CELL COUNTING BREAKOUT SESSION

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Qualification / Validation of Cell Counting 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 Cell Counting Assays

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

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

### Recommended Options
- Spiking
- Check against alternative method
Acceptance Criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Variance</th>
</tr>
</thead>
<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
Troubleshooting
Cause and Effect Examples

Material Preparation
- Toxicity
- Cell aggregates
- Homogeneity
- Buffer/Dye consistency
- Dust / Debris / Particles

Sampling
- Sampling location
- Mixing
- Homogeneity
- Accuracy pipetting
- Adherence

Counting
- Settings
- Focal plane
- Math-Recording errors
- Instrument-to-instrument inconsistencies

Look for Patterns Contributing to Data Artifacts
END