The 23rd Congress of the Japanese Society for Regenerative Medicine
Symposium 16 “Standards -A tool to connect academia and industry-

Cell counting and cell viability

Sysmex Corporation
Masakazu Kadowaki

Together for a better
healthcare journey

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The lead presenter has no COI to disclose regarding the presentation of this topic in the past year (Jan. to Dec.).

Introduction

Value chain of RM and Standards

Material cell

General requirements for Biobanking

ISO 20387:2018; General requirements for **biobanking**

Supply facility of Cellular starting materials

（under consideration）

Supporting Industry

Ancillary material J

ISO 20399:2022

Ancillary materials present during the production of cellular therapeutic products and gene therapy products

Packaging J

ISO 20404:2023

General requirements for the **design of packaging** to contain cells for therapeutic use

Gene Delivery Systems

Vocabulary

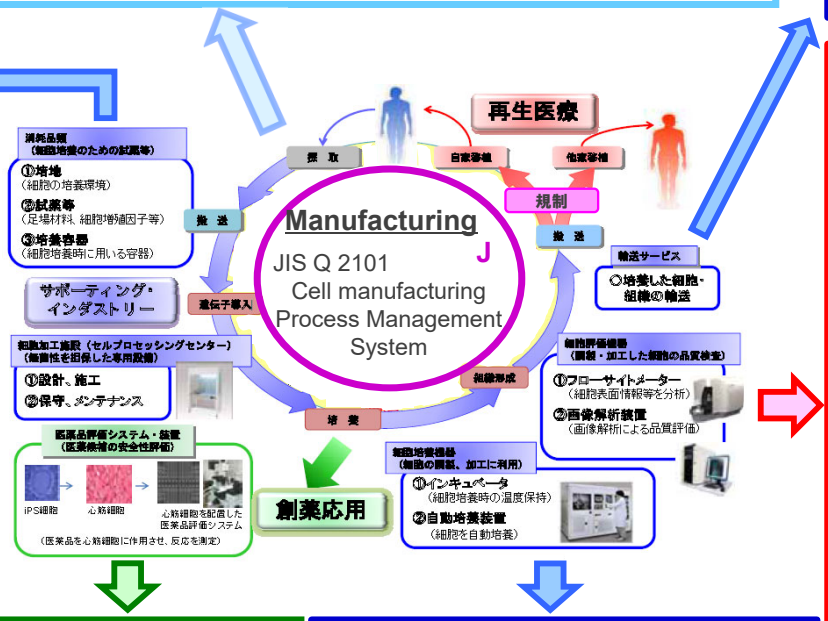
ISO/WD 16921-1：Gene Delivery Systems — Part 1: Vocabulary

Viral Vector

ISO/WD 16921-2：Gene Delivery Systems — Part 2: Guide for Methods for the Qualification of Viral Vectors

mRNA-Lipid Nanoparticles

ISO/PWI 16921-3：Gene Delivery Systems — Part 3: Guide for Methods for the Measurements of mRNA-Lipid Nanoparticles



Related field

Drug discovery J

Extracellular vesicle

(started development) J

Manufacturing equipment

Equipment system

ISO/TS 23565：2021

General requirements and considerations for **equipment systems** used in the manufacturing of cells for therapeutic use

Supporting Industry

transportation J

ISO 21973:2020：General requirements for **transportation** of cells for therapeutic use

Analytical method

testing and characterization J

ISO 23033:2021：General requirements and considerations for the **testing and characterization** of cellular therapeutic products

Cell counting - General guidance

ISO 20391-1:2018：**Cell counting** -- Part 1: **General guidance** on cell counting methods

Cell counting - Experimental design

ISO 20391-2:2019：**Experimental design and statistical analysis** to quantify counting method performance

Rapid microbial detection

ISO 24190:2023：Risk based approach for method selection and validation of methods for **rapid microbial detection** in bioprocesses

Cell viability

ISO/CD 8934：General considerations and requirements for **cell viability** analytical methods — Part 1: Mammalian cells

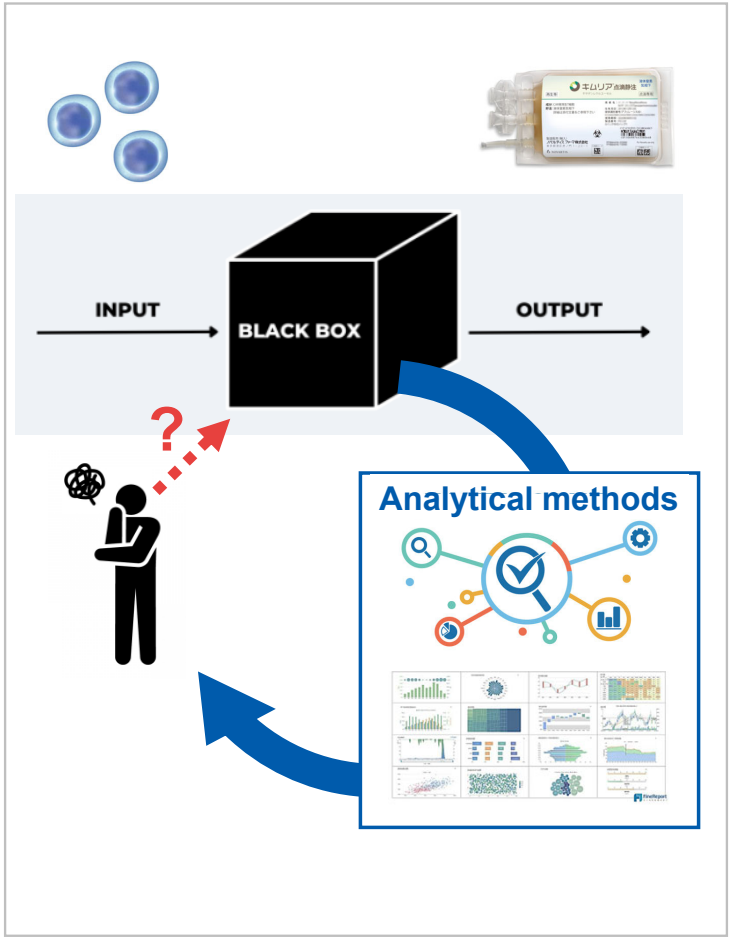
Cellular morphology J

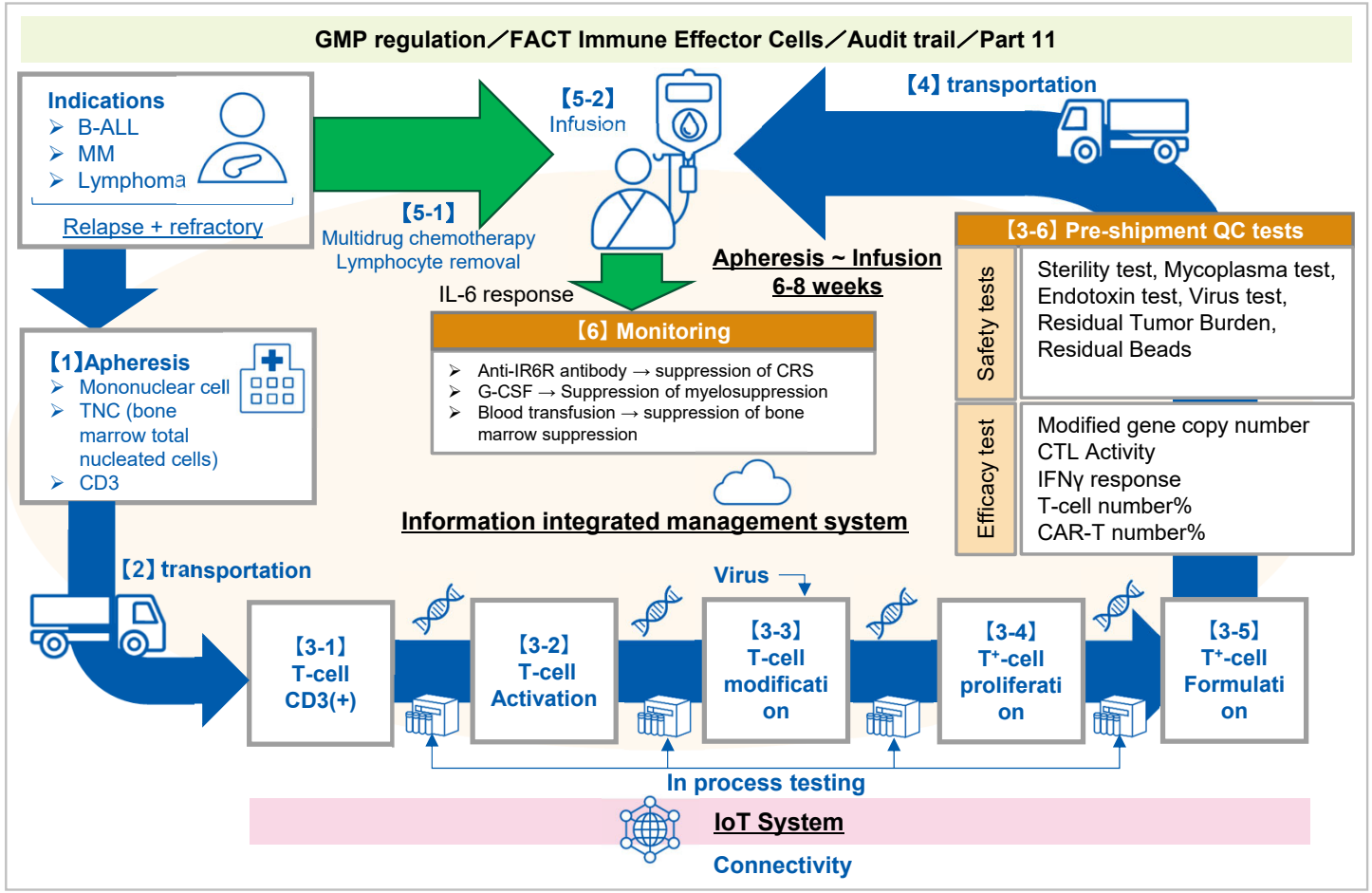
ISO/DIS 24479：Minimum requirements for cellular morphological analysis

Red：Published、**Blue**：To be published by FY2024, J：Developed by Japan as PL

Benefits of international standardization activities

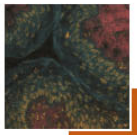
Analytical methods in Regenerative Medicine and Cell therapy





Direction of standardization

Suite of standards to address all sectors of emerging biotechnology



By molecules & entities

- Nucleic acids*
- Protein
- Cells*
- Virus/viral vectors*
- Others

By attribute

- Identity*
- Count*
- Purity*
- Viability*
- Activity*

By method

- qPCR/ddPCR*
- NGS*
- Genome Editing*
- Photometric*
- Microscopy*
- Flow cytometry
- ELISA

By application

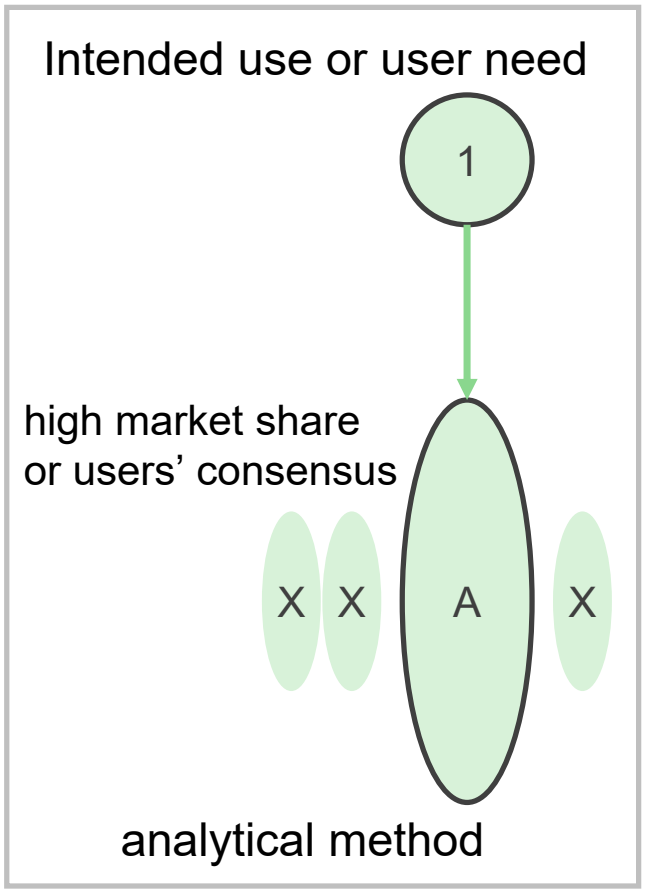
- Cellular therapeutics*
- Biotechnology
- Biodiversity
- Infectious disease*
- Synthetic/ engineering biology

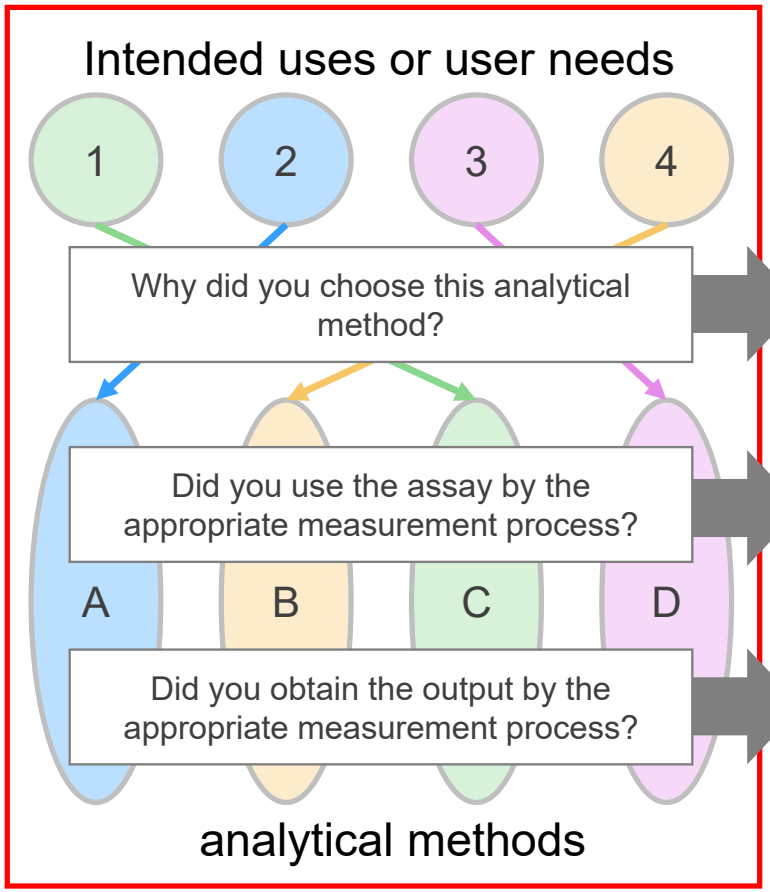
*Ongoing efforts or completed standards

Version Jun 16, 2023

Advantages of international standardization
(1) Users (Academia or Pharma)

The process of selecting an analytical method is carried out appropriately according to the Intended uses or user needs.





Clarifying how to select the analytical method

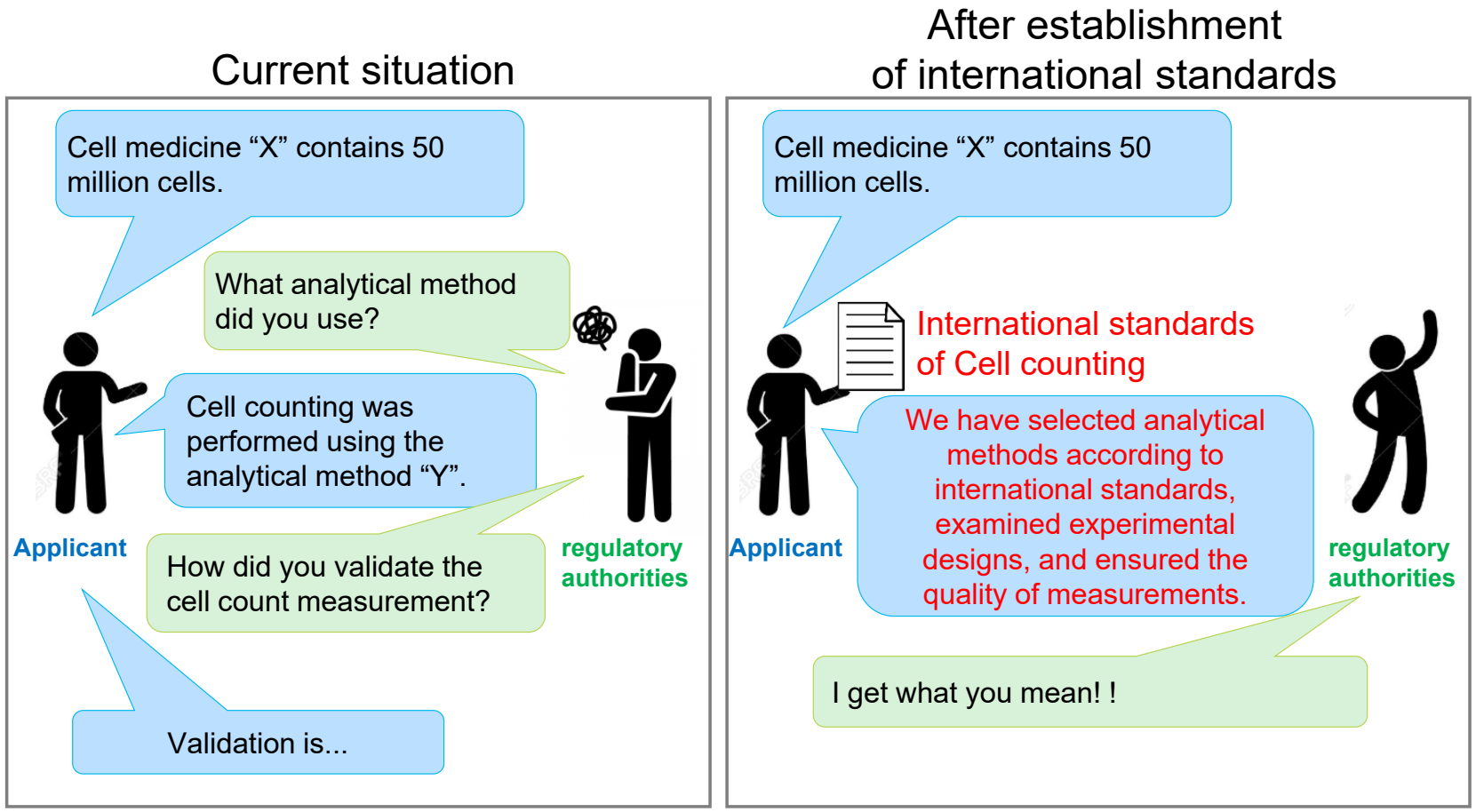
Setting the minimum and common required specifications

Setting the unified index

Advantages by international standardization

(2) Regulatory authorities and (3) Standardization organizations

When international standards are widely recognized, the process by regulatory authorities will proceed smoothly and quality will be guaranteed.

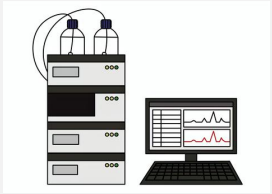


Advantages by international standardization
(4) Device/reagent manufacturers

By participating in international standardization activities, they can quickly detect trends in market needs and regulations, and utilize them in product planning.

Under international standards, it is not possible to use a specific company name or product name to promote sales, but...

e.g.) Automated cell counter

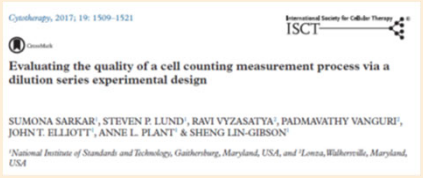


Software that achieves requirements

[Statistical method]

- ✓ Setting acceptance criteria
- ✓ mean-variance model
- ✓ Regression line
- ✓ formula for calculating residuals
- ✓ Smoothing with polynomials
- ✓ Scaling
- ✓ Non-parametric bootstraps

Submission of papers according to international standards

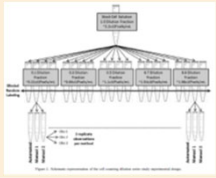

Cytotherapy, 2017; 19: 1509–1521

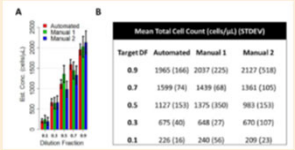
ISCT

Evaluating the quality of a cell counting measurement process via a dilution series experimental design

SUMONA SARKAR[1], STEVEN P. LUND[2], RAVI VYZASATYA[2], PADMAVATHY VANGURI[2], JOHN T. ELLIOTT[1], ANNE L. PLANT[1] & SHENG LIN-GIBSON[1]

[1]National Institute of Standards and Technology, Gaithersburg, Maryland, USA, and [2]Lonza, Walkersville, Maryland, USA



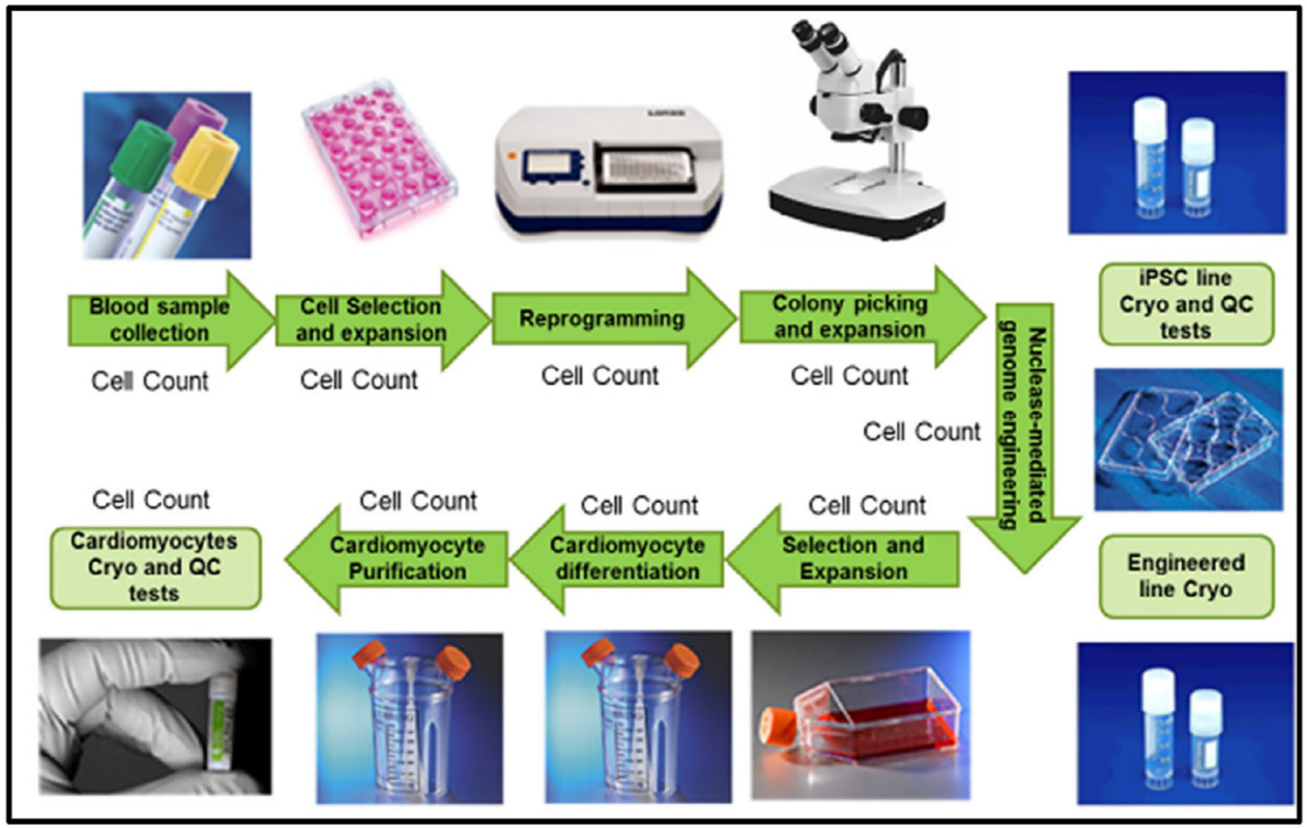


Example 1:

International standards for cell counting

Overview of cell counting

Cell counting is one of the important analytical methods for quality control of cell medicines and is widely used regardless of treatment method or cell type.



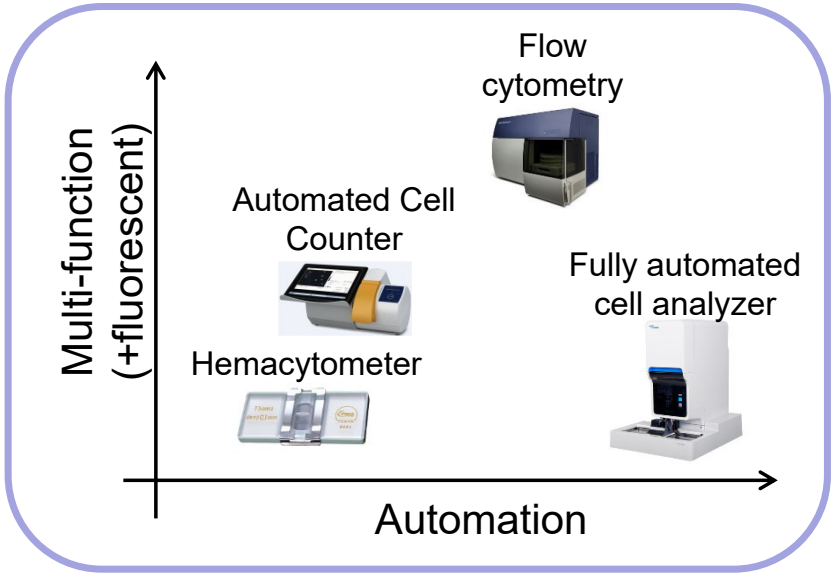
Summary of the National Institute of Standards and Technology and US Food And Drug Administration cell counting workshop: Sharing practices in cell counting measurements (Cytotherapy; Volume 20, Issue 6, June 2018, Pages 785-795)

Market needs of cell counting

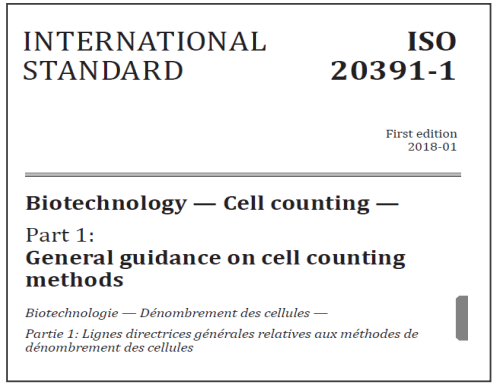
No reference materials or standard measurement methods have been established, and different analytical methods are used in each situation.

① How can we prove the performance and suitability of the selected analytical method?

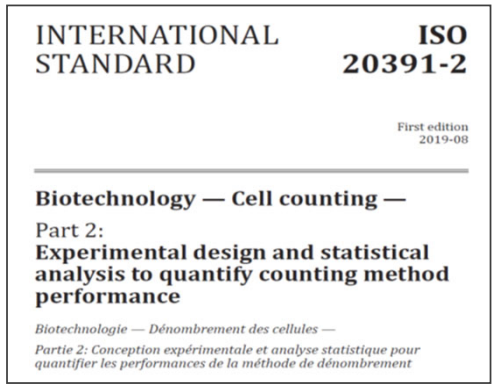
② How can we ensure the quality level of the selected analytical method?



Cell counting Part1:
General guidance on cell counting


INTERNATIONAL STANDARD
ISO 20391-1
First edition 2018-01
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

Cell counting Part2:
Experimental design and statistical analysis to quantify cell counting method performance


INTERNATIONAL STANDARD
ISO 20391-2
First edition 2019-08
Biotechnology — Cell counting —
Part 2:
Experimental design and statistical analysis to quantify counting method performance
Biotechnologie — Dénombrement des cellules —
Partie 2: Conception expérimentale et analyse statistique pour quantifier les performances de la méthode de dénombrement

Cell counting Part1:
General guidance on cell counting

Four categories are defined in cell counting

Direct:
the recording of a signal or a set of signals from each cell

Indirect:
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)

Total:
the measurement of all cells, independent of the attribute(s) of the cell

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

Direct / Total	Indirect / Total
Direct / Differential	Indirect / Differential

Cell counting Part1:
General guidance on cell counting

Analytical methods require qualification and method validation / verification

1) Instrument qualification

- The measurement instrument shall be qualified per predefined specifications. Qualification protocols shall be documented prior to conducting instrument qualification and results shall be documented.

2) Method validation and verification

- The cell counting measurement method shall be validated. Method performance parameters should be provided to give evidence that the method produces results that are suitable for the intended purpose.

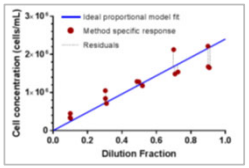
3) Reference materials

- Reference materials should be used to ensure measurement traceability, enable comparison, and/or verify a measurement process.

1) DQ, IQ, OQ, and PQ

2) Validation of analytical procedures

Specificity
Sensitivity
Range
Linearity or proportionality
Repeatability
Accuracy
Available proficiency tests

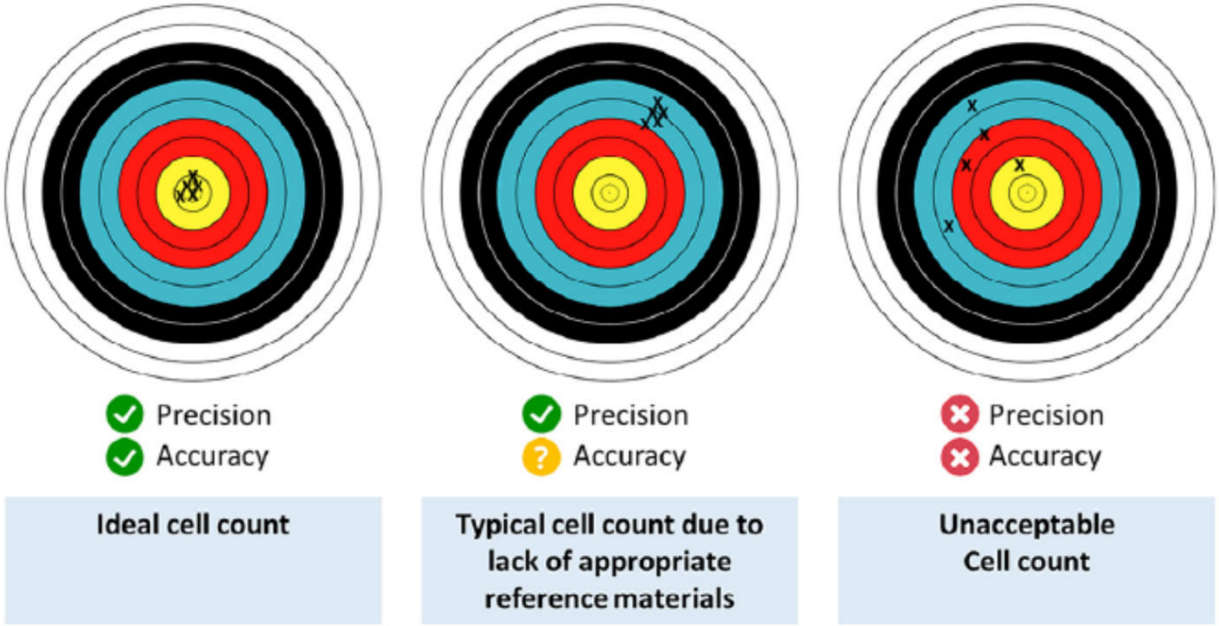


Cell counting Part2: Experimental design and statistical analysis to quantify cell counting method performance

Assuring the quality of measurement based on evaluating repeatability of the measurement and proportional response to dilution.

Scope of this international standard

- This method is especially applicable in cases where an appropriate reference material to assess accuracy is not readily available.
- This method does not directly provide the accuracy of the cell count.



Lin-Gibson S, Sarkar S, Elliott JT.
Cytotherapy. 2018 Jun;20(6):785-795. doi: 10.1016/j.jcyt.2018.03.031. Epub 2018 Apr 24.

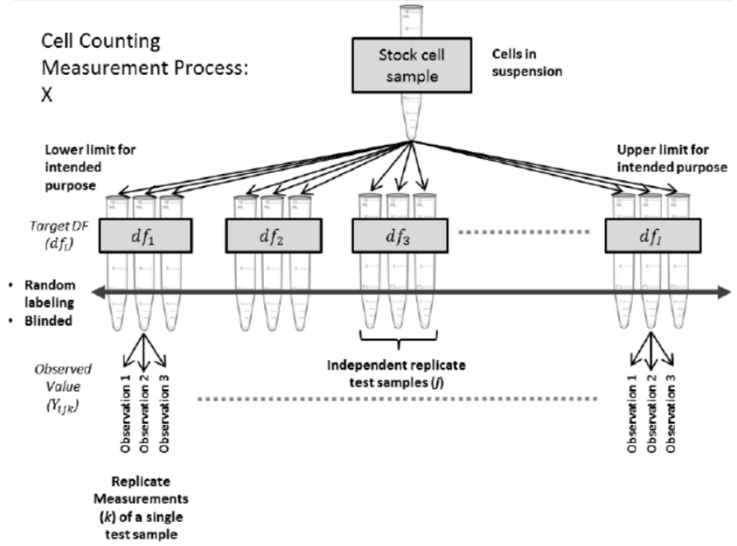
Cell counting Part2: Experimental design and statistical analysis to quantify cell counting method performance

Assuring the quality of measurement based on evaluating repeatability of the measurement and proportional response to dilution.

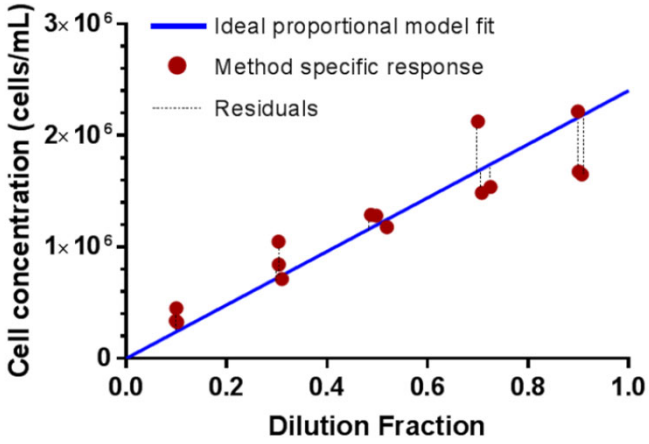
Setting quality indicators, that can be used for general purposes

- A variety of analysis methods and cell types are used.
- A serial dilution of the cell suspension is prepared, measured with a specific method and equipment, and evaluated repeatability of the measurement and proportional response to dilution.

Dilution fraction experimental design



Repeatability and deviation from proportionality

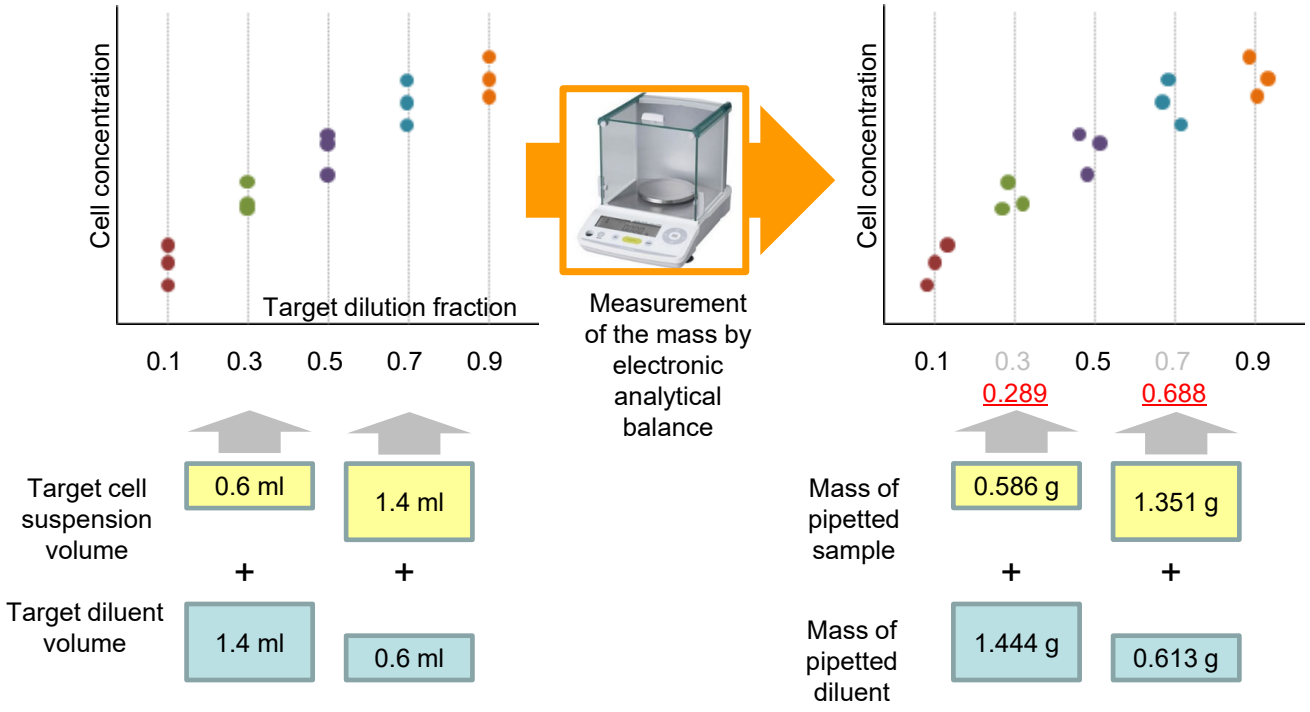


Cell counting Part2: Experimental design and statistical analysis to quantify cell counting method performance

Evaluate processes including pipetting errors and report statistical analysis results

Assessing pipetting error contributions to dilution integrity

- Contributions of pipetting error to dilution integrity shall be addressed.
- Statistical processing considering the difference between the actual measurement value and the theoretical value.

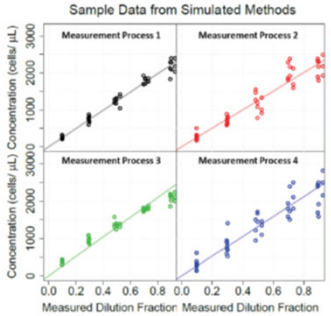


Cell counting Part2: Experimental design and statistical analysis to quantify cell counting method performance

Use for process optimization, confirmation of Between-day reproducibility, evaluation and selection of multiple analytical methods, etc.

Use case 1

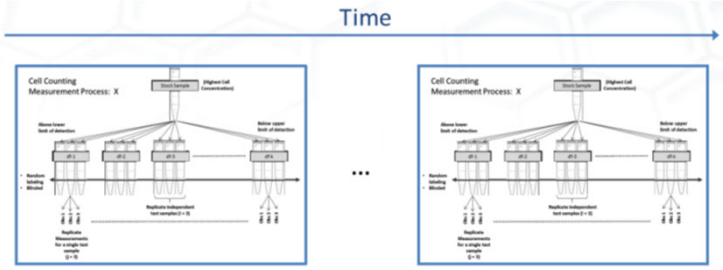
Optimization of cell counting process, etc.



	Process 1	Process 2	Process 3	Process 4
CV	0.0992	0.1712	0.0728	0.229
R^2 (Proportional Model Fit)	0.999	0.985	0.978	0.988
Smoothed-Residual	0.291 (CI)	1.064 (CI)	2.676 (CI)	1.002 (CI)

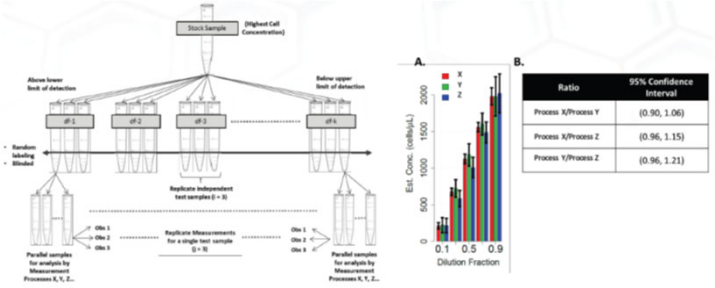
Use case 2

Evaluation index for quality control of cell counting (Between-day reproducibility, etc.)



Use case 3

Compare two or more analytical methods or select the best method



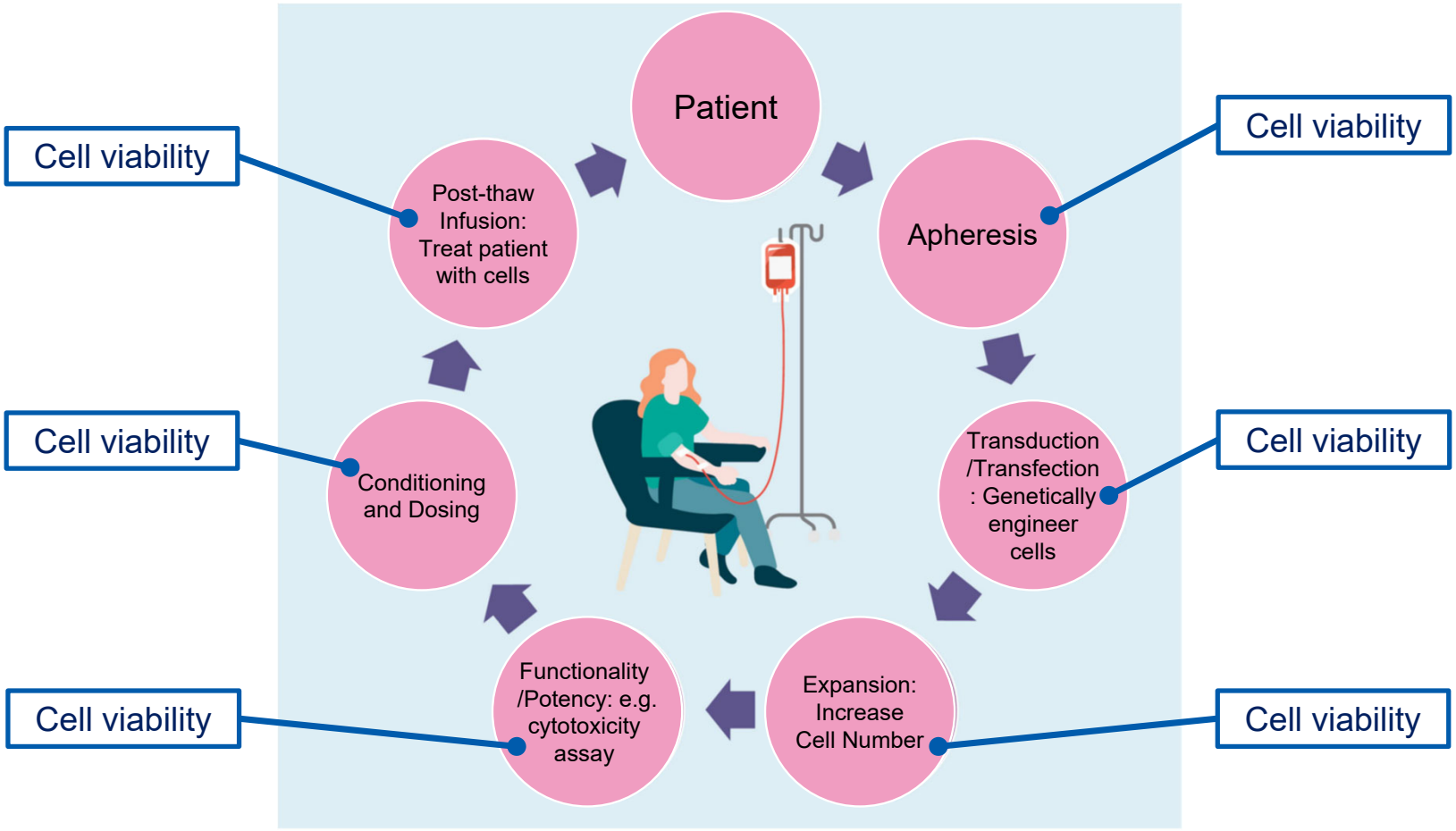
Ratio	95% Confidence Interval
Process X/Process Y	(0.90, 1.06)
Process X/Process Z	(0.96, 1.15)
Process Y/Process Z	(0.96, 1.21)

Example 2:

International standard for cell viability

Overview of cell viability

Cell viability is one of the important analytical methods for quality control of cell medicines, and is performed to check manufacturing processes and characterize products.



Market needs of cell viability

No reference materials or standard measurement methods have been established, and different analytical methods are used in each situation.

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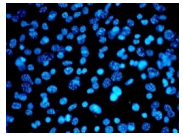
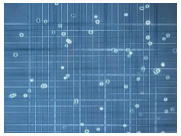
② How can we ensure the quality level of the selected analytical method?

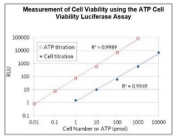
DAPI



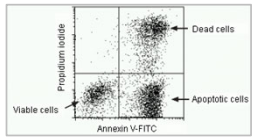
Trypan Blue

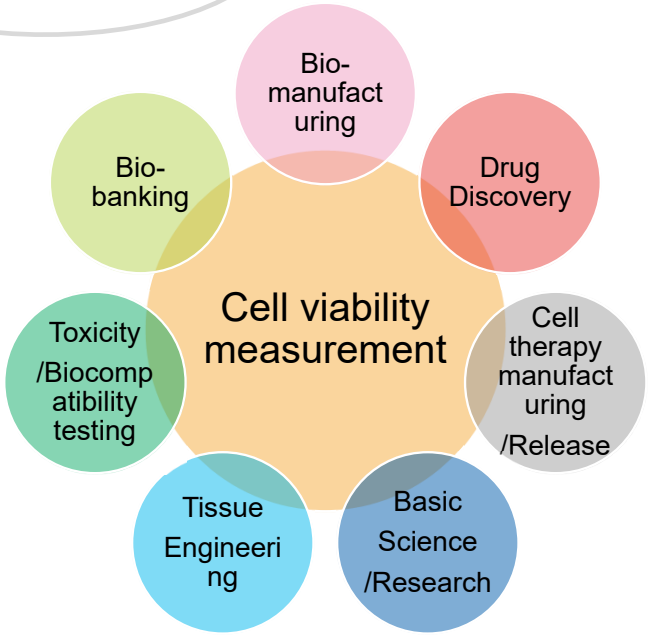


ATP

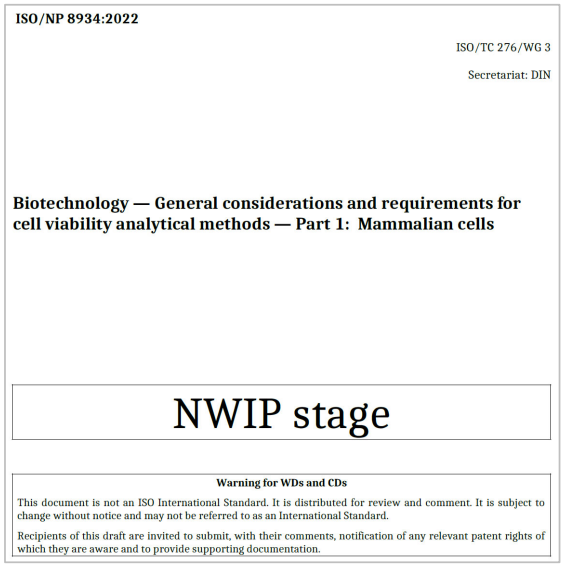


Annexin V





【General considerations and requirements for cell viability analytical methods】



ISO/NP 8934:2022

ISO/TC 276/WG 3

Secretariat: DIN

Biotechnology — General considerations and requirements for cell viability analytical methods — Part 1: Mammalian cells

NWIP stage

Warning for WDs and CDs

This document is not an ISO International Standard. It is distributed for review and comment. It is subject to change without notice and may not be referred to as an International Standard.

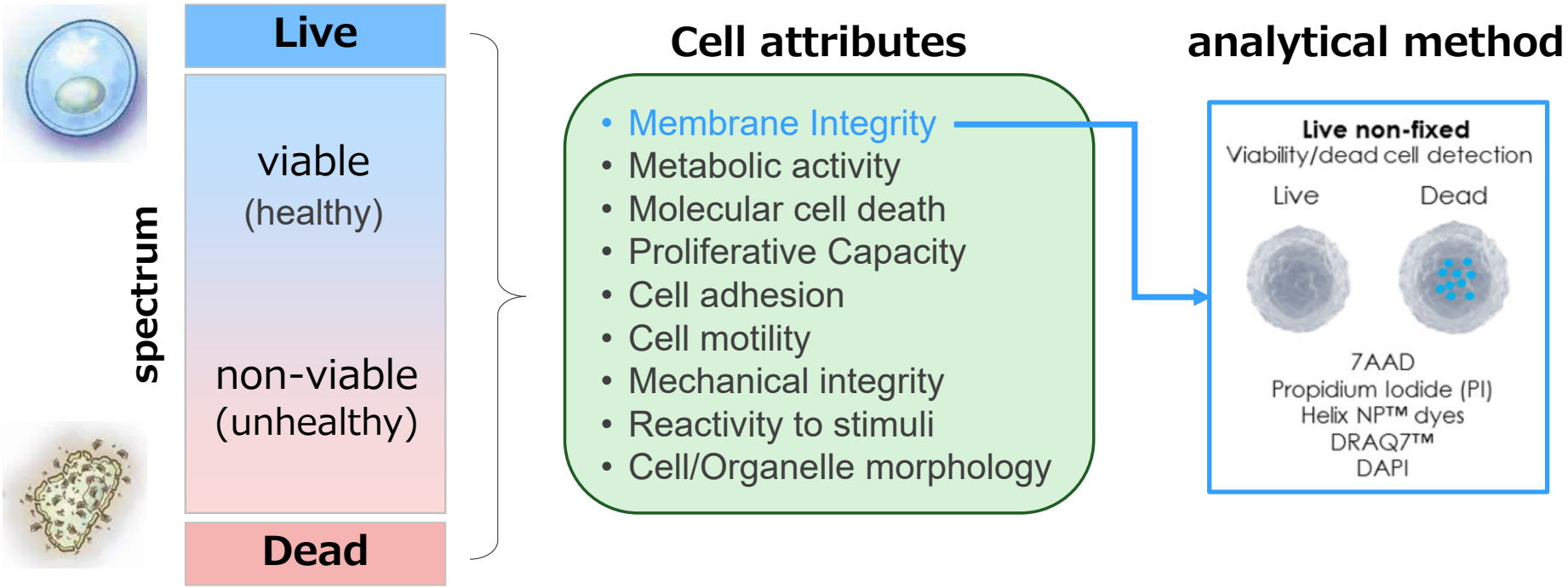
Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

General considerations and requirements for cell viability analytical methods

Cell viability is defined by the broader concept of “Viable state”

Definition of “viable state”

- The viable state can be described by a spectrum of attributes related to vital cellular function and cell death.



General considerations and requirements for cell viability analytical methods

Select the appropriate method from the cell viability analytical methods

The cell viability analytical methods for measuring different biological properties

- The definition of viable and non-viable cells can be based on physical, biochemical, and biomolecular properties related to the viable state of the cells.
- A fit-for-purpose approach should be followed when selecting and designing cell viability analytical methods.

FIGURE 2

Examples of viability measurements and the quality attribute being measured.

Membrane Integrity	Metabolism	Molecular Markers	Ability to Proliferate
• Trypan Blue • AO/DAPI • AO/PI • Calcein-AM • Impedance • Lactate dehydrogenase	• Calcein-AM • MTT • Alamar blue • ATP • Glucose consumption	• Annexin V • Caspases • TUNEL	• Count over time • Colony forming unit (CFU) assays • BrdU/EdU • CM-FDA

AM: Acetoxymethyl; AO: Acridine orange; ATP: Adenosine triphosphate; BrdU: Bromodeoxyuridine; CM-FDA: 5-chloromethylfluorescein diacetate; DAPI = 4′,6-diamidino-2-phenylindole; EdU: 5-ethynyl-2′-deoxyuridine; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, PI: Propidium iodide; TUNEL: Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling.

Select the appropriate analytical method by fit-for-purpose approach

1. Select viable cell state attribute
2. Consider the properties of the cell sample
3. Consider the measurement process.

General considerations and requirements for cell viability analytical methods

Consider and clarify the sources of measurement variability for specific cell viability analytical methods.

Managing sources of variability in a cell viability measurement

- Sources of variability in cell viability measurements should be evaluated and documented.
- The importance of individual factors varies depending on the type of analytical method deployed. A design of experiments (DOE) approach can be applied to identify sensitivity of the viability analytical method to different sources of variability.

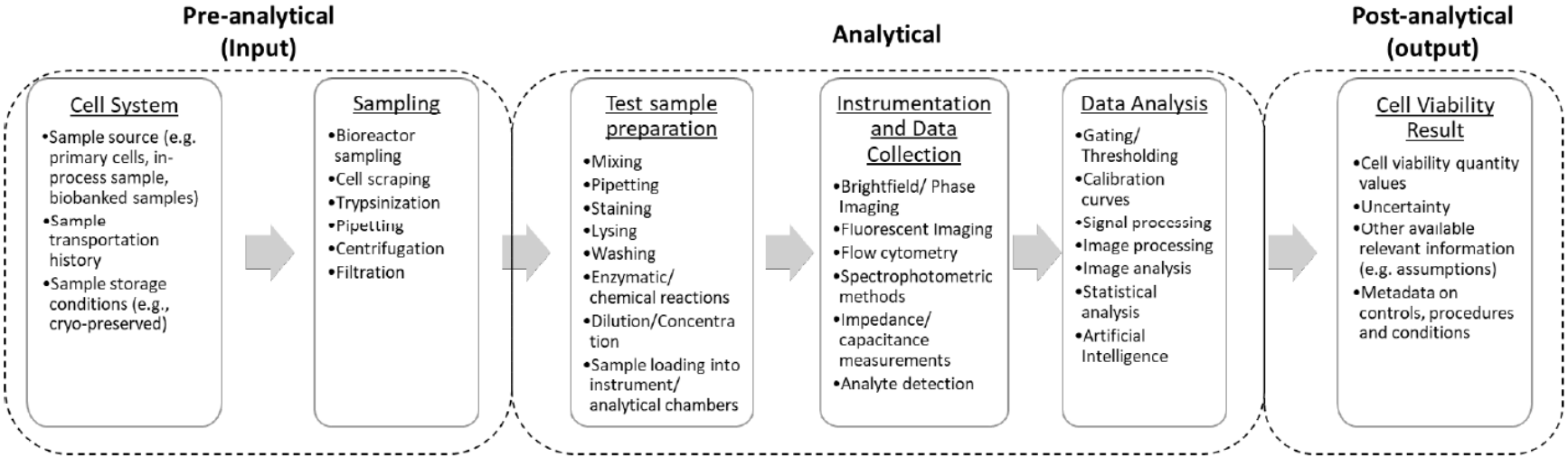


Figure 3 — A general cell viability measurement process with examples of processes and considerations that can affect the results of cell viability analytical methods.

Conclusion

Benefits of international standardization activities

- The process of selecting an analytical method is carried out appropriately according to the Intended uses or user needs.
- The process by regulatory authorities will proceed smoothly and quality will be guaranteed.
- The manufacturers can quickly detect trends in market needs and regulations and utilize them in product planning.

Purpose of the international standards for cell counting

- This method is especially applicable in cases where an appropriate reference material to assess accuracy is not readily available.
- A serial dilution of the cell suspension is prepared, measured with a specific method and equipment, and evaluated repeatability of the measurement and proportional response to dilution.

Purpose of the international standard for cell viability

- The cell viability analytical method should be selected based on the biological property.
- Identify points that cause variation in measurement results, implement them appropriately, and manage data.

Acknowledgement

■ Cell counting

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■ Cell viability

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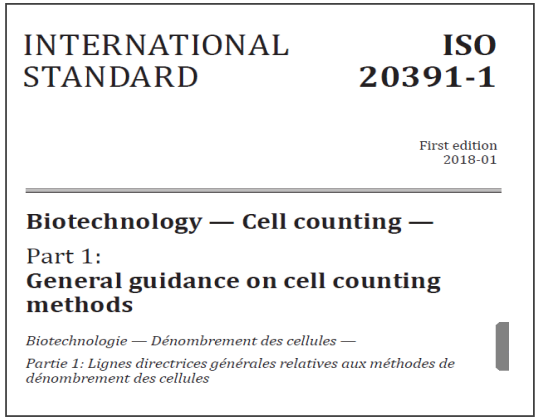

INTERNATIONAL STANDARD

ISO 20391-1

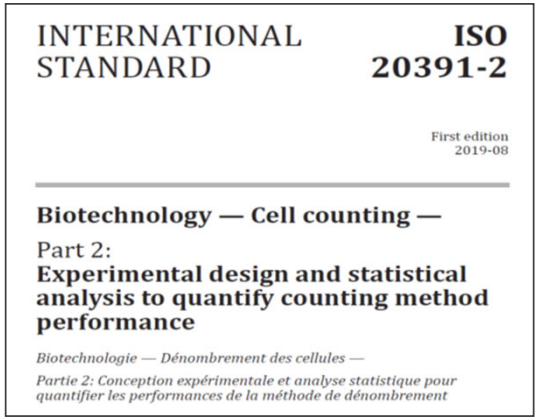
First edition 2018-01

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


INTERNATIONAL STANDARD

ISO 20391-2

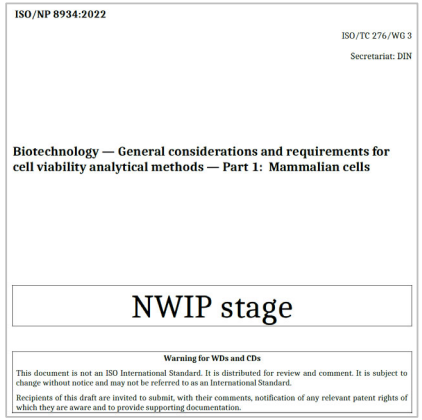
First edition 2019-08

Biotechnology — Cell counting —

Part 2:
Experimental design and statistical analysis to quantify counting method performance

Biotechnologie — Dénombrement des cellules —

Partie 2: Conception expérimentale et analyse statistique pour quantifier les performances de la méthode de dénombrement


ISO/NP 8934:2022

ISO/TC 276/WG 3

Secretariat: DIN

Biotechnology — General considerations and requirements for cell viability analytical methods — Part 1: Mammalian cells

NWIP stage

Warning for WDs and CDs

This document is not an ISO International Standard. It is distributed for review and comment. It is subject to change without notice and may not be referred to as an International Standard.

Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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healthcare journey

Experimental design and statistical analysis to quantify cell counting method performance

Document experimental design parameters and statistical analysis method

Reporting

- The report shall contain information on the experimental design and statistical analysis.

7.1 Reporting of quality indicators

(Shall; requirement)

Mean cell count
Measurement Precision (%CV)
Coefficient of determination (R^2)
Proportionality index (PI)

7.2 Documentation of experimental design parameters and statistical analysis method

(Shall; requirement)

a) Description of the cell counting measurement system(s) investigated
b) Dilution fraction experimental design elements
c) Method for evaluating pipetting error contributions to dilution integrity
d) The assumed mean-variance relationship used in the proportional model fit
e) Formula for the proportional model fit
f) Method for calculating PI

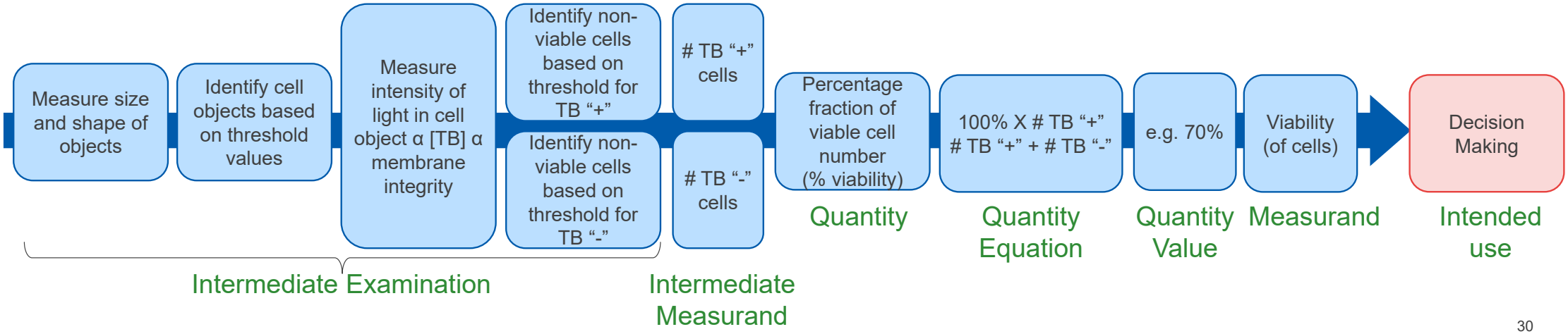
General considerations and requirements for cell viability analytical methods

Document the cell viability quantity
and the information related to the measurement method

Reporting and documentation

A) the cell viability quantity with appropriate units
B) the attribute, related to viable state, used for evaluating cell viability;
C) the measurement method, where the measurement method shall indicate the biological property used in the analytical method.
D) any relevant intermediate measurands that were used in the calculation of the cell viability quantity value

Measurand chart: Semi-automated image-based Trypan Blue Dye Exclusion Cell Viability



Q&A

アカデミアと産業をつなぐツールとしての意義
臨床応用への橋渡しであることを強調

分析法はブラックボックスの細胞製造において、データを提供するための極めて重要なツール
そのツールの信頼性が担保されないと大きな問題となる

【パネルディスカッション】
標準を開発してどう活かしていくのか？
標準を実装するための施策は？
⇒　まずは発刊された国際標準に全てに従う必要はない　日本として利用価値のある標準を採用していく
　FIRM認証の仕組みを活用する
　そもそもは、分析法を提供しているメーカー、規制当局、標準推進機構が連携して、より良い標準を作っていく
　標準の作成・改訂作業に積極的に参加していく
　ある程度、お墨付きという要素を踏まえて、国際標準の価値を説明していく　それが普及につながる
　目的に応じて、より良い分析法・商品が選択されて、医薬品開発や薬事承認がスムーズに進む　臨床まで早く届く