitations are understood, covers a procedure for both the enumeration and measurement of size distribution of most all cell types. The instrumentation allows for user-selectable cell size ~~settings, hence, this test method is not restricted to specific~~ settings and is applicable to a wide range of cell types. The method works best for spherical cells, and may be less accurate if cells are not spherical, such as for discoid cells or budding yeast. The method is appropriate for suspension as well as adherent cell cultures (**1**).[2] ~~This is a quantitative laboratory method not intended for on-line or field use.~~ Results may be reported as number of cells per ~~millilitre~~milliliter or total number of cells per volume of cell suspension analyzed. ~~Both count and size~~ Size distribution may be expressed in cell ~~micron~~ diameter or ~~volume, femtolitres.~~volume.

1.2 Cells commonly used in tissue-engineered medical products (**2**) ~~routinely~~ are ~~analyzed.~~ analyzed routinely. Examples are chondrocytes (**3**), fibroblasts (**4**), and keratinocytes (**5**). Szabo et ~~al~~al. used the method for both pancreatic islet number and volume measurements (**6**). In addition, instrumentation using the electrical sensing zone technology was used for both count and size distribution analyses of porcine hepatocytes placed into hollow fiber cartridge extracorporeal liver assist systems. In this study (**7**), and others (**6, 8**), the automated electrical sensing zone method was ~~clearly~~ validated for ~~superior accuracy and~~ precision when compared to the conventional ~~manual method,~~ visual cell counting under a microscope using a hemocytometer. ~~This validation has been demonstrated over a wide variety of cell types. In addition, the automated procedure is rapid, rugged, and cost effective; it also minimizes operator-to-operator variability inherent in manual techniques.~~Currently, it is not possible to validate cell counting devices for accuracy, since there not a way to produce a reference sample that has a known number of cells. The electrical sensing zone method shall be validated each time it is implemented in a new laboratory, it is used on a new cell type, or the cell counting procedure is modified.

1.3 ~~This instrumentation~~ Electrical sensing zone instrumentation (commonly referred to as a Coulter counter) is manufactured by a variety of ~~companies; however, the principle used in all~~ is companies and is based upon electrical impedance. This test method, for cell counting and sizing, is based on the detection and measurement of changes in electrical resistance produced by a cell, suspended in a conductive liquid, traversing through a small aperture (see Fig. 1(**9**)). When cells are suspended in a conductive liquid, phosphate-buffered saline for instance, they function as discrete insulators. When the cell suspension is drawn through a small cylindrical aperture, the passage of each cell changes the impedance of the electrical path between two submerged electrodes located on each side of the aperture. An electrical pulse, suitable for both counting and sizing, results from the passage of each cell through the aperture. The path through the aperture, in which the cell is detected, is known as the "electronic sensing zone." This test method permits the selective counting of cells within ~~very~~ narrow size distribution ranges by electronic selection of the generated pulses. While the number of pulses indicates cell count, the amplitude of the electrical pulse produced depends on the cell's volume. The baseline resistance between the electrodes is due to the resistance of the conductive liquid within the boundaries of the aperture. The presence of cells within the "electronic sensing zone" raises the resistance of the conductive pathway that depends on the volume of the cell. Analyses of the behavior of cells within the aperture demonstrates that the height of the pulse produced by the cell is the parameter that most nearly shows proportionality to the cell volume.

1.4 Limitations are discussed as follows:

1.4.1 *Coincidence*—Occasionally, more than a single cell transverses the aperture simultaneously. Only a single larger pulse, as opposed to two individual pulses, is generated. The result is a lower cell count and higher cell volume measurement. The frequency of coincidence is a statistically predictable function of cell concentration that is corrected by the instrument. This is called

[1] This test method is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.43 on Cells and Tissue Engineered Constructs for TEMPs.

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

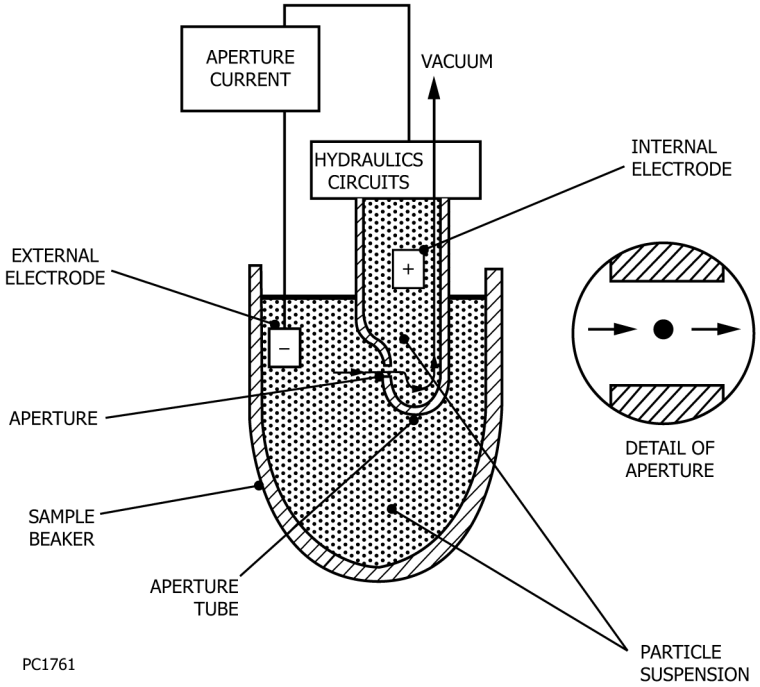

FIG. 1 Cell, Suspended in a Conductive Fluid, Traversing Through a Small Aperture

coincidence correction (**8**). This phenomenon may be ~~minimized, thus ensuring greater result accuracy, by using relatively low cell concentrations, around the 5 % level.~~reduced by using lower cell concentrations.

1.4.2 *Viability*—~~Automated~~ Electrical sensing zone cell counting enumerates both viable and nonviable ~~cells. It does not measure percent cell viability. To measure the percent cell viability, either a vital dye or nonvital dye, such as trypan blue, procedure must be performed.~~cells and cannot determine percent viable cells. A separate test, such as Trypan blue, is required to determine percent viable cells.

1.4.3 ~~*Size Variation of the Cell Sample—*~~*Cell Diameter*—~~Up to 30 to 1 by cell diameter in microns; 27 000 to 1 by cell volume. This is simply~~ This is a function of the size range capability of the ~~particular~~ aperture size selected. ~~Using this technology, measurements~~ Measurements may be made in the cell diameter range of ~~about~~ 0.6 µm to 1200 µm. ~~The lower size limit is restricted only by thermal and electronic noise.~~ Setting the counting size range on the instrument can affect the test results, especially if the cell size has a large distribution, and should be carefully controlled to help achieve repeatability.

1.4.4 *Size Range of the Aperture*—The size range for a single aperture is proportional to its ~~diameter,~~diameter. ~~D.~~The response has been found to depend linearly on ~~D~~diameter over a range from ~~0.02~~2 ~~D~~% to ~~0.80~~80 ~~D%; however, the~~ of the diameter. However, the aperture tube may become prone to blockage at levels greater than ~~0.60~~60 ~~D.~~% ~~The~~ of diameter. Therefore, the practical operating ~~range, therefore,~~ range of the aperture is considered to be 2 % to 60 % of the diameter.

1.4.5 *Humidity*—10 % to 85 %.

1.4.6 *Temperature*—10 °C to ~~35°C.~~35 °C.

1.4.7 *Electrolyte Solution*—The diluent for cell suspension ~~must~~shall provide conductivity and have ~~no~~minimal effect on cell size. The electrolyte of choice is ~~most often physiologic phosphate buffered~~ commonly phosphate-buffered saline.

2. Terminology

2.1 *Definitions:*

2.1.1 *channelyzer, n*—a pulse height analyzer; places voltage pulses into appropriate size bins for the size distribution data.

2.1.2 *coincidence, n*—more than one cell transversing the aperture at the same time.

2.1.3 *corrected count, n*—the cell count corrected for coincidence.

2.1.4 *electrolyte, n*—diluent, offering slight conductivity, in which cells are suspended.

2.1.5 ~~*femtolitre,*~~*femtoliter, n*—a cubic ~~micron;~~micrometer; a measurement of cell volume.

~~2.1.6 *micron (µ), n*—0.001 mm, also known as a micrometre; measurement of cell diameter.~~

2.1.6 *raw count, n*—the enumeration of the cell population not corrected for coincidence.

2.1.7 *ruggedness, n*—the degree of reproducibility of the same sample under a variety of normal conditions; for example, different operators.