



BLOG

ISO 20391

Understanding Cell Counting Standards: ISO 20391-1

General Guidance on Cell Counting Methods



Introduction

Why Are Standards for Cell Counting Necessary?

Cell counting is one of the most fundamental yet critical processes in life science research and clinical diagnostics. Whether a researcher is verifying the effect of a new drug, analyzing immune cell counts in a patient's blood sample, or monitoring the culture state in stem cell research, every step begins with cell counting. In other words, cell counting data are not just numbers—they are core data that determine the reliability of both research and clinical practice.

The problem is that this process is not always accurate. Even when measuring the same sample, results can differ from one researcher or laboratory to another. This variability arises from differences in counting methods, instruments, reagents, and environmental conditions. Incorrect data can distort research outcomes, undermine reproducibility, and, in clinical settings, pose serious risks to patient safety.

In recent years, the use of automated cell counting instruments has grown rapidly. These tools are increasingly adopted by pharmaceutical companies, research laboratories, and clinical institutions because they improve efficiency and reduce human error. Moreover, in the manufacturing of advanced therapies such as cell-based treatments under GMP (Good Manufacturing Practice) conditions, cell counting data have become not just research outputs but also critical quality control data directly tied to regulatory review and patient treatment.

As the impact of cell counting data has grown, researchers and regulatory bodies worldwide have long grappled with the question: "How can cell counting results be standardized and validated?" The answer came in the form of the ISO 20391 series. ISO 20391 provides a common language and validation framework for cell counting methods. In particular, ISO 20391-1 offers general guidance on cell counting, defining the fundamental factors that researchers must take into account. In this article, we will explore the key concepts and structure of ISO 20391-1 and discuss its practical significance in research settings.

Purpose of ISO 20391-1

The primary purpose of ISO 20391-1 is to provide internationally consistent standards that ensure the reliability and reproducibility of cell counting data.

A cell growth curve typically follows four distinct phases:

- **Establishing a common language** : Clearly defining terms such as accuracy, precision, and uncertainty so that researchers, instrument manufacturers, and regulatory bodies can communicate with a shared understanding.
- **Improving research reproducibility** : Providing criteria that allow the same sample to yield comparable results across different laboratories
- **Supporting regulatory and academic credibility** : Enabling cell counting data to be internationally recognized in contexts such as clinical research and drug development.
- **Providing an instrument validation framework** : Outlining a stepwise qualification process (IQ/OQ/PQ) from installation to actual experimental application, empowering researchers to independently validate instrument performance.

In summary, the purpose of ISO 20391-1 is to serve as the minimum standard for data reliability, as well as the foundational language and validation framework for achieving international credibility.

Key Concepts of ISO 20391-1

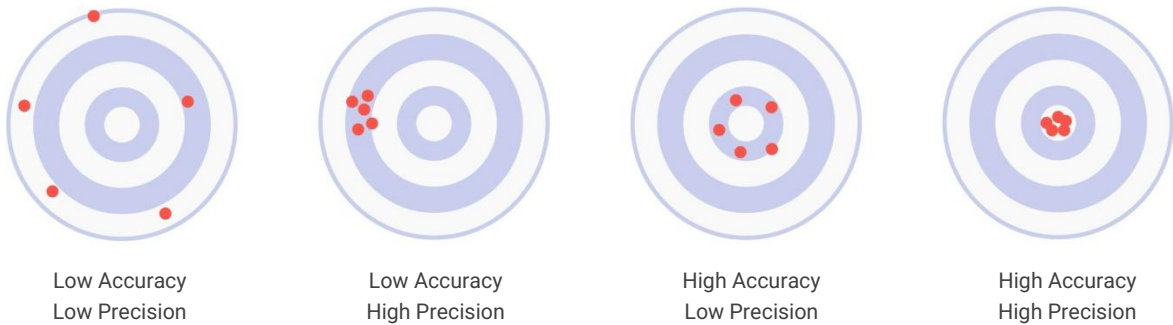
ISO 20391-1 defines three essential concepts that must be considered in quality control (QC) of cell counting.

1. Accuracy

- Definition : How close a measurement is to the true value.
- Example : If the true cell concentration is 1×10^6 cells/mL :
 - A measurement of 0.95×10^6 → high accuracy.
 - A measurement of 0.70×10^6 → low accuracy.

2. Precision

- Definition : The degree of consistency when the same sample is measured repeatedly.
- Example : When measuring the same sample five times :
 - Results of 0.98, 1.00, 1.01, 0.99, and 1.02×10^6 cells/mL → clustered closely together → high precision.
 - Results of 0.98, 1.00, 1.01, 0.99, and 1.02×10^6 cells/mL → clustered closely together → high precision.



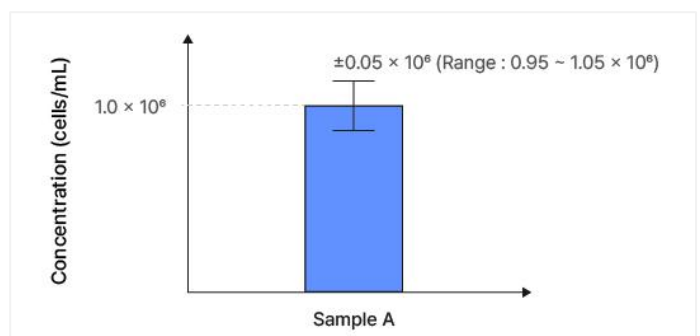
*Conceptual difference between Accuracy and Precision :
Accuracy = closeness to the true value, Precision = consistency among repeated measurements.*

3. Uncertainty

- Definition : The concept that all measurement values inherently contain an error margin.
- Note : ISO recommends expressing this uncertainty numerically.
- Example : If the measured cell concentration is $1.0 \times 10^6 \pm 0.05 \times 10^6$ cells/mL:
 - The actual value is most likely within the range of 0.95 to 1.05×10^6 cells/mL.

Thus, uncertainty provides information not about the value itself, but about the range in which the true value is likely to exist.

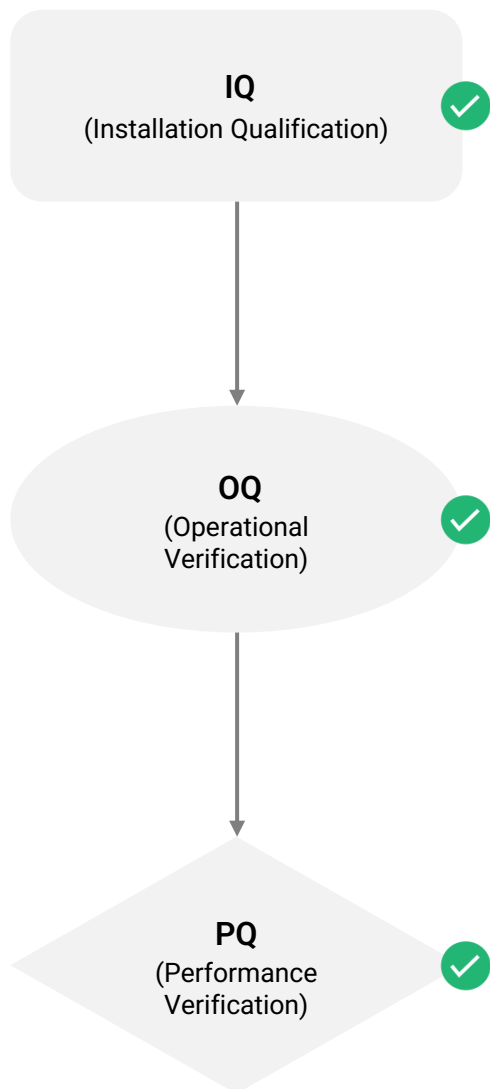
In short, accuracy means “how correct the result is,” precision means “how consistent the results are,” and uncertainty refers to “how wide the error margin is.” These three concepts form the basic language for describing data reliability and are central to understanding ISO 20391-1.



Example of Uncertainty: Measurement (1.0×10^6 cells/mL) with error bars ($\pm 0.05 \times 10^6$), showing the possible range of true values.

ISO 20391-1 Procedures (IQ/OQ/PQ)

ISO 20391-1 outlines three sequential procedures to ensure that a cell counting instrument operates properly in the research environment.



1. IQ (Installation Qualification)

– Installation Verification

◦ Confirms that the instrument has been set up correctly in the intended environment.

◦ **Example** : Checking power supply, temperature, software version, and installation space.

◦ **Why it matters** : If errors occur at the installation stage, those errors will accumulate and affect all subsequent results.

2. OQ (Operational Qualification)

– Operational Verification

◦ Confirms that the instrument operates correctly as intended.

◦ **Example** : Running built-in standard tests, checking image capture, and verifying analysis functions.

◦ **Why it matters** : Ensures basic functionality before experiments, preventing errors in advance.

3. PQ (Performance Qualification)

– Performance Verification

◦ Confirms that the instrument delivers the expected performance under actual experimental conditions.

◦ **Example** : Measuring various cell samples to verify that accuracy, precision, and uncertainty remain within defined ranges.

◦ **Why it matters** : Only when performance is guaranteed under real conditions can data be trusted for research or clinical application.

IQ/OQ/PQ process: stepwise quality assurance from installation to performance validation.

The IQ/OQ/PQ framework is a three-stage assurance process that moves from simply asking, "Does the instrument turn on?" to confirming, "Does it deliver reliable data fit for research purposes?"

The Importance of Reference Material

ISO 20391-1 recommends the use of Reference Materials to objectively validate cell counting results.

- Reference Materials are test samples with clearly defined cell concentration and viability, serving as benchmarks to determine whether an instrument is producing correct results.
- For example, if a specific Reference Material is defined as having an actual concentration of 1.0×10^6 cells/mL and a viability of 90%, researchers can evaluate instrument performance by checking how closely the measured results match these defined values.

However, there are clear practical limitations:

- **Cost burden** : Reference Materials are specially produced and often expensive, placing significant pressure on research budgets.
- **Supply constraints** : They are not easily accessible in every laboratory, and in some regions or institutions, procurement is particularly challenging.
- **Storage difficulties** : Reference Materials are sensitive to conditions such as temperature and container type, making long-term use impractical.

In summary, while the Reference Material–based validation method proposed by ISO is ideal in principle, there are significant practical challenges for researchers applying it in everyday laboratory work. To overcome these limitations, more practical alternatives such as Validation Slides have recently gained attention.

Strengths and Limitations of ISO 20391-1

Strengths

- Provides a unified international language for cell counting QC.
- Defines key concepts such as accuracy, precision, and uncertainty, making communication between researchers easier.
- Expands the possibility of data comparability, enabling results to be shared across different laboratories or institutions in a standardized way.

Limitations

- The guidelines are principle-based, leaving the actual design and execution of QC procedures to the discretion of researchers.
- Obtaining and managing Reference Materials can be practically challenging.
- Researchers bear the burden of directly carrying out repetitive validation procedures (e.g., experimental design, statistical analysis).

Conclusion

ISO 20391-1 is an international standard that serves as the starting point for quality control (QC) in cell counting.

By establishing the core concepts of Accuracy, Precision, and Uncertainty, and by outlining the IQ/OQ/PQ procedures, it enables researchers to manage their data in alignment with internationally recognized criteria.

However, due to the practical challenges of securing and implementing Reference Materials, researchers should treat ISO 20391-1 as a foundational principle and design QC strategies tailored to their specific laboratory environments. In this process, alternative approaches such as Validation Slides can play an important role, bridging ISO requirements with practical procedures that can be readily applied in real laboratory settings.

Ultimately, ISO 20391-1 is not merely a recommendation but a fundamental standard for ensuring the quality of cell counting data. And its value becomes even more powerful when combined with verification tools that researchers can routinely and practically employ.

Frequently Asked Questions (FAQ)

Q1. How does ISO 20391-1 help researchers

A. ISO 20391-1 clearly defines the key concepts of accuracy, precision, and uncertainty in cell counting, and provides a framework for stepwise equipment verification through IQ/OQ/PQ procedures. This allows researchers to manage their data according to internationally recognized standards of reliability.

Q2. Is the use of Reference Material mandatory?

A. While ISO recommends the use of Reference Material, there are significant challenges in terms of cost, supply, and management. Therefore, it is not mandatory. Researchers may use it when feasible, or consider alternatives such as Validation Slides.

Q3. Can ISO 20391-1 be applied immediately in the laboratory?

A. ISO 20391-1 presents a conceptual and principle-based framework. In other words, it defines what needs to be considered but does not provide a step-by-step QC protocol. Researchers must therefore use these principles as a reference and design QC protocols tailored to their own laboratory environments.

Q4. Why is ISO 20391-1 especially important now?

A. The use of automated cell counting devices has been rapidly increasing, and in GMP environments, cell counting data are directly linked to the manufacturing of cell therapies. Because data reliability is closely tied to patient safety, following internationally harmonized standards is becoming increasingly critical.