Session 3: Needs and Approaches for overcoming gaps in obtaining sufficient measurement assurance for cell counting

Regulatory considerations for cell counting
Steven Bauer / FDA

Measurement assurance strategies
John Elliott / NIST

Round Table Discussion

- Common methods/best practices
- Reference materials
- In process controls for the measurement process
- Other strategies for measurement assurance
- Documentary standards

Round Table Discussion

Moderator: Sumona Sarkar, NIST

Some Considerations

What are the gaps in the existing resources for cell counting?

What are the best practices for establishing validated cell counting methods?

How can we improve our confidence in cell count measurements?

How can we assure comparability of cell counting measurements?

Are their pre-competitive activities the community can engage in to assure the quality of cell counting measurements?

What are common practices across cell counting

Identifying appropriate control materials

Sources of variability

Method Comparability

Gating Practices

Collaboration to draft consensus practices

What set of cell types for instrument validation

Improving variability in cell counts

Method Transfer

Dealing with sample artifacts (bubbles, debris etc.)

Method acceptance criteria

Control Experiments prior to a run

Approaches for limiting user variability

Influence of Count on Bioassays

Sample Stability during Counting

Good Study Designs

Standard for live/dead assessment and counting

Instrument-instrument variability

Accuracy/ True Counts

Counting irregular cells

Additional Information

Workshop Goals

- Raise awareness of the importance and challenges associated with cell counting measurements
- Develop and document best practices for cell counting
- Discuss options to address measurement challenges through collaborative studies (NIIMBL)
- Workshop outcomes to support the development of international standards and more specific measurement challenges

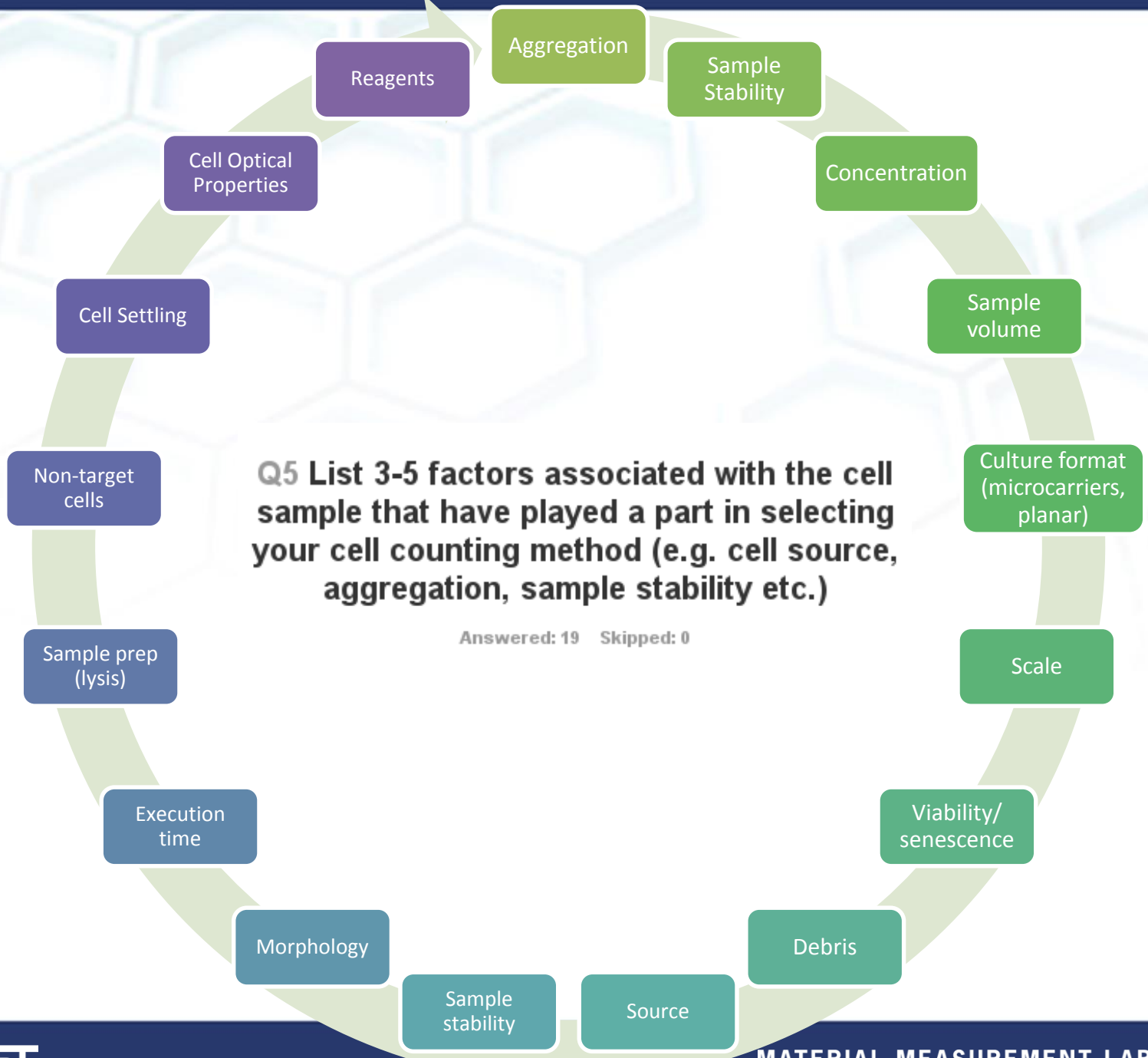
Workshop Participants: Resources

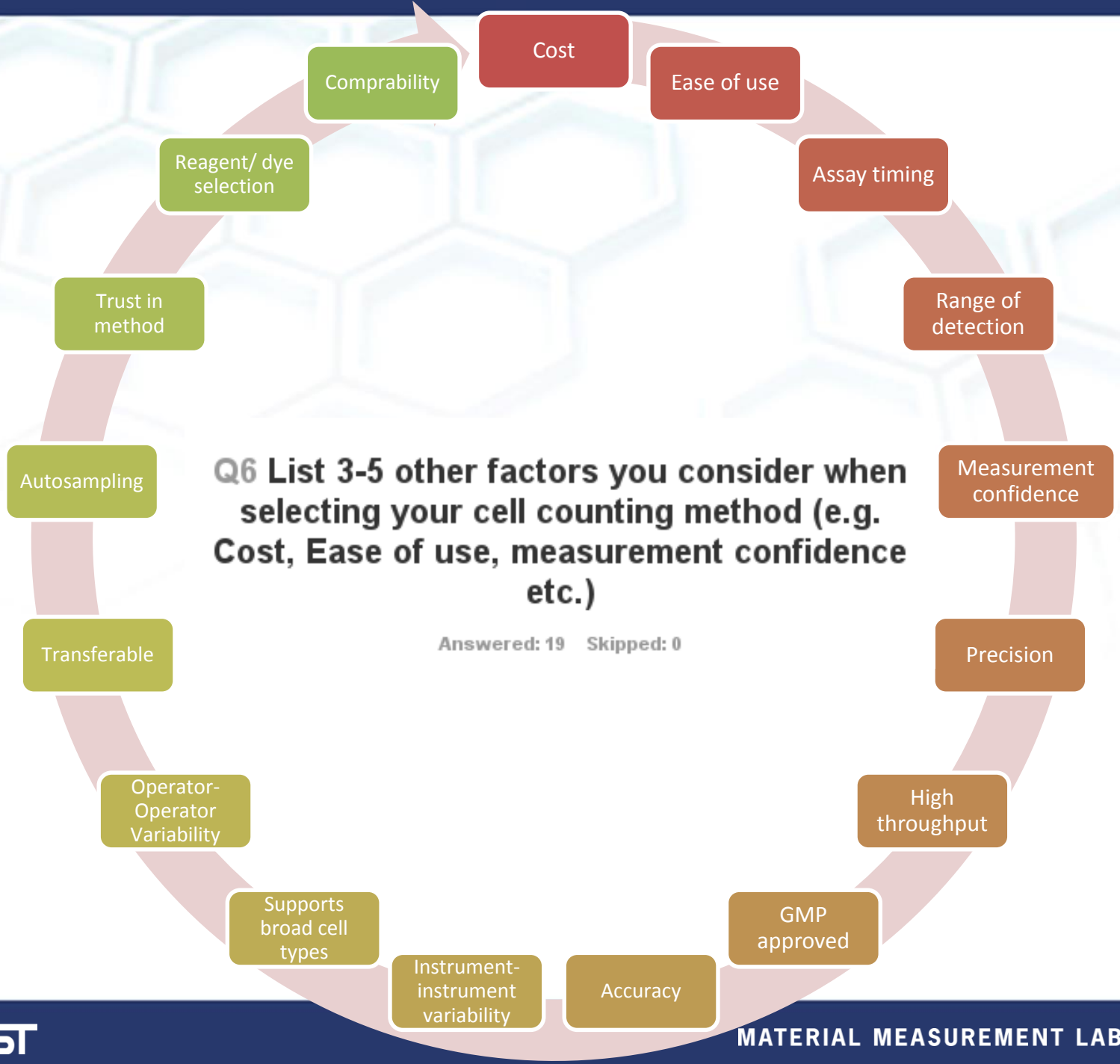
- Bartlow, P. Importance of a Cell Counting Method to Develop a Robust Cell Therapy Process. Podium presentation, Cell Therapy Bioprocessing and Commercialization. Annual Meeting in Alexandria, VA, October 2015.
- Cytometry B Clin Cytom. 2007 Sep;72(5):427-32. CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY,May 1997, p. 309–313
- Accurate measurement of peripheral blood mononuclear cell concentration using image cytometry to eliminate RBC-induced counting error
- Morphological observation and analysis using automated image cytometry for the comparison of trypan blue and fluorescence-based viability detection method
- A high-throughput AO/PI-based cell concentration and viability detection method using the Celigo image cytometry
- Biotechnology Report 7 (2015) 9-16. Tran, SL, et al. PLoS One September 2011 6(9): e22876 Warren Strober, Current Protocols in Immunology, 1997, A.3B.1
- Cell Gene Therapy Insights 2016;2(6),663-673.
- USP 1034 Analysis of Biological Assays
- A Comparison of Assay Performance Measures in Screening Assays: Signal Window, Z' Factor, and Assay Variability Ratio ARTICLE in JOURNAL OF BIOMOLECULAR SCREENING · MAY 2006
- Global Procedure for Lab Investigations - Phase 2b Cryopreserved Drug Product (CDP) - Phase 2b Working Cell Bank (WCB). - GMP guidance for method: accuracy, intermediate precision, repeatability precision, linearity, range, and stability indicating properties.
- FDA guidance on method validations: https://www.fda.gov/downloads/drugs/guidances/ucm386366.pdf
- Validation of three viable-cell counting methods: Manual, semi-automated, and automated: Cadena-Herrera, D. etal.
- ICH Validation of Analytical Procedures: Text and Methodology Q2(R1)
- ICH Guidance for Industry Q2B Validation of Analytical Procedures: Methodology
- 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.
- Lin-Gibson, S., Sarkar, S., & Ito, Y. (2016). Defining quality attributes to enable measurement assurance for cell therapy products. *Cytotherapy*, 18(10), 1241-1244.
- Lin-Gibson, S., Sarkar, S., Elliott, J. T., & Plant, A. L. (2016). Understanding and managing sources of variability in cell measurements. *Cell Gene Therapy Insights* 2016;2(6),663-673.
- ISO/WD 20391-1 [Under development] Biotechnology -- Cell Counting -- Part 1: General guidance on cell counting methods
- ISO/WD 20391-2 [Under development] Biotechnology -- Cell Counting -- Part 2: Experimental design and statistical analysis to quantify counting method performance

Workshop Participants

What Types of Follow-on Activities/ Projects would be valuable from this workshop?

- Case studies that exemplify cell counting method evaluation
- Protocols/ Best Practices Documents
- White Paper
- Establishing written consensus standards
- Collaborative/ Inter-lab studies


Q5 List 3-5 factors associated with the cell sample that have played a part in selecting your cell counting method (e.g. cell source, aggregation, sample stability etc.)
Answered: 19 Skipped: 0
Aggregation
Sample Stability
Concentration
Sample volume
Culture format (microcarriers, planar)
Scale
Viability/ senescence
Debris
Source
Sample stability
Morphology
Execution time
Sample prep (lysis)
Non-target cells
Cell Settling
Cell Optical Properties
Reagents


Cost
Ease of use
Assay timing
Range of detection
Measurement confidence
Precision
High throughput
GMP approved
Accuracy
Instrument-instrument variability
Supports broad cell types
Operator-Operator Variability
Transferable
Autosampling
Trust in method
Reagent/ dye selection
Comprability
Q6 List 3-5 other factors you consider when selecting your cell counting method (e.g. Cost, Ease of use, measurement confidence etc.)
Answered: 19 Skipped: 0