Towards more accurate, reproducible, and robust measurement and analysis of biological samples: International Standardization Efforts within ISO/TC 276 *Biotechnology*

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Chair of US Mirror Committee to ISO/TC 276

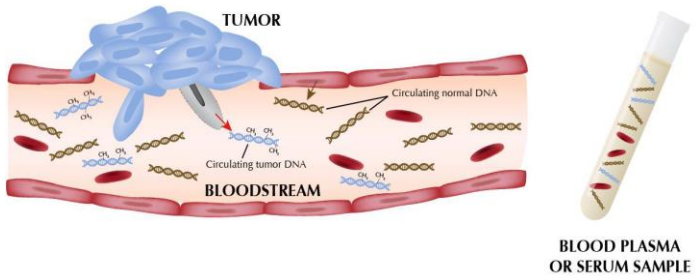
September 11, 2018

Key take-away messages

- ISO/TC 276 is developing a suite of analytical measurement standards assessing the quality of biological samples
- ISO/TC276 and partners are working to better articulate "fit for purpose" and clarify actions needed for a specific purpose
- Expert input is needed to ensure that standards are balanced and address a need without becoming over burdensome

Example of a NIST laboratory program

Focus on Liquid Biopsy (LB)



I. Measurements of Tumor DNA in LB: reference materials / interlaboratory evaluation of measurement quality

II. Measurements of Methylated DNA in LB

III. Measurements of Gene Copy Number Variants in LB

IV. Future work with exosomes

https://www.nist.gov/mml/bbd

Working to solve the following challenges

- Low concentration of mutations in high background of wild-type DNA
- Degraded in molecular weight
- Many mutations, which ones and what kinds to focus on
- Measuring assay performance: limits of detection, specificity, ranges…
- Process controls (pre-analytical process steps)
- Calibrators (standards) that work with different methods and instruments (commutability)

Scope of ISO/TC 276 *Biotechnology*

Standardization in the field of biotechnology processes that includes the following topics:

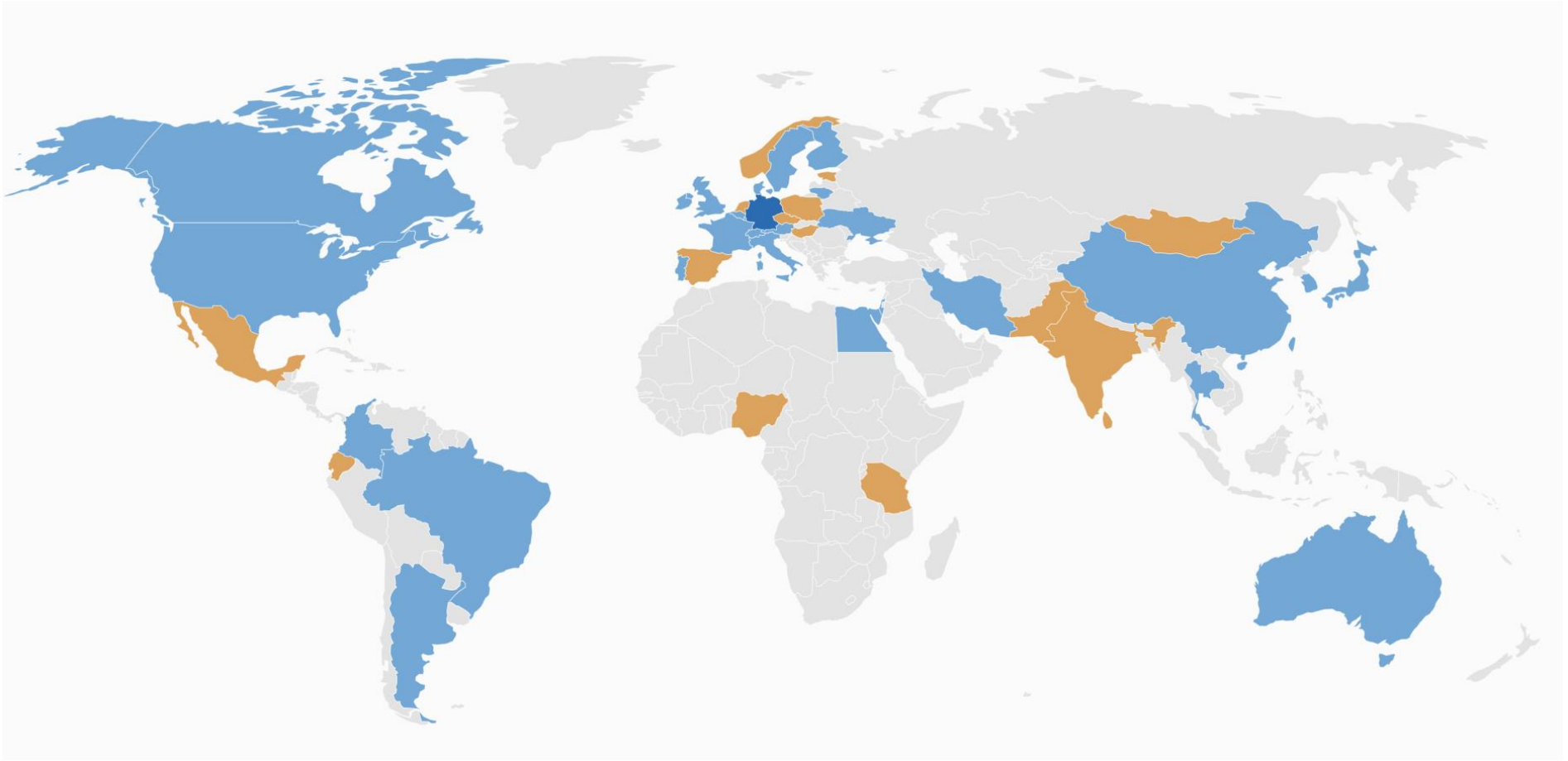
- Terms and definitions;
- biobanks and bioresources;
- analytical methods;
- bioprocessing;
- data processing including annotation, analysis, validation, comparability and integration;
- metrology.

ISO/TC 276 Biotechnology will work closely with related committees in order to identify standardization needs and gaps, and collaborate with other organisations to avoid duplications and overlapping standardization activities.

Structure | Liaisons | Meetings

Reference	Title
ISO/TC 276/WG 1	Terminology
ISO/TC 276/WG 2	Biobanks and bioresources
ISO/TC 276/WG 3	Analytical methods
ISO/TC 276/WG 4	Bioprocessing
ISO/TC 276/WG 5	Data processing and integration

Participants of ISO/TC 276



Participating Members (29) ■ Observing Members (15) ■ Various Liaisons, including BBMRI-ERIC

New Biobanking Standard published by ISO/TC276/WG2

ISO 20387:2018 *Biotechnology -- Biobanking -- General requirements for biobanking*

This document specifies general requirements for the competence, impartiality and consistent operation of biobanks including quality control requirements to ensure biological material and data collections of appropriate quality.

- Competence + quality management principles tailored to biobanks
- Menu of processes covering general and specific requirements

Biobanking Standards and Best Practices Development

Quality & quality attributes of biological material

Functions

Collect & Process
Store & Maintain
Distribute & Deploy

Perform functions
Characterization
Control & Track

Activities

Standards

Management standard
General requirements
Technical standards

Scope of ISO TC/276 WG3 *Analytical Methods*

- The Analytical Methods Working Group aims to develop standards for accurate, reproducible and robust measurement and analysis in support of biotechnologies.
- WG 3 will develop a package of International Standards for biologically relevant molecules and entities, including nucleic acids, proteins, and cells.
- This WG will develop horizontal standards and, when applicable, vertical / particular standards for industry sectors.

Current Work Programme of WG3

	Analytical Methods for Cells
ISO/CD 20391-1	*Biotechnology - Cell Counting – Part 1. General guidance on cell counting methods*
ISO/CD 20391-2	*Biotechnology - Cell Counting – Part 2. Experimental Design and Statistical Analysis to Quantify Counting Method Performance*
ISO/WD 23033	*Cell Characterization – General guide for characterization of human cells for therapeutic applications*
ISO/PWI 23511	*Biotechnology – General guidance on detection methods of cell cross-contamination*

Current Programme of WG3 (cont.)

	Analytical Methods for Nucleic Acids
ISO/CD 20688-1	Biotechnology - Nucleic acid synthesis – Part 1: General definitions and requirements for the quality control of synthesized oligonucleotides
ISO/PWI 20688-2	Biotechnology – Nucleic acid synthesis – Part 2: General definitions and requirements for the production and quality control of synthesized gene fragments, genes and genomes
ISO/CD 20395-1	Biotechnology - Guidelines for evaluating the performance of quantification methods for nucleic acid target sequences - Part 1: qPCR and dPCR
ISO/PWI 20397-1	Biotechnology - General requirements for massive parallel sequencing - Part 1: Upstream processes
ISO/WD 20397-2	Biotechnology - General requirements for massive parallel sequencing - Part 2: Methods to evaluate the quality of sequencing data

Do I need to / should I adopt standards from ISO Analytical Methods?

It depends → Fit for purpose

- Ex: Does sample meet the required qualities for national/multinational study?
- Ex: Has my sample changed / degraded over time?
- Ex: Does my current measurement provide sufficient information, and can I practically adopt a new method?

Fitness for Purpose (FFP)

- Defined as "in line with prearranged requirements for an intended use" (ISO 20387)
- Fundamental to effective, efficient biobank operations
- Applies to biological material, its associated data, or the biobank itself

A bit more on Fitness for Purpose (FFP) for biological material

- FFP Parameters will be context-specific:
  - Analytical methodologies / approaches /studies to be employed by end users
- Quality is only one aspect of FFP:
  - Evaluation based on intended purpose(s) or methodology
  - "Sufficient quality" for one methodology or set of specimens / data may not be sufficient for another use
  - Other FFP considerations could include consent details and appropriate access policies for specific needs

Ongoing ISO/TC 276/WG3 *Analytical Methods* Standards

NOTE: only scope, outline, and materials already in the public domain shown. BBMRI members should contact Liaison for full documents under development

Standards for cell counting

Cell count is a fundamental measurement in biosciences and biotechnology.

Cell number can effect:

- Bioassays that are normalized by activity per cell
- Manufacturing process for cells and biopharmaceutical products
- Potency and efficacy of a cell therapy treatment (dosing)

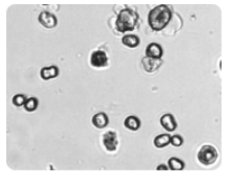
Cell count is often the first measurement to check the "quality" of cells received from a biobank

Challenges for Cell Counting, a Fundamental Measurement

Wide Range of Cell Properties

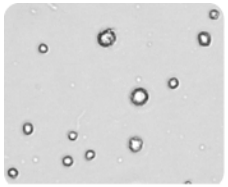
Cell Suspensions with Debris (PBMCs)

Irregularly Shaped Cells (Primary Hepatocytes)

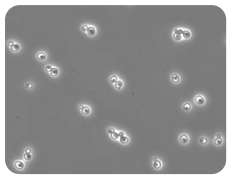


Clumpy Cells (MCF-7 breast cancer cell line)

Cells of varying size (MSCs)



Budding Cells (Yeast Cells)

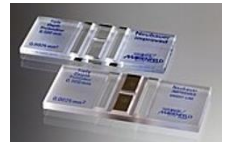


How can we provide confidence in cell counting methods in the absence of

- Reference Method
- Physical Standard
- Ground Truth

How can we compare results from different methods and platforms?

Wide Range of Counting Devices



Manual Light Microscopy

Automated Light Microscopy

Automated Fluorescent Microscopy

Coulter (impedance) Counters

Flow Cytometers

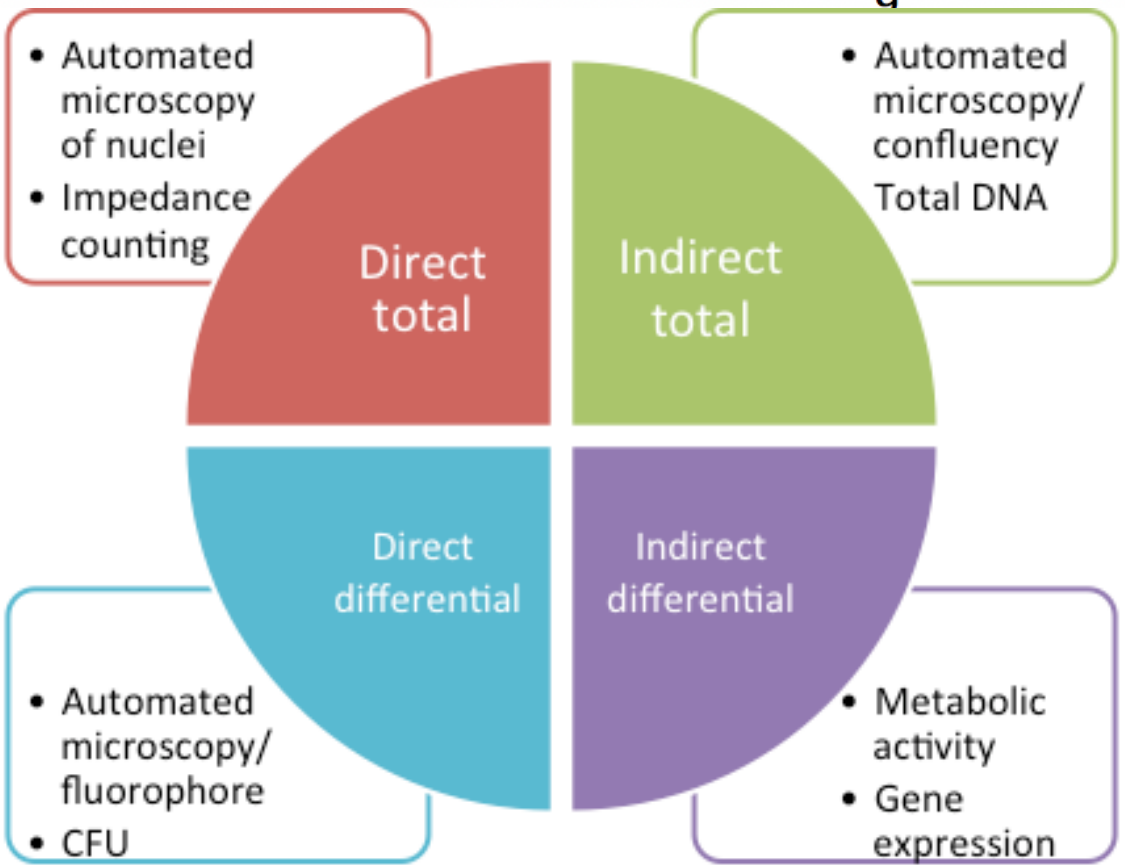
New/Custom Counters

ISO 20391-1: 2018 *Biotechnology - Cell Counting – Part 1. General guidance on cell counting methods*

Scope

- This document defines terms related to cell counting for biotechnology. It describes counting of cells in suspension (generally cell concentration) and cells adhered to a substrate (generally area density of cells). It provides key considerations for general counting methods (including total and differential counting, and direct and indirect counting) as well as for method selection, 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.
- This document is not intended for counting of cells while in a tissue section or a biomaterial matrix.
- Several sector/application-specific international and national standards for cell counting currently exist. When applicable, the user can consult existing standards when operating within their scope (specific measurement techniques and/or applications).

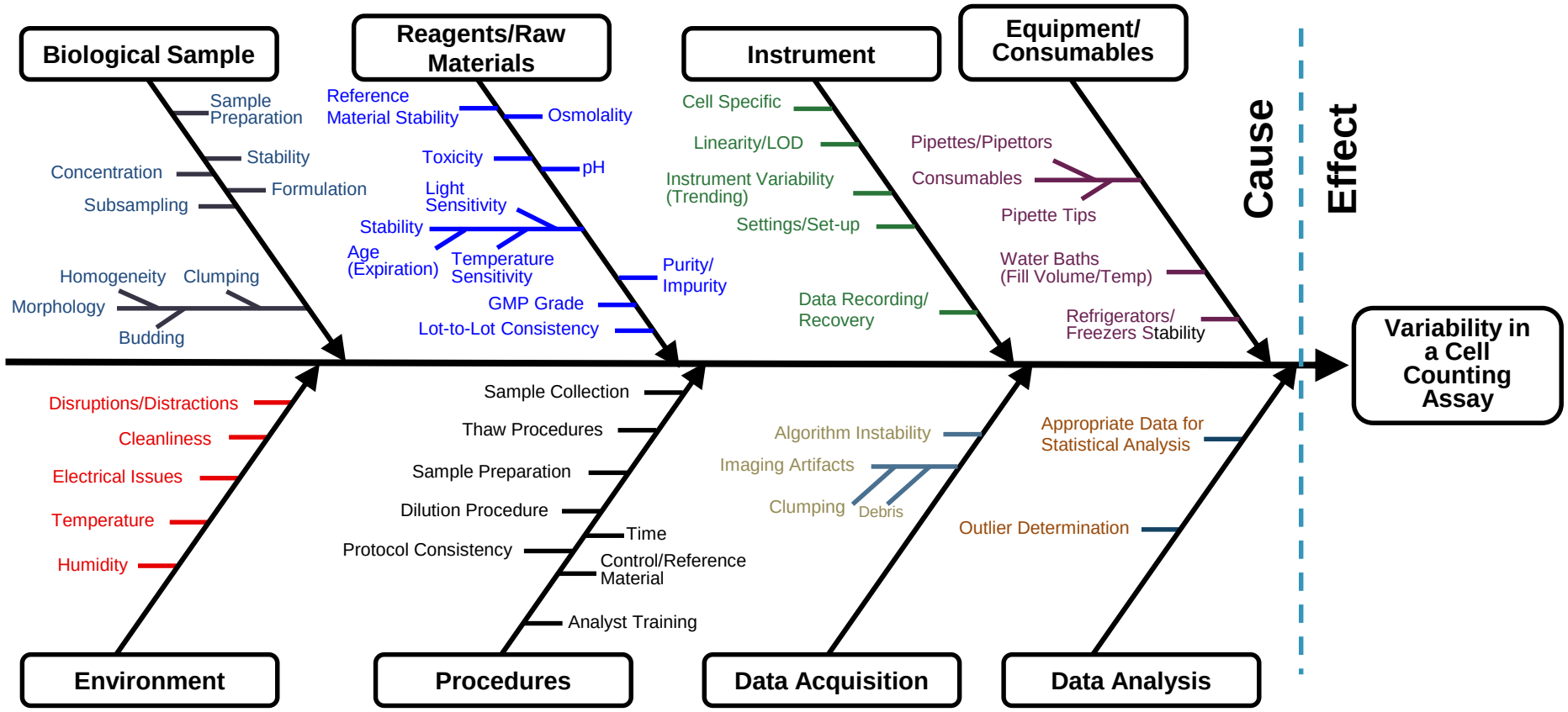
ISO 20391-1: 2018 *Biotechnology - Cell Counting – Part 1. General guidance on cell counting methods*



ISO 20391-1: 2018 *Biotechnology - Cell Counting – Part 1. General guidance on cell counting methods* (cont.)

ISO 20391-1: 2018 *Biotechnology - Cell Counting – Part 1. General guidance on cell counting methods* (cont.)

Sources of variability in a cell counting measurement



Developed based on NIST Measurement Assurance Workshop, May 2015

Simon, C. G., Lin-Gibson, S., Elliott, J. T., Sarkar, S., & Plant, A. L. (2016). Strategies for Achieving Measurement Assurance for Cell Therapy Products.*Stem Cells Translational Medicine*, *5*(6), 705-708.

Still, there is a need for a practical method to assess the quality of cell counting measurements

How can we provide confidence in cell counting methods in the absence of reference method, physical standard, or ground truth

How can we compare results from different methods and platforms?

MEASUREMENT PROCESS FOR CELL COUNTING

INPUT

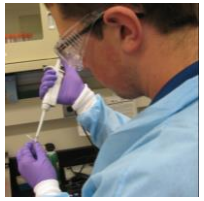
- **Cell Sample**
- **Range of Properties**

Formulation

Composition

Biological Variability

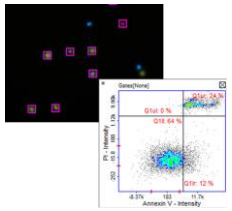
Cell Counting Measurement Process



Sample preparation

Cell Counting Instrument

Data collection



Data analysis

Decision making/ user defined specification

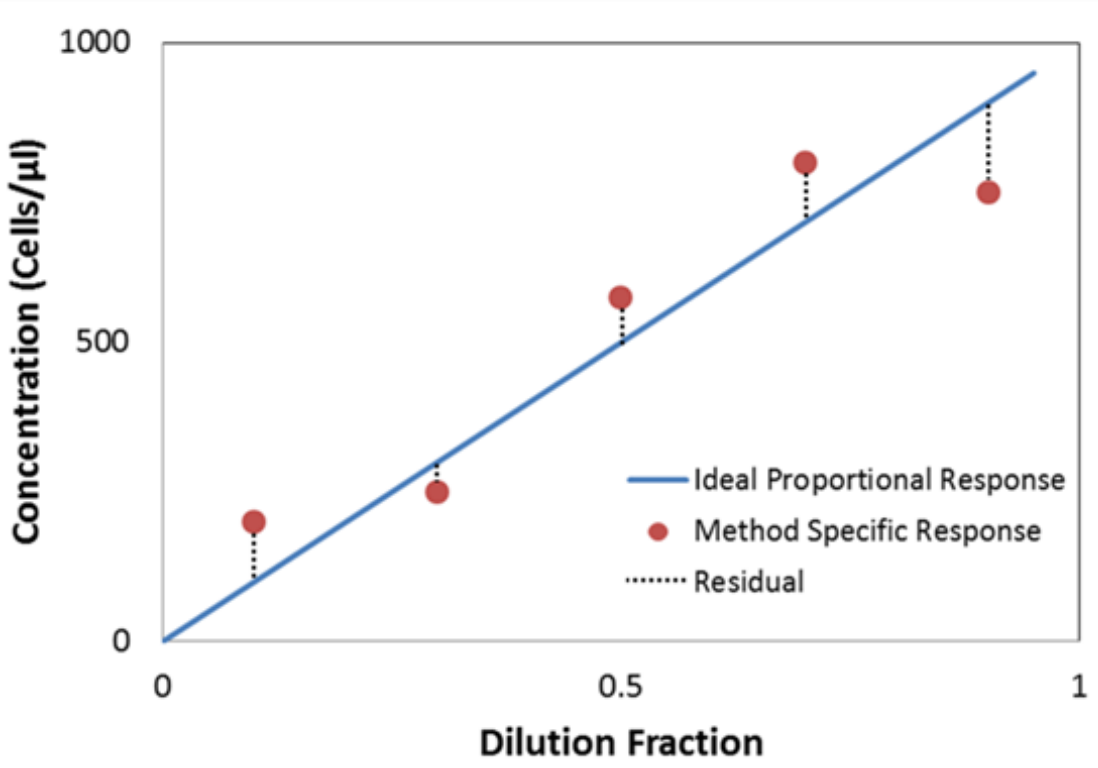
Need confidence in the measurement process over the range of samples that are intended to be measured in order to enable decision making based on cell count

ISO/CD 20391-2 *Biotechnology - Cell Counting – Part 2. Experimental Design Statistical Analysis to Quantify Counting Method Performance*

The principle of proportionality is used to evaluate the quality of a cell counting measurement process

Proportionality serves as an internal control

- In a proportional system, change in dilution (DF) is always accompanied by a proportional change in the measured cell quantity
- Proportionality must hold true for an accurate cell counting process; deviation from proportionality indicates measurement error.



Concepts within Cell Counting Part 2 standard

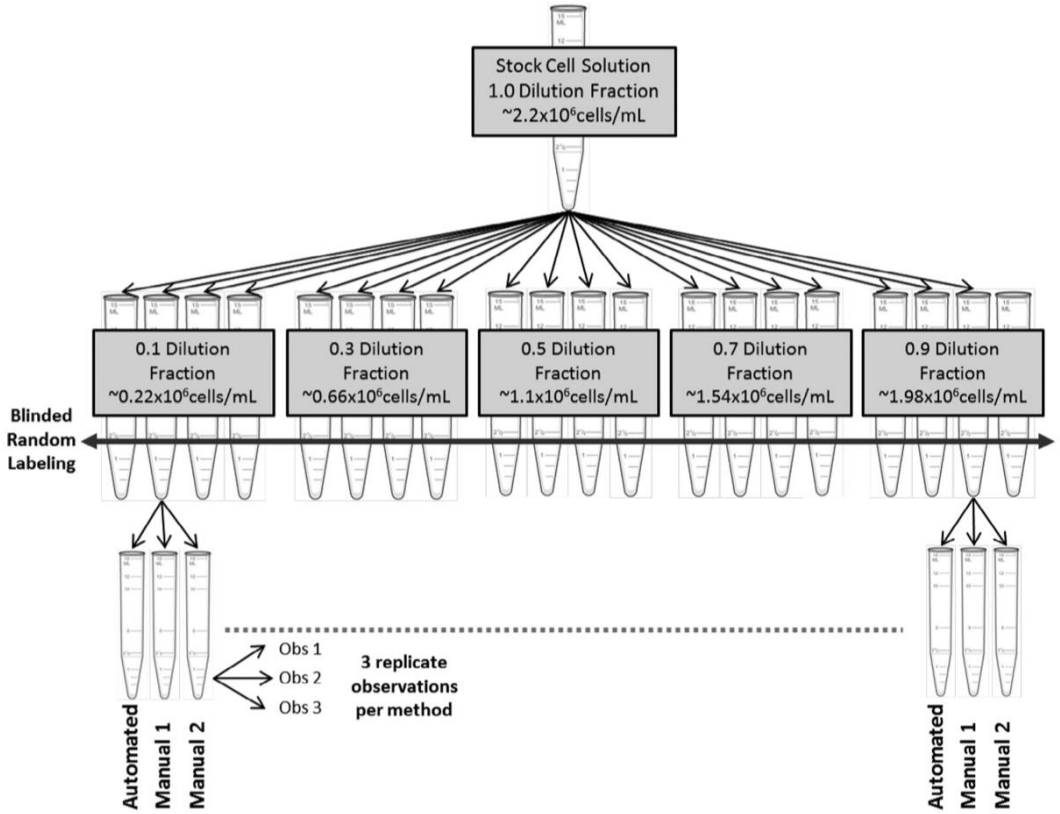
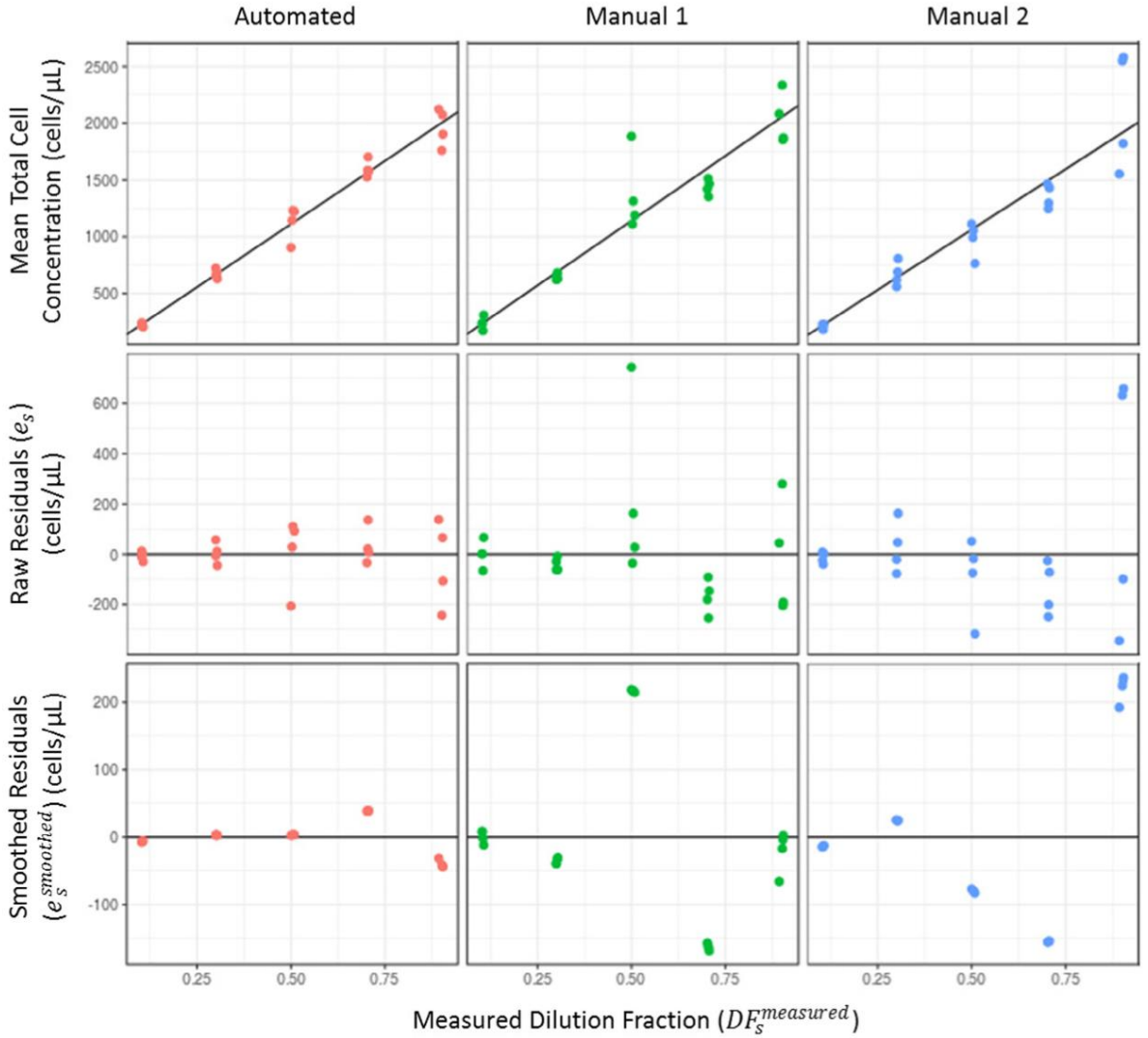


Figure 2. Schematic representation of the cell counting dilution series study experimental design.

Experimental design with a data structure to enable robust statistical analysis while also taking into consideration sample stability/ availability and operational constraints

Evaluating the quality of a cell counting measurement process via a dilution series experimental design. Cytotherapy 2017 Dec;19(12):1509-1521. doi: 10.1016/j.jcyt.2017.08.014. Epub 2017 Oct 14. Sarkar, Lin-Gibson, et al

Concepts within Cell Counting Part 2 standard



Deviation from proportionality on smoothed data **(i.e., smoothed-residual)**

- Reduces the contribution of imprecision
- PI summarizes the smoothed residual information

Using an experimental design involving a dilution series study, quantitative indicators of repeatability (CV) and deviation from proportionality (PI and R 2) can be calculated to provide an assessment of measurement quality.

Cytotherapy 2017 Sarkar, Lin-Gibson, et al

Next Steps for Cell Counting Part 2 standard

- DIS ballot in process
- Publication date anticipated late 2019

Authentication of Human Cell Lines

- China proposed to develop an ISO standard on cell line authentication
- The ATCC SDO published a US National standard, **ANSI/ATCC ASN-0002: Authentication of Human Cell Lines: Standardization of STR Profiling.**
- Agreement to jointly develop an international standard. Project planning under way.

ISO/WD 23033 *Cell Characterization – General guide for characterization of human cells for therapeutic applications*

Scope

- This International Standard defines terms and serves as a general guide for the characterization of cellular therapeutic products intended for human use. Aspects of this document are also applicable to intermediates of cellular therapeutic products.
- It provides considerations for general cell characterization, including processes to define critical quality attributes (i.e., identity, quantity, purity, potency, viability, stability and sterility) and approaches to select and design measurement methods that are fit-for-purpose.
- This standard is applicable for the characterization of cellular components of tissue engineered products.
- NOTE Requirements for safety testing of cells and cell therapy products are addressed in detail in relevant legislation and guidance documents.

ISO/DIS 20395-1 *Biotechnology — Requirements for evaluating the performance of quantification methods for nucleic acid target sequences — Part 1: qPCR and dPCR*

1 Scope

This document provides generic guidelines for evaluating the performance and ensuring the quality of methods used for the quantification of specific nucleic acid sequences (targets). This will serve to ensure confidence in the data generated and demonstrate that a selected method is fit for its intended purpose.

The document is applicable to the quantification of DNA and RNA target sequences using either digital (dPCR) or quantitative real-time PCR (qPCR) amplification technologies. It applies to target sequences present in nucleic acid molecules including double-stranded DNA (dsDNA) such as genomic DNA (gDNA) and plasmid DNA, single stranded DNA (ssDNA), complementary DNA (cDNA), and single stranded RNA (ssRNA) including ribosomal RNA (rRNA), messenger RNA (mRNA), and long and short non-coding RNA (microRNAs (miRNAs) and short interfering RNAs (siRNAs)), as well as double-stranded RNA (dsRNA).

The document applies to nucleic acids derived from biological sources such as viruses, prokaryotic and eukaryotic cells, cell-free biological fluids (e.g. plasma or cell media) or *in vitro* sources (e.g. oligonucleotides, synthetic gene constructs and *in vitro* transcribed (IVT) RNA). This document is not applicable to quantification of very short oligonucleotides.

qPCR and dPCR

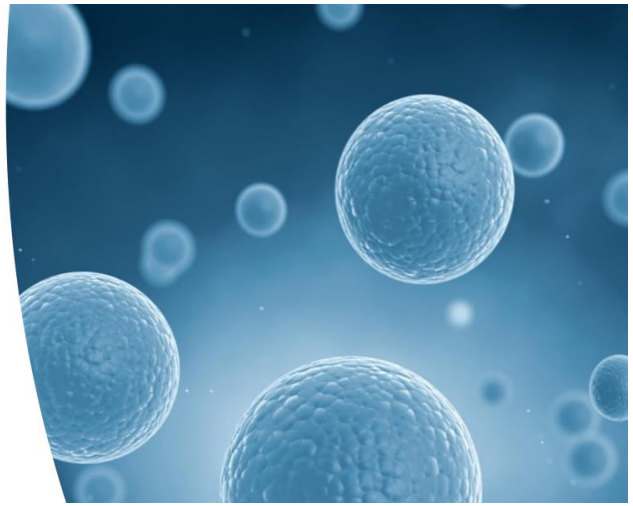
- Current status: Translation period closes and DIS ballot opens this week
- All experts can review the draft by contacting their National Standards Body; BBMRI member can access document via their liaison status.

Other Projects under discussion

Needs for Standards in Rapid Microbial Testing

8th meeting of ISO/TC 276 WG3
Beijing, China
6/15/18

Dawn Henke
Standards Coordinating Body



Justification for Development of this Standard

- Lead to decrease in time to validate new rapid microbial testing methods
- Lead to decrease in time to develop new biologic products
- Lead to decreased cost for manufacturers of biologic products
- Lead to the development of novel RMTM strategies
- Streamline the use and validation process of new rapid microbial methods
- Maintain or improve safety and efficacy of biologic products

Key take-away messages

- ISO/TC 276 is developing a suite analytical measurement standards assessing the quality of biological samples
- TC276 and partners are working to better articulate "fit for purpose" and clarify actions needed for a specific purpose
- Expert input is needed to ensure that standards are balanced and address a need without overburdensome

Selected Resources for Biobanks

Tools

- ISBER Self- Assessment Tool (SAT)
- Integrated Biobank of Luxembourg's (IBBL) Biorepository Proficiency Testing Program
- Biospecimen Stability Testing Calculator (STABCAL)
- Pre-analytical Biorepository External Quality Assessment (EQA) Survey
- Standard Pre-Analytical Code (SPREC)
- International Repository Locator (IRL)
- Neurological Disease Metadata

Best Practices

- ISBER Best Practices for Repositories: Collection, Storage, Retrieval and Distribution of Biological Materials for Research
- OECD Guidelines for Human Biobanks and Genetic Research Databases
- OECD Best Practice Guidelines for Biological Resource Centres
- National Cancer Institute (NCI) Best Practices for Biospecimen Resources

Standardization

- International Standards Organization
  - –ISO 20387 *General Requirements for Biobanks*
  - –ISO 9001:2000 *Quality Management Systems -- Requirements*
  - –ISO/IEC 17025 *General Requirements for the Competence of Testing and Calibration Laboratories*
  - –ISO TC 276 *Biotechnology*
- College of American Pathologists Biorepository Accreditation Program
- CTRNet Biobank Certification Program

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Laboratory collaborations via CRADA, consortium, etc.

Participate in NIST workshops

Participate in standards development efforts

Learn more @ www.nist.gov/mml/bbd or search NIST Advanced Therapies