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Second edition
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Biotechnology — Cell counting —

Part 1: General requirements and recommendations for cell counting analytical methods

Biotechnologie — Dénombrement des cellules —

Partie 1: Exigences générales et recommandations relatives aux méthodes analytiques 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 document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 276, *Biotechnology*, Subcommittee SC 1, *Analytical Methods*.

This second edition cancels and replaces the first edition (ISO 20391-1:2018), which has been technically revised.

The main changes are as follows:

— Title updated;

— Scope clarified to not specifically exclude cells in biomaterial matrix;

— additional examples added for differential cell counting and indirect cell counting;

— subclause (4.2) added describing cell counting as a measurement process with Figure 2 providing examples of steps in the measurement process for cell counting;

— subclause (4.3) added describing cell count as a measurand, explaining intermediate examinations and intermediate measurements that can occur as a part of a cell counting analytical method (new Annex C), and describing quantity values for cell count;

— new requirement was added in 4.3 regarding documenting intermediate examination criteria (e.g. gating strategy, threshold values);

— Clause 5 was updated to address the concept of fit for purpose considerations for method selection;

— organization of the document was updated to include Clause 6 on sources of variability in cell counting analytical methods with a new figure, Figure 3; Clause 6 incorporates ISO 20391-1:2018, 5.3, 5.4, and 5.5;

— requirement in ISO 2039101:2018 that cell counting be performed using validated procedures was removed and replaced with the requirement that the cell counting method be at minimum performed using qualified procedures;

— Clause 7 of this document (previously ISO 20391-1:2018, Clause 6) was updated for clarity and consistency with other biotechnology analytical method standards;

— requirement in ISO 20391-1:2018 that the cell counting measurement method be validated, was removed and replaced with requirements for documentation of method qualification strategies;

— requirements in ISO 2039101:2018 that a validation plan and results be documented and maintained and include method performance parameters for the intended use were removed and replaced with similar requirements for qualification instead;

— requirement regarding the establishment of reference values for in-house reference materials was added in 7.3.3;

— reporting clause was updated with new requirements for reporting cell count values.

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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 (Part 1 of the ISO 20391 series on cell counting) defines terms and provides general requirements and recommendations for cell counting, including fit for purpose method selection, sources of variability, measurement, qualification and validation, and data analysis and reporting.

Biotechnology — Cell counting —

Part 1: General requirements and recommendations for cell counting analytical methods

1 Scope

This document provides standard terminology related to cell counting for biotechnology. This document describes counting of cells in suspension (generally cell concentration) and cells adhered to a substrate (generally area density of cells). This document provides key considerations for general counting methods (including total and differential counting, and direct and indirect counting) as well as for fit for purpose method selection, sources of variability in the 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.

NOTE Several sector or application-specific international and national standards for cell counting currently exist. When applicable, the user can consult existing standards when operating within their scope (e.g. specific measurement techniques 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 terminology databases for use in standardization at the following addresses:

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

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

3.1
accuracy
closeness of agreement between a measured *quantity value* (3.28) and a true *quantity value* (3.28) of a *measurand* (3.23)

Note 1 to entry: The concept of "measurement accuracy" is not a *quantity* (3.27) and is not given a numerical *quantity value* (3.28). 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* (3.28) that are being attributed to the *measurand* (3.23).

[SOURCE: ISO/IEC Guide 99:2007, 2.13, modified — Note 2 was removed.]

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
analytical method
investigative procedure for qualitatively or quantitatively measuring or assessing the presence, amount, or functional activity of a target entity (the analyte)

[SOURCE: ISO 23033:2021, 3.3]

3.5
area density
<cells> *cell count* (3.10) of adherent cells on a surface, typically expressed as number of cells per unit area

3.6
attribute
physical, chemical, biological or microbiological property or characteristic

3.7
calibration
operation that, under specified conditions, in a first step, establishes a relation between the *quantity values* (3.28) with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication

Note 1 to entry: A calibration may be expressed by a statement, calibration function, calibration diagram, *calibration curve* (3.8), or calibration table. In some cases, it may consist of an additive or multiplicative correction of the indication with associated *measurement uncertainty* (3.37).

[SOURCE: ISO/IEC Guide 99:2007, 2.39, modified — Notes 2 and 3 to entry were removed.]

3.8
calibration curve
expression of the relation between indication and corresponding measured *quantity value* (3.28)

[SOURCE: ISO/IEC Guide 99:2007, 4.31, modified — Note to entry removed.]

3.9
cell concentration
cell count (3.10) per volume

Note 1 to entry: Typically used for cells in suspension (e.g. cell number per ml).

Note 2 to entry: Cell concentration can refer to the *total cell count* (3.35) or the count of a specific subset of cells within the volume (e.g. *viable cell* (3.39) number per ml).

3.10
cell count
discrete number of cells

Note 1 to entry: Cell count for cells in suspension is typically expressed as *cell concentration* (3.9) or *area density* (3.5).

3.11
cell counting
measurement process to determine the *cell count* (3.10)

3.12
cell suspension
single cells or aggregates of cells dispersed in a liquid matrix

3.13
debris
<in *cell suspensions* (3.12)> fragments of cells or other particles of biological or non-biological origin

3.14
differential cell count
cell count (3.10) of a subset of cells, which have been distinguished from other cell subpopulations by at least one distinct cell *attribute* (3.6) 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.

3.15
differential cell counting
cell counting (3.11) method in which a *differential cell count* (3.14) is evaluated.

3.16
direct cell counting
cell counting (3.11) 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.

3.17
examination
<*nominal property* (3.25)> process of experimentally obtaining one or more values that can reasonably be attributed to a *nominal property* (3.25) together with any other available relevant information

Note 1 to entry: Definition based on Reference [1], NORDIN G, et al. (2018) Vocabulary on *nominal property* (3.25), examination, and related concepts for clinical laboratory sciences (IFCC-IUPAC Recommendations 2017). Pure Appl. Chem. 2018, 90(5), 913-935.

3.18
fit for purpose
fitness for the intended purpose
in line with prearranged requirements for an *intended use* (3.20)

[SOURCE: ISO 20387:2018, 3.24, modified — Note to entry deleted and "fitness for the intended purpose" given as an admitted term.]

3.19
indirect cell counting
cell counting (3.11) 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* (3.8))

3.20
intended use
intended purpose
use for which a product, process, or service is intended according to the specifications, instructions, information or multiple of them provided by the manufacturer or user

[SOURCE: ISO 23033:2021, 3.26]