



ISO 20391-1

**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*

**Second edition
2026-07**

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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 20391-1:2018 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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- 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.

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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.