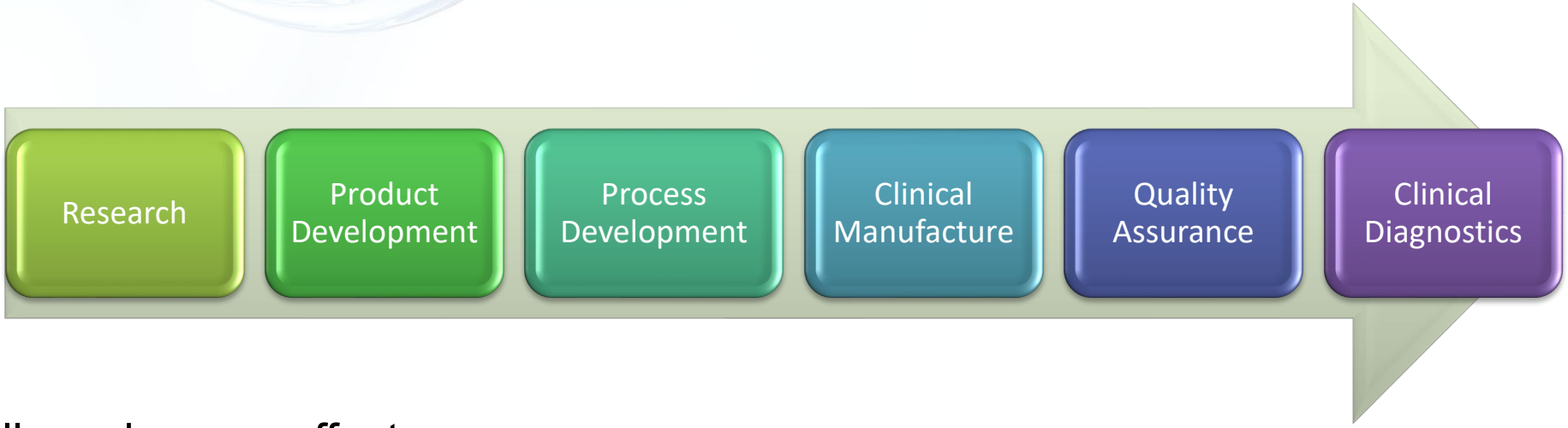
MEASUREMENT ASSURANCE FOR CELL COUNTING

Cell Count is one of the most fundamental metrics of a cell sample and underpins key decisions in the manufacturing and commercialization of cell-based products

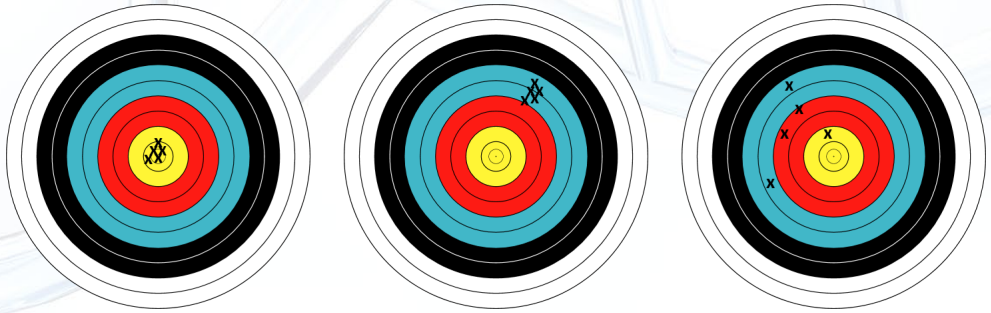


Cell number can affect:

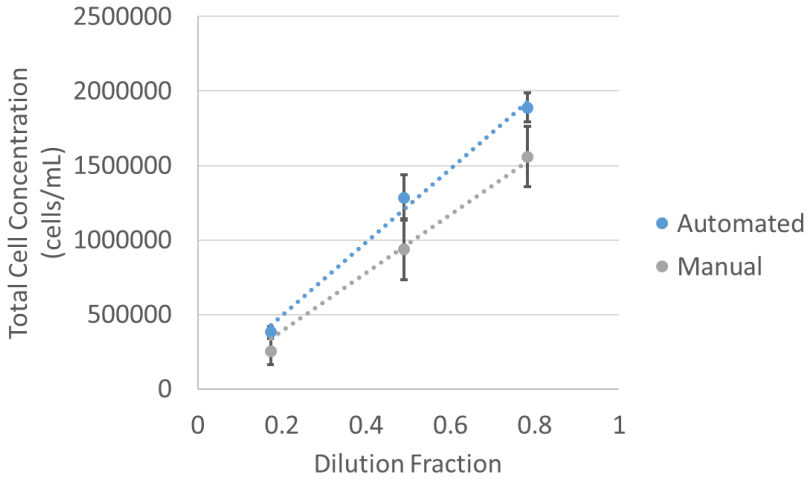
- Potency and efficacy of a cell therapy treatment (dosing)
- Manufacturing process for cells and biopharmaceutical products
- Rate of growth of regenerated tissue in a biomaterial scaffold
- Bioassays

Contact: Sumona Sarkar (Sumona.sarkar@nist.gov)

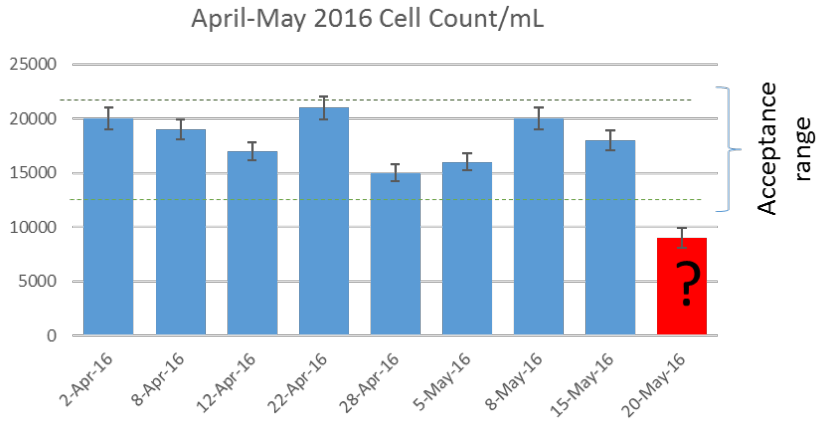
HOW RELIABLE ARE THE CELL COUNT & VIABILITY MEASUREMENTS?



What do you do when cell counting methods give different results?



When a cell count falls out of specification, is the problem in the measurement or the sample?

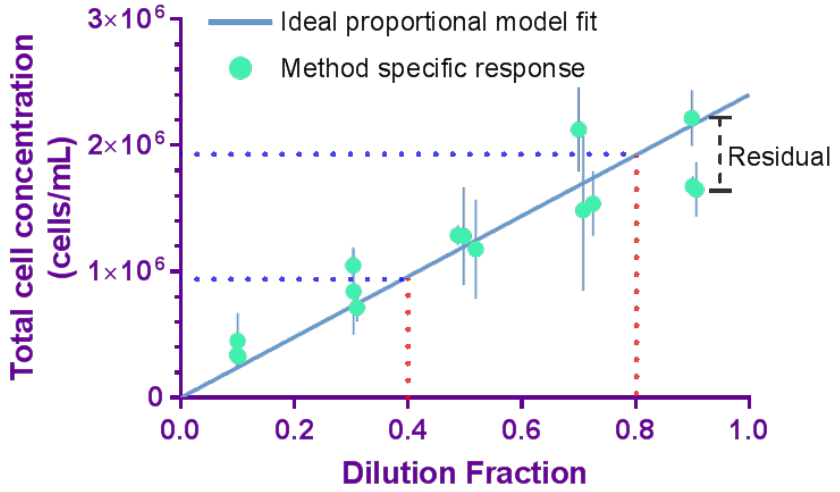


Lin-Gibson S, Sarkar S, Elliott JT. Summary of the National Institute of Standards and Technology and US Food And Drug Administration cell counting workshop: Sharing practices in cell counting measurements. Cytotherapy. 2018 Apr 24.

ICS › 07 › 07.080

ISO 20391-2:2019

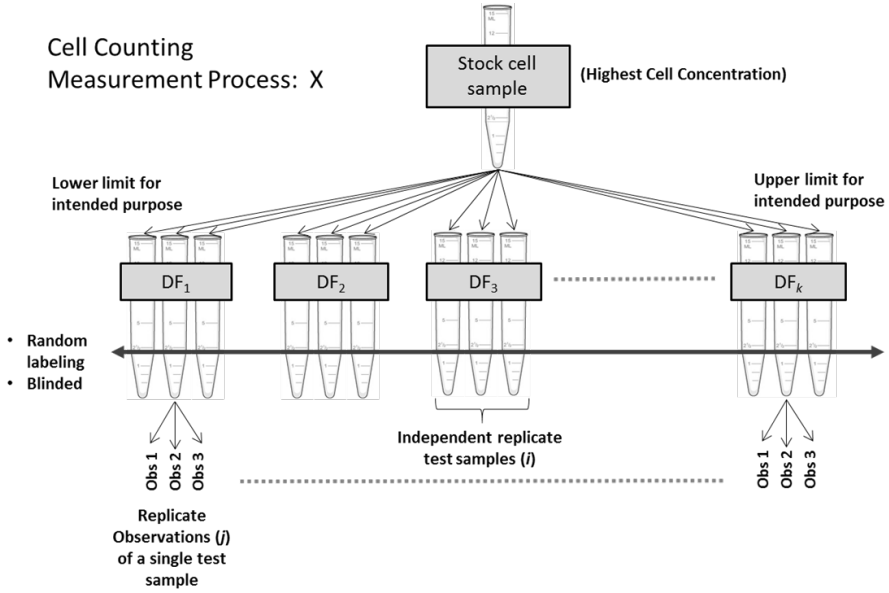
Biotechnology — Cell counting — Part 2: Experimental design and statistical analysis to quantify counting method performance



Principle of Proportionality Serves as an Internal Control to Evaluate Quality

- Proportionality must hold true for an accurate cell counting process; deviation from proportionality indicates measurement error.
- Approach does not require a reference material and is measurement platform independent

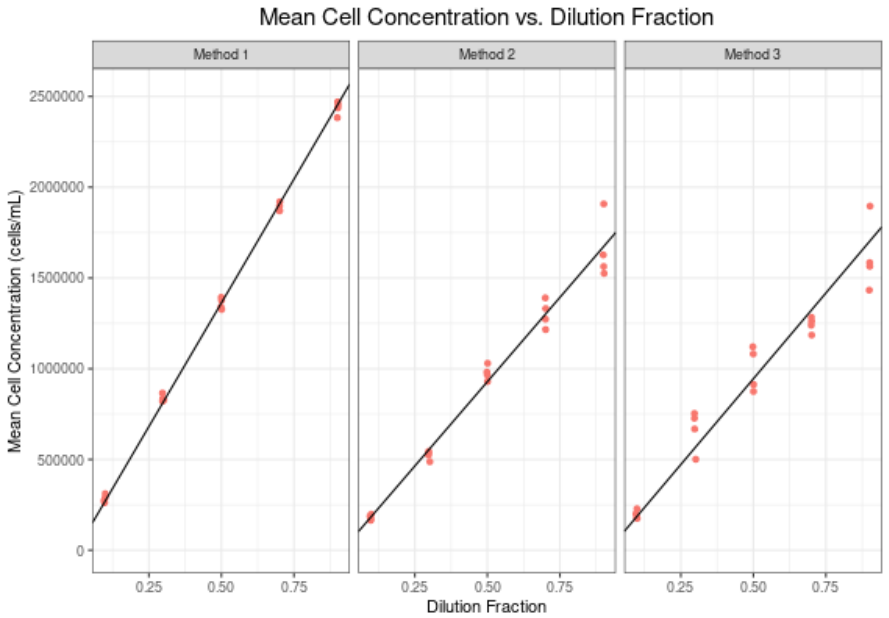
- Homogeneous Stock Solution
- Independent dilution fractions (DF)
- Replicate samples per DF
- Weight-verified pipetting
- Replicate Observations per Sample
- Blinded random labeling and randomized measurement order
- Recording of analyst and time elapsed



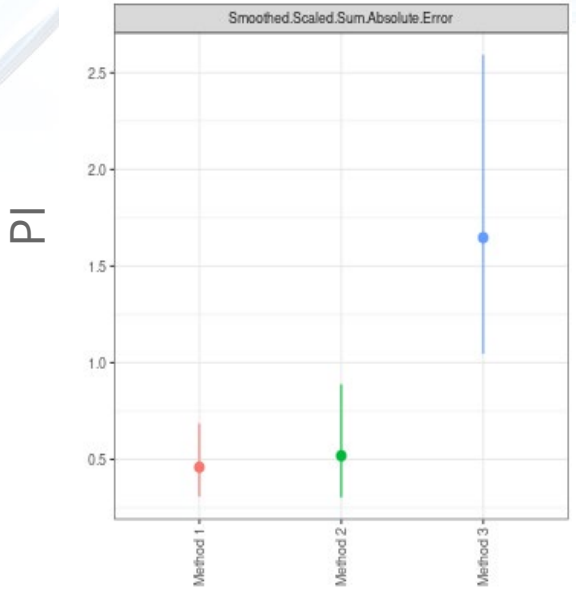
Quality indicators %CV, R^2, Proportionality Index (PI) are calculated to evaluate the quality of the measurement process

COMET DEMO: COMPARISON OF THREE CELL COUNTING METHODS FOR A JURKAT CELL SAMPLE

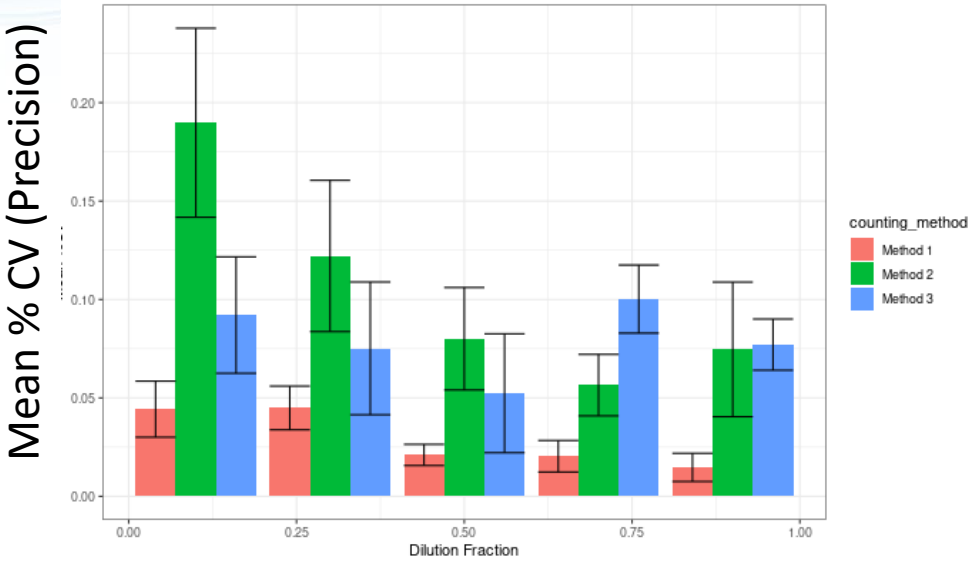
Dilution Series Design



Proportionality



Precision



The vertical bars represent bootstrap confidence intervals computed at the requested confidence level.

Bias

	Method 1	Method 2	%Bias	Bootstrap Lower CL	Bootstrap Upper CL	Significant
1	Method 1	Method 2	-46.7	-50	-42.7	yes
2	Method 1	Method 3	-44.2	-48.4	-39.7	yes
3	Method 2	Method 3	1.7	-2.5	5.3	no

Sarkar, S., Pierce, L., Lin-Gibson, S., Lund, SP., Cell & Gene Therapy Insights 2019; 5(1), 117–131

Concluding Statements

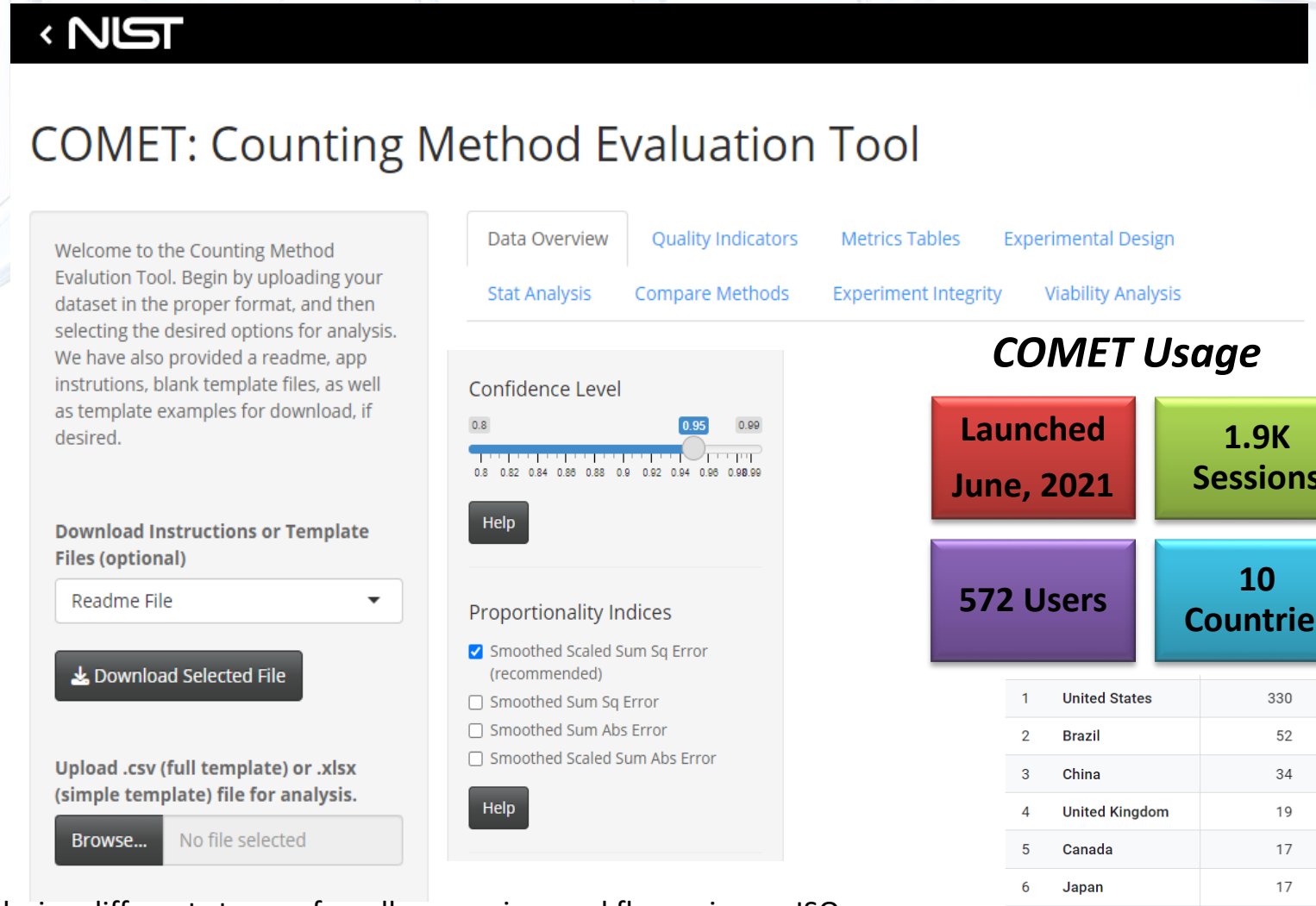
- Methods 1 and 2 demonstrate a high degree of performance with respect to proportionality and precision when compared to method 3
- A significant bias is detected between method 1 and method 2, however performance is similar; therefore, method selection will need to incorporate other important fit-for-purpose factors such as correlation to other biological phenomenon, considerations for intermediate precision, ease of use, etc.

COMET: AN ONLINE OPEN-SOURCE 20391-2 IMPLEMENTATION TOOL

Available at:
https://www.nist.gov/itl/sed/products-services/statistical-software)

COMET Outputs (Everything Needed for Reporting in ISO 20391-2):

- *Quality Indicators*
- *Reporting Requirements (experimental design, stats equations, pipetting error)*
- *Interpretation (comparison of methods, experiment integrity)*
- *Additional Tools (cell viability analysis, discrimination plots)*



COMET Usage

Launched June, 2021

1.9K Sessions

572 Users

10 Countries

1	United States	330
2	Brazil	52
3	China	34
4	United Kingdom	19
5	Canada	17
6	Japan	17
7	Australia	14
8	Germany	9
9	Denmark	7
10	France	7

Recent Publications using COMET:

- "Assessing the suitability of cell counting methods during different stages of a cell processing workflow using an ISO 20391-2 guided study design and analysis." *Frontiers in Bioengineering and Biotechnology*, 11, 1223227. (2023)
- "A guided demonstration of the Counting Method Evaluation Tool (COMET) for implementing the ISO 20391-2 Cell Counting standard." Cell & Gene Therapy Insights 2023; 9(5), 581–609 (NIST)

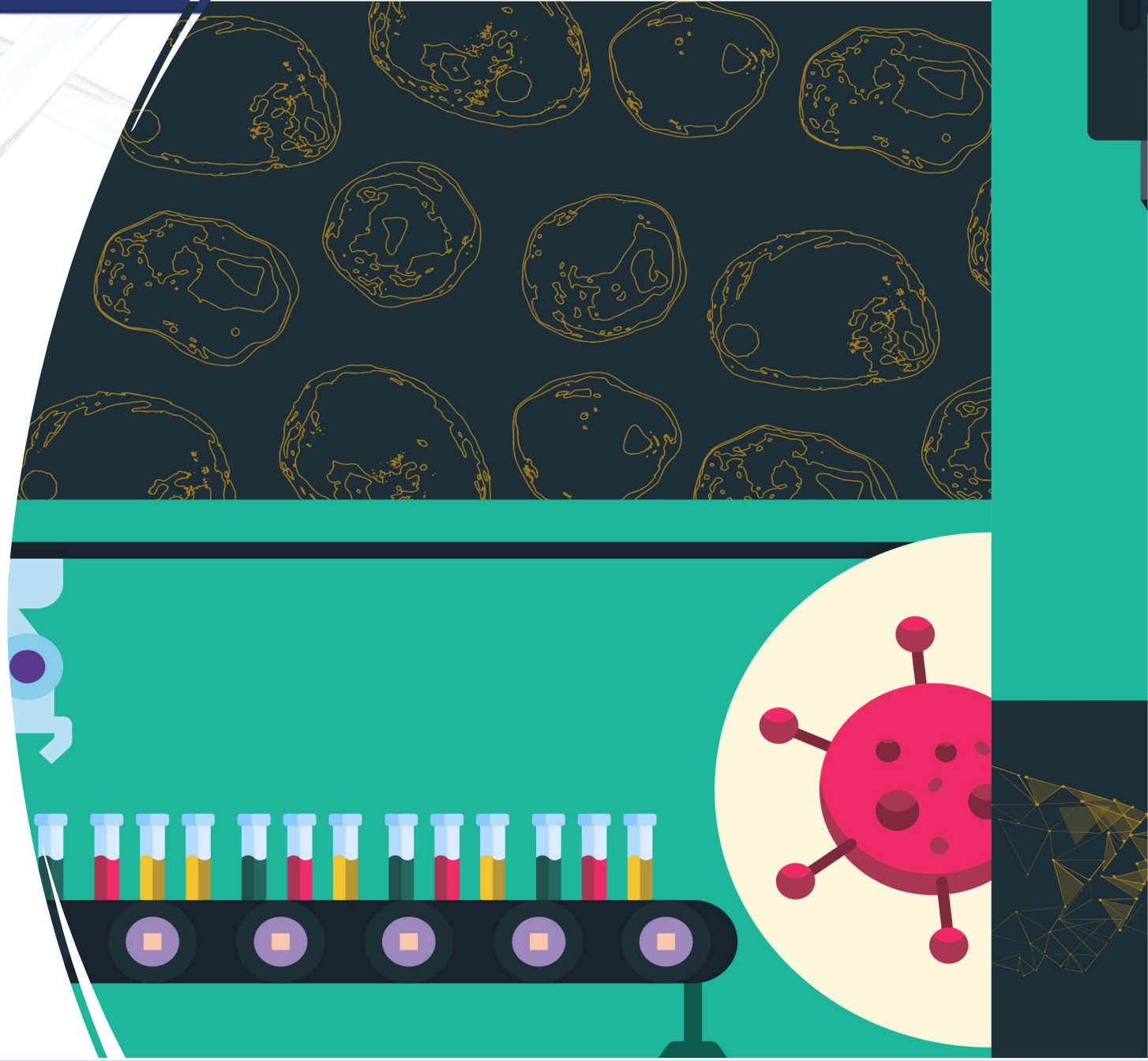
NIST Flow Cytometry Standards Consortium

https://www.nist.gov/programs-projects/nist-flow-cytometry-standards-consortium

A public-private partnership through cost sharing to address the measurements and standards needed to increase confidence and comparability of flow cytometry data in research and commercial products.

MISSION: *Convene stakeholders in the pre-competitive space to accelerate the adoption of quantitative flow cytometry in biomanufacturing of cell and gene therapies.*

Contact: Lili Wang (lili.wang@nist.gov)



Consortium Goals

- **Equivalent Number of Reference Fluorophores (ERF) Measurement Service**: Develop reference standards including reference materials, reference data, reference methods, and measurement service for assigning ERF units to calibration microspheres and assessing the associated uncertainties and utilities.

- **Reference Material Selection and Design**: Develop candidate reference standards including biological reference materials, reference data, reference methods, and evaluate them via interlaboratory studies.

- **Assay and Protocol Selection and Design:** Establish an inventory of existing protocols, data, and standards; Generate new SOPs/methods for cross platform assay standardization and data analysis; test robustness of assays and associated uncertainties.

- **Data Infrastructure and Applications**: Establish, curate, and maintain a highly structured data repository and associated metadata standards; enable applications and evaluation of AI/ML approaches with refer e datasets generated from consortium interlaboratory studies.

Consortium Outputs

- Created a community that jointly addresses measurement challenges and collaborates on pre-competitive solutions with specific focus areas distributed across four active consortium working groups (WGs).
- Created a forum to discuss emerging measurement needs through monthly meetings and annual workshops.
- Completed two, large-scale interlaboratory comparison studies, one for instrument standardization and the other for cell counting and cell viability assay standardization.
- Established instrument harmonization across multiple instrument platforms, sites and calibration materials/standards.
- Developed robust data repository infrastructure containing well qualified, structured, FAIR datasets functioning as a flow cytometry “data commons”.
- Developed five standard operating procedures (SOPs) for methods included in the interlaboratory comparison studies.
- Supported collaborating organizations through improving flow cytometry expertise and competencies.

Flow Cytometry Standards Consortium Members Represent Broad Perspectives and Expertise

Bristol Myers Squibb™
SANOFI
Kite A GILEAD Company
AstraZeneca
CaringCross CREATING ACCESS TO CURES
ascend
REGENERON

ThermoFisher SCIENTIFIC
BECKMAN COULTER Life Sciences
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Miltenyi Biotec
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QOGNIT
Spherotech Let the Possibilities Flow...
JCVI J. CRAIG VENTER INSTITUTE®
SLINGSHOT
ELLARCUS BIOSCIENCES
AHEAD
Raytheon BBN
HEMATOCE

ISAC INTERNATIONAL SOCIETY FOR ADVANCEMENT OF CYTOMETRY
BCS Boston Cell Standards
NIMBL The National Institute for Innovation in Manufacturing Biopharmaceuticals
STANDARDS COORDINATING BODY REGENERATIVE MEDICINE

CURIOX BIOLOGY AT ITS BEST
UNIVERSITY OF DELAWARE
THE UNIVERSITY OF WESTERN AUSTRALIA
MiFtek
Bruce H Davis MD

FDA CBER CDRH
NIST
NIBSC Confidence in Biological Medicines
NIH NATIONAL CANCER INSTITUTE
WRAIR Walter Reed Army Institute of Research Soldier Health ★ World Health
KRISS Korea Research Institute of Standards and Science