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Biotechnology — Cell counting —

Part 1: General guidance on cell counting methods

Biotechnologie — Dénombrement des cellules —

Partie 1: Lignes directrices générales relatives aux méthodes de dénombrement des cellules

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2. www.iso.org/directives

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This document was prepared by ISO/TC 276, *Biotechnology*.

A list of all the parts of ISO 20391 can be found on the ISO website.

Introduction

Cell counting (or cell enumeration) is a fundamental measurement that broadly impacts many aspects of biotechnology, from biomanufacturing to advanced therapy. The cell count (or discrete number of cells) is often expressed as cell concentration (i.e. cell count per volume) when in suspension and area density of cells (i.e. cell count per unit area) when adhered to a surface. Cell count is critical in evaluating potency and efficacy for cell-based therapy. The cell concentration within a bioreactor can serve as a quality assurance metric in cell-based manufacturing processes. Many cell-based bioassays need to be normalized to the respective cell count to allow data inter-comparability. This document (which is Part 1 of a multi-part standard on cell counting) defines terms and provides general guidance for the cell counting measurement process, including method selection, sample preparation, measurement, qualification and validation, and data analysis and reporting.

Biotechnology — Cell counting —

Part 1:
General guidance on cell counting methods

1 Scope

This document defines terms related to cell counting for biotechnology. It describes counting of cells in suspension (generally cell concentration) and cells adhered to a substrate (generally area density of cells). It provides key considerations for general counting methods (including total and differential counting, and direct and indirect counting) as well as for method selection, measurement process, and data analysis and reporting.

This document is applicable to the counting of all cell types – mammalian and non-mammalian (e.g. bacteria, yeast) cells.

This document is not intended for counting of cells while in a tissue section or a biomaterial matrix.

Several sector/application-specific international and national standards for cell counting currently exist. When applicable, the user can consult existing standards when operating within their scope (specific measurement techniques and/or applications).

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
accuracy
closeness of agreement between a measured quantity value and a true quantity value of a measurand

Note 1 to entry: The concept of “measurement accuracy” is not a quantity and is not given a numerical quantity value. A measurement is said to be more accurate when it offers a smaller measurement error.

Note 2 to entry: “Measurement accuracy” is sometimes understood as closeness of agreement between measured quantity values that are being attributed to the measurand.

[SOURCE: ISO/IEC Guide 99:2007, 2.13, modified]

3.2
agglomerate
<cells> two or more cells clustered weakly together and detected as a larger object

Note 1 to entry: Agglomerates of cells can be separated into nominally single cells without causing significant damage to the cell.

3.3
aggregate
<cells> two or more cells clustered together (tightly or loosely) and detected as a larger object

Note 1 to entry: Aggregates of cells are generally more difficult to be separated into single cells.

3.4
area density
<cells> cell count of adherent cells on a surface, typically expressed as number of cells per unit area

3.5
attribute
physical, chemical, biological or microbiological property or characteristic

3.6
cell concentration
cell count per volume

Note 1 to entry: Typically used for cells in suspension.

3.7
cell count
discrete number of cells

Note 1 to entry: Cell count is typically expressed as *cell concentration* (3.6) or *area density* (3.4).

3.8
cell counting
measurement process to determine the cell count

3.9
cell suspension
cells dispersed in a liquid matrix

3.10
debris
<in cell suspensions> fragments of cells and/or particles of biological or non-biological origin

3.11
differential cell count
number of a subset of cells, which have been distinguished from other cell subpopulations by at least one distinct cell attribute identified in the measurement

Note 1 to entry: The concentrations derived from a differential cell count can be expressed in absolute concentration or as a relative measure (i.e. percentage) with respect to the total cell number or another predefined population.

3.12
direct cell counting
counting method in which one signal is (or several signals are) detected for each single event

Note 1 to entry: Each single event should represent a single cell in an idealized measurement.

3.13
indirect cell counting
counting method during which a signal (or a set of signals) is measured from a population of cells and that signal is then related to cell number based on a measurement-specific mathematical model (e.g. calibration curve)

3.14
limit of quantitation
LoQ
lowest amount of analyte in a sample that can be quantitatively determined with a suitable precision and accuracy using a specific analytical method

Note 1 to entry: The limit of quantitation describes quantitative assay for low levels of cells in sample matrices.

3.15
linearity
ability to elicit test results that are directly, or indirectly by means of well-defined mathematical transformations, proportional to cell count within a given range

3.16
measurand
quantity intended to be measured

[SOURCE: ISO/IEC Guide 99:2007, 2.3, modified]

3.17
precision
measurement precision
closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions

[SOURCE: ISO/IEC Guide 99:2007, 2.15, modified]

3.18
proportionality
characteristic exhibited by a collection of measurements in which the ratio of the expected value of the measurement to the value of the input parameter at which the measurements were taken remains constant as the value of the input parameter changes (while all other inputs and measurement conditions are held constant)

Note 1 to entry: When a set of measurements exhibits proportionality over a range of a given input, the expected value of the measurements can be expressed as the input parameter multiplied by a fixed constant, with no bias term.

3.19
reagent
substance used in chemical/biochemical analysis or other reactions

3.20
reference material
material sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties

Note 1 to entry: Reference materials with or without assigned quantity values can be used for measurement precision control whereas only reference materials with assigned quantity values can be used for calibration or measurement trueness control.

[SOURCE: ISO/IEC Guide 99:2007, 5.13, modified]

3.21
reference method
thoroughly investigated measurement procedure shown to yield values having an uncertainty in measurement commensurate with its intended use, especially in assessing the trueness of other measurement procedures for the same quantity and in characterizing reference material

[SOURCE: ISO 17511:2003, 3.29, modified]

3.22
repeatability
<results of measurement> measurement precision under defined conditions of measurement

[SOURCE: ISO/IEC Guide 99:2007, 2.21, modified]

3.23
ruggedness
measure of a method's capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during normal usage

[SOURCE: ICH Harmonised Tripartite Guideline, 1994]

3.24
selectivity
property of a measuring system, used with a specified measurement procedure, whereby it provides measured quantity values for one or more measurands such that the values of each measurand are independent of other measurands or other quantities in the phenomenon, body, or substance being investigated

[SOURCE: ISO/IEC Guide 99:2007, 4.13, modified]

3.25
total cell count
count of all cells, independent of the attribute(s) of the cell

3.26
uncertainty
<measurement> non-negative parameter characterizing the dispersion of values attributed to a measurand, based on the information used

[SOURCE: ISO/IEC Guide 99:2007, 2.26, modified]

3.27
validation
confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

[SOURCE: ISO 9000:2015, 3.8.13, modified]

3.28
verification
confirmation, through the provision of objective evidence, that specified requirements have been fulfilled

[SOURCE: ISO 9000:2015, 3.8.12, modified]

3.29
viable cells
cells within a sample that have an attribute of being alive (e.g. metabolically active, capable of reproduction, possessed of intact cell membrane, or with the capacity to resume these functions) defined based on the intended use

4 General concepts of cell counting

4.1 General

Various cell counting methods (as described in Annex A) can be broadly categorized as total or differential cell counting, and direct or indirect cell counting.

4.2 Total cell counting

Total cell counting involves the measurement of all cells, independent of the attribute(s) of the cell.

Criteria should be applied to distinguish cells from debris (cellular and non-cellular in origin).

4.3 Differential cell counting

Differential cell counting involves the measurement of a subset of cells that have been distinguished from other cells by at least one distinct cell attribute.

EXAMPLE Differential cell counting includes viable cell counting, counting of cells that express a specific surface marker, or counting of cells that exhibit specific cell morphology.

4.4 Direct cell counting

Direct cell counting involves the recording of a signal or a set of signals from each cell (3.12). In this context, the signal(s) can be electrical (as in impedance), optical (as in fluorescent or colorimetric), or mechanical. The signal can be recorded manually by a user or automatically by an instrument. Due to the large number of cells in a typical sample, certain direct cell counting methods require dilution of samples. The cell count is then extrapolated based on a dilution factor.

4.5 Indirect cell counting

Indirect cell counting involves the recording of a signal or a set of signals from all cells or a subset of cells in the sample and then relating that signal to a cell count based on measurement specific mathematical model(s) (e.g. calibration curve) (3.13).

EXAMPLE Indirect cell counting includes measurement of total cell mass, total DNA, and metabolic activity.

NOTE Uncertainty in the cell counts derived from indirect cell counting can arise from the mathematical model(s) (e.g. calibration curve), in addition to other sources of measurement errors.

5 Considerations for cell counting measurements

5.1 Selection of a cell counting method

Many cell counting methods exist (see Annex A); these methods can be used to measure total or differential cell count via direct or indirect cell counting (Figure 1 and Annex B).

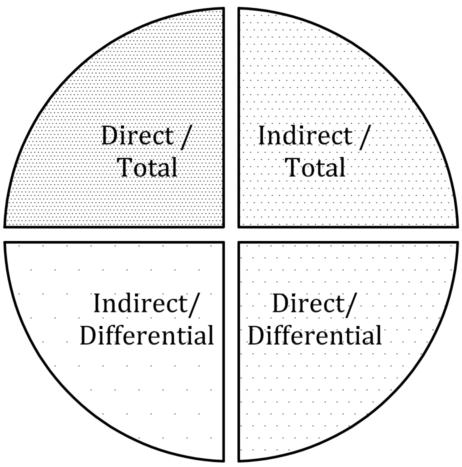

Figure 1 — Cell counting categories