Strategy to develop cell characterization standards

ISO/TC 276  WG3

version June 15, 2015
Modified by slg
Standards Efforts for Cell Therapy

Many ongoing efforts, include several that aims to develop standard methods
In early 2014, a number of needs and gaps related to cell characterization were identified by ISO/TC276 TG3: Analytical Methods.

In late 2014, TG3 converted to WG3 with a number of standardization proposals consistent with the following scope:

- Develop standards for accurate, reproducible and robust measurement and analysis in support of biotechnologies.
- Biologically relevant molecules and entities, including nucleic acids, proteins, and cells.
- Develop horizontal standards and, when applicable, vertical / particular standards for industry sectors.

Currently, 2 approved work items and 4 approved preliminary work items.
List of Current ISO/TC 276 WG3 Work Items

ISO/WD xxxxx – Biotechnology – Cell Counting – Part 1: General guidance on cell counting method

ISO/WD xxxxx – Biotechnology – Cell Counting – Part 2: Experimental design and statistical analysis to quantify counting method performance

ISO/PWI 20395 – Quality considerations for targeted nucleic acid quantification methods

ISO/PWI 20396 – Methods to determine the concentration of total nucleic acids

ISO/PWI 20397 – Methods to evaluate the quality of the massive sequencing data

ISO/PWI 20688 – Oligonucleotide Quality Control
Cell Counting Standards

Industry needs:
• Cell type independent
• Measurement platform independent
• Amenable to changes in measurement process

Proposed solutions:
• An approach to assess the measurement confidence for a specific cell count measurement process where a reference material is not readily available
Overall Cell Counting Standardization Strategy

Part 1: General Guidance on cell counting methods

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

Part 3 and above or independent standards:
- Application of reference materials and benchmarks
- Differential cell counting methods, i.e., viable cell counting
- Method to address morphology or manufacturing processes

Instead, a series of documents will be developed to provide guidance on various aspects of cell counting challenges.

There isn’t a readily available reference cell for “calibration”.

There isn’t a single “protocol” that can address all cell counting issues.
Project Management of Cell Counting Standards

1. NP (new work item proposal)
2. Building expert consensus
3. Consensus building within TC/SC
4. Enquiry on DIS (Draft International Standard)
5. Formal vote on FDIS (proof check by secretariat)
6. Publication of International Standard

TC/SC route

Deliverables
- First CD (Committee draft)
- or ISO/PAS (Publicly Available Specification)
- DIS or ISO/TS (Technical Specification)
- ISO/TR (Technical Report) for non-normative documents
- Final text for processing as FDIS (Final Draft International Standard)
- Final text of International Standard
- ISO International Standard

June 2015

October 2015

June 2016

June 2017

June 2018 or earlier
Need for Cell Characterization Standards
Discussion from US TAG meeting March 2015

• Potential vertical standard with critical quality attributes (CQAs) essential to determine product quality, final release, and in-process testing
  • Cell number, viability, identity, purity/impurity, stability, potency/biological activity

• Industry in need of standardized approaches and standardized methodologies for cell characterization
  • Cell characterization and potency assays to guide process improvement and ensure intellectual property boundaries
  • Standardized approaches and standardized methodologies to help streamline regulatory review/approval

Slide adopted from ARM presentation
Proposal from Apr2015 ISO/TC 276 WG3 Meeting

Matrix Approach to Analytical Method Standards

- Method Standards for general analytical methods
- General guides
  - Reference specific method standards
  - Sector/application specific standards
### Needs and Gaps

<table>
<thead>
<tr>
<th>Cell Characterization</th>
<th>Cell counting</th>
<th>Viability</th>
<th>qPCR</th>
<th>Total NA</th>
<th>NGS</th>
<th>Protein</th>
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**Biobanks & Bioresources**

**Bioprocessing**

**Biopharmaceutical**

**Application x**

**General Guide**

**Horizontal Standard**
Resolutions from ISO/TC 276 WG3 Meeting April 16 2015

• Recommendation 2/2015/01 taken by ISO/TC 276/WG3 on 16 April 2015: A “small committee” consisting of experts from US, Japan, Korea, France, UK and China will work to develop/refine the cell characterization strategy and report at the next WG3 meeting.

How should we develop international standard(s) on potency/cell viability?

**Industry needs:**

- Cell type independent
- Measurement platform independent
- Amenable to changes in measurement process
• We conducted an inventory of industry needs for cell characterization

• We reviewed many of the existing standards and regulatory guidelines to avoid conflict/duplications

• We reviewed existing framework within TC276 and WG3 to come up with a reasonable structure

• We had input from the recent NIST measurement assurance workshop, individual discussions with stakeholders, discussion with the Japan team, and others.
Cell and Gene Therapy Product Characterization

Biological assays
Non-biological analytical assays
or Multiple assays (assay matrix)

Molecular
Biochemical
Immunologic
Phenotypic
Physical
Biological

Source: Guidance for industry - Potency Test for Cellular and Gene Therapy Products; FDA 2011
Existing Standards and Guidelines

Existing guidelines with applicability to cell-based medicinal products:

- **ICH guidelines (August 2011)**
- **EMA guidelines (October 2012)**
- **FDA guidance (October 2012)**
- **Guidelines related to Reference Materials (February 2012)**
- **Comparability guidelines (October 2011)**

Guides produced by BSI:

- **PAS 83:2012 – Developing Human Cells for Clinical Applications in the European Union and the United States of America**
- **PAS 93:2011 – Characterization of Human Cells for Clinical Applications**
- **Glossary**
  - **PAS 84:2012 – Cell Therapy and Regenerative Medicine Glossary (January 2013)**

Existing Standards and Guidelines (cont.)

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

Potency Test for Cellular and Gene Therapy Products; FDA 2011

Proposed Standards for Cell Characterization

Proposed NWIP - Cell characterization - Part 1 - Guide and definition
- Define critical quality attributes (also see BSI PAS 93)

Proposed NWIP - Cell Characterization - Guide for Measurement Process (see below)

Proposed NWIP - Cell Characterization - Design of Analytical Methods
  • Using QbD based principles (under development)
### Possible Cell Measurement Strategy

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<tr>
<td>Count</td>
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<td></td>
<td>Cell Counting - Part 1, Cell Counting - Part 2</td>
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<tr>
<td>Identity</td>
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<tr>
<td>Purity</td>
<td>Potential NWIP - See US</td>
<td>Potential NWIP - content under development</td>
<td>Potential NWIP - defines critical quality attributes</td>
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<td>Biological activity</td>
<td>Presentation</td>
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<td>Viability</td>
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<tr>
<td>Sterility</td>
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<tr>
<td>Others</td>
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<td>TBD</td>
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</tbody>
</table>
Proposal: Considerations for Assay Validation Plan

Analysis and validation of all relevant assay parameters
- Accuracy, precision, specificity, linearity and range, system suitability, and robustness

Statistical design and analysis
- Apply sound statistical methods
- Consider potential sources of assay variability and variations form replicates

Validation of qualitative assays
- Determine assay suitability
- Validate all parameters relevant to qualitative assay and provide a rationale for those parameters that are not relevant
- When possible demonstrate accuracy and precision with data or proper assay design.
- Establish acceptance criteria for the control and/or RM

Based in part on: Guidance for industry - Potency Test for Cellular and Gene Therapy Products; FDA 2011
Typical Cell Measurement Process

Upstream Considerations for Assay Development
- Intended Use
- Material Choice (Population Definition)
- Choice of Instrument (IQ/OQ/PQ)

Collection/storage/transportation → Sample Preparation → Sampling → Measurement/Data collection → Reporting → Data Analysis

Instrument set-up/validation

Process Controls
Control of Raw & Consumable

Developed based on NIST Measurement Assurance Workshop, May 2015
Proposed Outline

Scope
Normative references
Terms and definitions
General concepts of cell characterization (ref slide 5),
  • when possible refer to specific method standards (slide 7)
  • Biological assays
  • Non-biological analytical assays
  • Multiple assays (assay matrix)
Upstream considerations for assay development
  • Intended use
  • Materials choice
  • Choice of instrumentation
Test Design
  • Assay validation plan (slide 11)
  • Design of experiment
  • Sampling
  • Measurement process and process controls
  • Qualification/validation of assays
    o Sensitivity
  • Acceptance criteria (slide 18)
Pre-examination
  • Raw material qualification (WG4)
  • Sample qualification
    o Sample homogeneity
    o Dilution factor
    o Identify the measurand
    o Additional considerations
  • Measurement device qualification
    o Selection of device/instrument
    o Instrument qualification
    o Measurement validation
      – Accuracy
      – Precision
      – Specificity
      – Detection Limit
      – Linearity
      – Range
  • Secondary validation
    o Inter-operator variability
    o Inter-device variability
    o Inter-day variability
    o Inter-batch variability
Examination
  • Sample preparation
  • Measurement
Post-examination
  • Collection of results
  • Decision based on
    o Evaluation of adequateness
    o Determination
  • Data analysis
  • Reporting
Potential Causes of Variability

**Cell source**
- Source
- Sample preparation
- Concentration
- Sample Volume
- Homogeneity
- Clumping
- Morphology
- Blebbing

**Settings in Advance (Process Controls)**
- 
- % CV range
- Linearity
- Stability
- Range
- Formulation
- Recovery after freeze

**Instrument**
- Specificity
- Specifications (range)
- Instrument variability (trending)
- Settings/Set-up
- Maintenance/Certification
- Data recording/recovery

**Equipment/Consumables**
- Pipettes/Pipettors
- Consumables
- Pipette tips
- Water baths (fill vol/temp)
- Refrigerators/freezers

**Environment**
- Disruptions/distractions
- Cleanliness
- Electrical issues
- Temperature
- Humidity
- Age (expiration)
- Purity/impurity
- Toxicity
- Ref material stability
- Light sensitivity
- Stability
- Temperature sensitivity
- GMP grade
- Control analysis

**Raw materials**
- Lot-to-lot consistency
- Sample collection
- Thaw procedures
- Sample Preparation
- Dilution procedure
- SOP (its order)
- Recording/verification
- pH
- Osmolality

**Procedures**
- Recording/verification
- Time
- Control/reference material
- Analyst training
- SOP (its order)
- Control reference material
- Time

**Data Acquisition**
- Algorithms
- Debris
- Clusters
- Statistical analysis
- Control analysis
- Outliers
- Upfront reporting parameters (i.e. variability)

**Data analysis**
- Outliers
- Control analysis
- Statistical analysis
- Upfront reporting parameters (i.e. variability)
2016 ISO/TC 276 Plenary and Working Group Meetings
Washington DC, USA
May 9-13, 2016

Venue:
Patriot's Plaza III
355 E STREET SW WASHINGTON, DC 20024.
United States
Meeting Venue for ISO/TC 276 Biotechnology
Extra Slides
QUALIFICATION / VALIDATION OF CELL ASSAYS

• What is the Purpose and Scope of the Method?

Fit-for-Purpose

• Consider the Intended Use of the Data

In-process Characterization

Formal Release/ Stability

Early Development QUALIFIED Assay

Late Development Commercial VALIDATED Assay
## Qualification / Validation of Assays

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>Ability to distinguish between analyte (specific cell type) and other substances (or other cell types) present</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Closeness of agreement between ‘true’ (reference) value and ‘found’ value</td>
</tr>
<tr>
<td>Precision</td>
<td>Closeness of agreement in a series of measurements</td>
</tr>
<tr>
<td></td>
<td>Short-term: repeatability; intra-assay; same conditions</td>
</tr>
<tr>
<td></td>
<td>Intermediate: different days; different analysts; different lots; different instruments</td>
</tr>
<tr>
<td>Linearity</td>
<td>Test results within a given range proportional to sample concentration [check observed vs. expected value]</td>
</tr>
<tr>
<td>Range</td>
<td>Interval between lowest and highest quantitative values that meet acceptance criteria for precision, accuracy, and linearity</td>
</tr>
<tr>
<td>Robustness</td>
<td>Degree of reproducibility under variety of conditions</td>
</tr>
</tbody>
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Also see: VALIDATION OF ANALYTICAL PROCEDURES: TEXT AND METHODOLOGY Q2(R1)
# Study Design

<table>
<thead>
<tr>
<th>Parameters to Test</th>
<th>Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample lots</td>
<td>• Minimum number • Inherent diversity</td>
</tr>
<tr>
<td>Instrument</td>
<td>• IQ/OQ/PQ • Settings</td>
</tr>
<tr>
<td>Analysts</td>
<td>• Minimum number • Experience</td>
</tr>
<tr>
<td>Environment</td>
<td>• Temperature &amp; Humidity (static)</td>
</tr>
<tr>
<td>Consumables</td>
<td>• Pipets/Tips: aperture &amp; retention  • Sampling: accuracy &amp; speed  • Dyes/Buffers: pH &amp; osmolality</td>
</tr>
<tr>
<td>Dilutions</td>
<td>• Sample linearity • Instrument linearity  • Independent prep • Mixing</td>
</tr>
<tr>
<td>Procedural Steps</td>
<td>• Timed • Verified</td>
</tr>
</tbody>
</table>

**Recommended Options**
- Spiking
- Check against alternative method
Look for Patterns Contributing to Data Artifacts

- Material Preparation
  - Toxicity
    - Cell aggregates
    - Homogeneity
    - Buffer/Dye consistency
    - Dust / Debris / Particles
  - Sampling location
    - Mixing
    - Homogeneity
    - Accuracy pipetting
    - Adherence
  - Measurement
    - Settings
      - Focal plane
      - Math-Recording errors
      - Instrument-to-instrument inconsistencies

Cause and Effect Examples
Acceptance Criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Variance</th>
</tr>
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<tbody>
<tr>
<td>Repeatability / precision</td>
<td>?? ≤ 30% ??</td>
</tr>
<tr>
<td>Specificity</td>
<td>?? ≤ 5% ??</td>
</tr>
<tr>
<td>Linearity</td>
<td>?? R² ≥ 0.95 ??</td>
</tr>
<tr>
<td>Range</td>
<td>Determined by instrument or method</td>
</tr>
</tbody>
</table>

**Considerations:**

- System suitability requirements
  - For the method
  - For the material
- Statistical measures of variance (RSD or %CV or both)
- Minimum number of measures to achieve a result
- Dealing with outlier results (USP<111> and Guidance for Industry)
  - Originating from the method
  - Originating from sampling
  - Originating from material
<table>
<thead>
<tr>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>taking slide 7 “Needs and gaps for cell characterization” into account, have people considered the two basic functions of all cell types, namely their ability to proliferate and differentiation. These two biological processes overlap with each other, but they cannot be measured using the same readout; different assays need to be used for each process. The type of assay used will depend on the goal to be achieved, but in general the accuracy of the assay that determines cells in either proliferation or differentiation is dependent upon the accuracy of measuring cell number and viability, the identity of the cells being measured and the degree of purity of the cell suspension or population being measured. All of these parameters will feed into the measurement of potency. If the biological process, i.e. proliferation or differentiation, is not defined with respect to how a cell population is characterized, the interpretation of the results will be incorrect and the conclusions will be wrong.</td>
</tr>
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<table>
<thead>
<tr>
<th>response</th>
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<tbody>
<tr>
<td>I completely agree with your assessment, which why I think it is so important to focus first on “primary” measures – identity, quantity (including sub-populations), and purity/impurity. We should then be able to more accurately assess things like vitality, proliferation, and differentiation.</td>
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ISO/TC 276: Biotechnology

Secretariat: DIN
Secretary: Katharina Lippert
Chairperson: Ricardo Gent
22 participating countries
13 observing countries

Structure:
Terms and Definition
Biobanking and Bioresources
Analytical Methods
Bioprocessing
Data Processing and Integration

U.S. TAG for ISO/TC 276

Secretariat: NIST
TAG Administrator: Clare Allocca
Chairperson: Sheng Lin-Gibson
46 Organizations
96 Individuals

Structure:
Terms and Definition
Biobanking and Bioresources
Analytical Methods
Bioprocessing
Data Processing and Integration

http://www.nist.gov/mml/bbd/iso.cfm
Contacts: slgibson@nist.gov
Measurement Assurance

• Facilitate regulatory approval and commerce
• Broadly applicable
• Do not impede innovation
CURRENT ISO/TC 276 WORK RELATED TO CELL THERAPY

WG3: Analytical Methods

ISO/PWI 20391: Methods to determine a relative accuracy for cell counting approaches
ISO/PWI 20395: Quality considerations for targeted nucleic acid quantification methods
ISO/PWI 20396: Methods to determine the concentration of total nucleic acids
ISO/PWI 20397: Methods to evaluate the quality of the massive sequencing data
ISO/PWI 20688: Oligonucleotide Quality Control

New project to develop a standard for cell viability
New project to develop a strategy to develop cell characterization standards

WG4: Bioprocessing

ISO/PWI 20398: Methods to control bioreactor processes for cell culturing
ISO/PWI 20399: Raw materials control for bioprocessing
ISO/PWI: Best practice in raw materials selection in the design of human cell therapy 1. manufacturing processes
Tools for achieving **measurement assurance/confidence** for measurement process

- Reference materials or controls
- Ishikawa (cause/effect) diagram
- Charting
- Process controls
- Experimental Design
- Quality by Design (QbD)
- Validation specifications
- Interlaboratory comparison

**Traceability**
Measurement uncertainty
Method validation
Possible Cell Measurement Strategy
Editable version

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<tbody>
<tr>
<td>Count</td>
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<td>Potential NWIP - See US Presentation</td>
<td>Potential NWIP - defines critical quality attributes</td>
<td>Cell Counting - Part 1</td>
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<tr>
<td>Identity</td>
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<td>Cell Counting - Part 2</td>
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<td>Biological activity</td>
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