CELL COUNTING BREAKOUT SESSION

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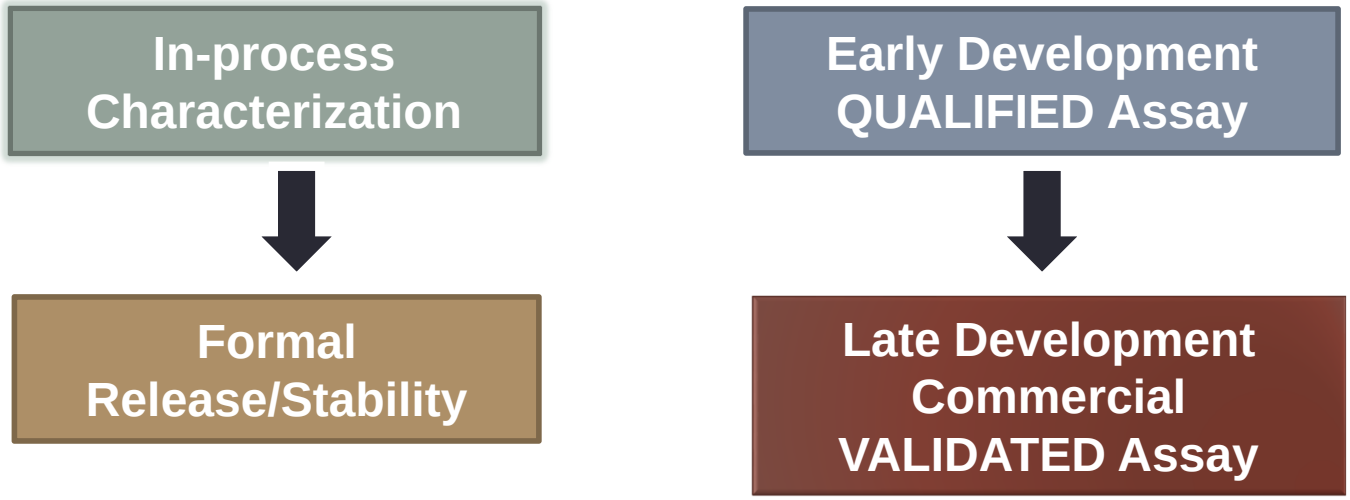
Qualification / Validation of Cell Counting Assays

- What is the Purpose and Scope of the Method?

Fit-for-Purpose

- Consider the Intended Use of the Data



Qualification / Validation of Cell Counting Assays

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

Study Design

Parameters to Test	Considerations
Sample lots	• Minimum number • Inherent diversity
Instrument	• IQ/OQ/PQ • Settings
Analysts	• Minimum number • Experience
Environment	• Temperature & Humidity (static)
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

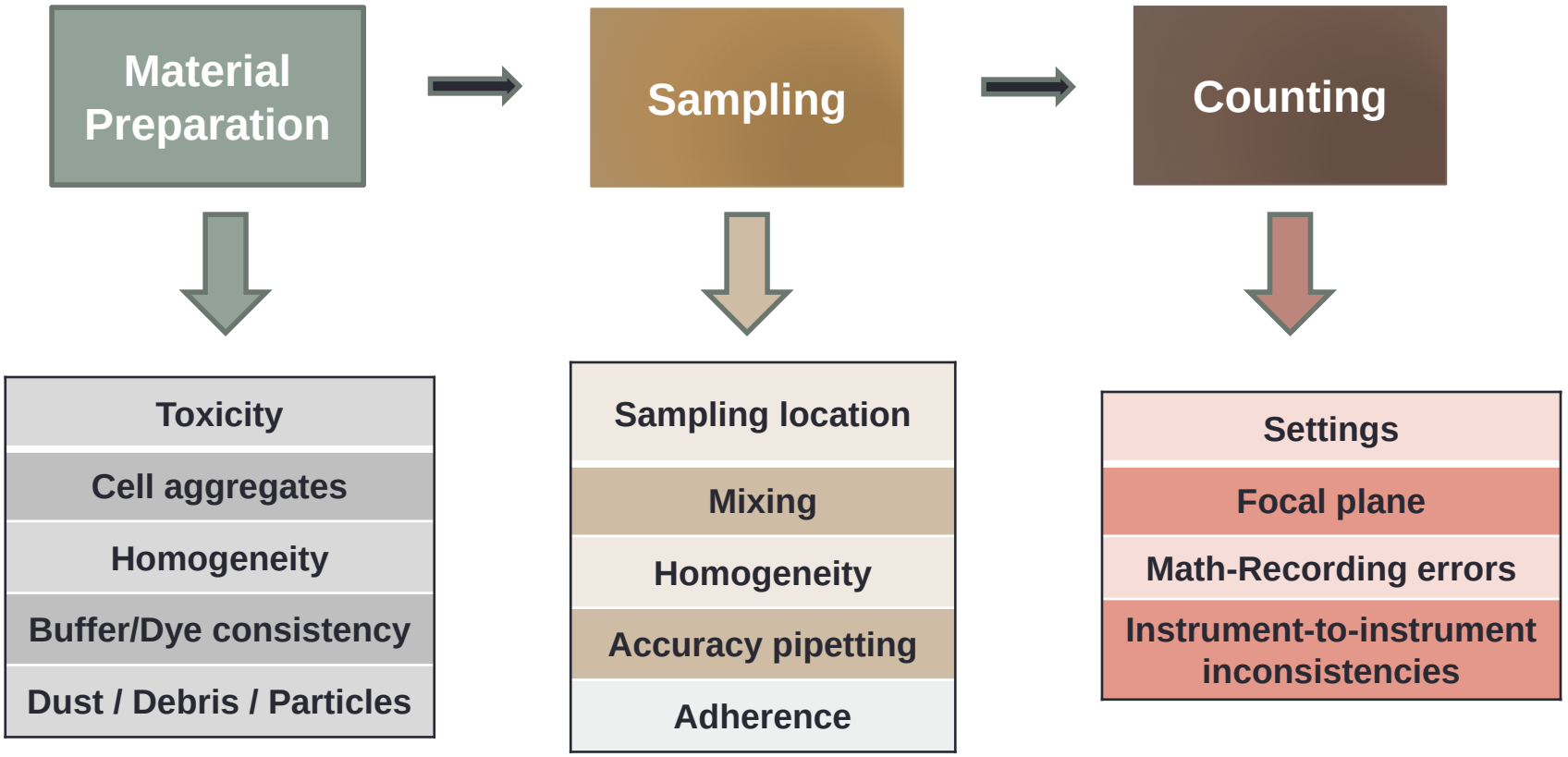
Criteria	Variance
Repeatability / precision	≤ 30%
Specificity	≤ 5%
Linearity	$R^2 \geq 0.95$
Range	Determined by instrument or method

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



Look for Patterns Contributing to Data Artifacts

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