SESSION 4: NEXT STEPS

Moderator: Sheng Lin-Gibson, NIST

- Meeting summary
- Key concepts / outline for whitepaper
- Other businesses

https://www.surveymonkey.com/r/CCW_idea_box
<table>
<thead>
<tr>
<th>Workshop Goals</th>
<th>Actions and potential solutions</th>
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| Raise awareness of the importance and challenges associated with cell counting measurements | • Whitepaper  
• Additional NIST-FDA workshops                                                                 |
| Develop and document best practices for cell counting                          | • Whitepaper  
• Develop methods of general use through appropriate forums                                        |
| Discuss options to address measurement challenges through collaborative studies (NIIMBL) | • Send R&D topics to NIIMBL/TAC for funding to advance biomanufacturing                           |
| Workshop outcomes to support the development of international standards and more specific measurement challenges | • Cell counting serves as a use case for upcoming ISO standard effort for cell characterization |
Recap

• Overarching theme: Counting is important
• Many use cases, important considerations, lessons learned, opportunities/new technologies
• FDA does not require prescriptive methods:
  – fit for purpose
  – system suitability
• Example of measurement assurance strategies for cell counting
Terminology

• What is required during qualification, validation, and verification
Fit for purpose

- I would like to count **AA** cells in media/matrix **BB** using **CC** methods for **DD** purpose

<table>
<thead>
<tr>
<th>AA</th>
<th>BB</th>
<th>CC</th>
<th>CC</th>
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<tbody>
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<td>MSC</td>
<td>Universal</td>
<td>Manual</td>
<td>Release</td>
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<td>iPSC</td>
<td>media</td>
<td>Automated</td>
<td>Dose</td>
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<td>Car-T</td>
<td>Cryo-preservant</td>
<td>Impedance</td>
<td>In process</td>
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- What is good enough – how to set specification using meaningful parameters
Various approaches

• General framework vs. individualized methods
• total cell count → → various stages of cell health
• Improving the quality of “gold standard” methods → → new counting technologies
3. ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

This is sometimes termed trueness.

Ref: VALIDATION OF ANALYTICAL PROCEDURES: TEXT AND METHODOLOGY Q2(R1)

Role of reference material/reference standards

• Beads and their roles in calibration, comparability
• Cells RM as an in process control, proficiency testing
• Opportunities for new, better reference materials/what is NIST doing
Evaluating the quality of cell counting methods in the absence of a ground truth

ISO/WD 20391-2 [Under development: 20.60]
Biotechnology -- Cell Counting -- Part 2: Experimental design and statistical analysis to quantify counting method performance

Cell Counting Results for 4 methods

- Comparability between methods
- Accuracy
- Precision
- Linearity (proportionality)
- Reproducibility

Proportionality Index (PI)
Tools to get to “good enough”

- Strategies for better measurements
- Education materials
- Reference materials/reference standards
- Bioprocessing control (equipment, reagent, etc.)
- Competency
- Method standards
What works well?
(ripe for standardization / best practices / SOP)
What are ongoing efforts

• Methods and funding to keep the discussions going
• NIST certified beads consortium

• ISO Cell Counting Part 1—definitions and general considerations
• ISO Cell Counting Part 2 – method to evaluate the quality of cell counting
What is needed?
(common understanding, methods, guidance, etc.)

• Deep dive on several case studies
• Best practices
• Spike in or other methods
• Minimum information for a specific method to provide confidence
• Inter-laboratory study / formation of consortium
Additional Topics for NIST-FDA workshop
FIGURE 2

An example of a generalized cell counting process that involves an automated imaging device, and where potential controls and standards for managing and minimizing sources of variability could be used.

- Assurance from service providers
- Validated methods/protocols
- Quality Management System
- Conformity assessment

- Instrument qualification (IQ,PQ)
- Validation studies to establish instrument sensitivity, LoD, robustness etc
- DOE to determine parameter space
- Design SOPs with appropriate in-process controls
- Proficiency testing

Sample collection
Sample storage & transportation
Reagents/Consumables/ Test kits

Reference beads for verification
Calibration via known standards

Measurement device
Data collection
Data analysis

Decision making/user defined specification

The central green box contains 3 steps of a typical measurement process; other aspects such as the composition of donor samples, reagent sourcing, and sample handling may contribute additional variability to the cell counting process.
Working with NIST

- Laboratory collaborations via joint studies, CRADA, consortium, post-doctoral opportunities, etc.
- Participate in NIST workshops
- Participate in standards development efforts, including inter-laboratory studies

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

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Outline of a whitepaper

• Brief recap of workshop
• Key concepts
  – What works well
  – What is ongoing
    • ISO Cell Counting Part 1 – definitions and general considerations
    • ISO Cell Counting Part 2 – method to evaluate the quality of cell counting
  – What is needed
    • Gaps in current guidance (communication, technical, guidance?)
    • Additional tools to improve measurement assurance
TC276 cell characterization standard effort
based on outcomes of Jan2017 US-Japan Workshop

Cell characterization “umbrella” standard – to enable common understanding

Cell characterization measurement process standard

By attribute
- Cell counting 1 & 2
- Viability ??

By technique
- Flow Cytometry
- Imaging

By purpose
- Cell therapy products
- Gene therapy products
- Drug discovery

By cell type
- ??
- ??

Cell Counting Standards are under development; all others are listed as examples