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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

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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;

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— 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.

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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.

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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.]

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