

# NIST-FDA Cell Counting Workshop: Challenges in Cell Counting

Sumona Sarkar

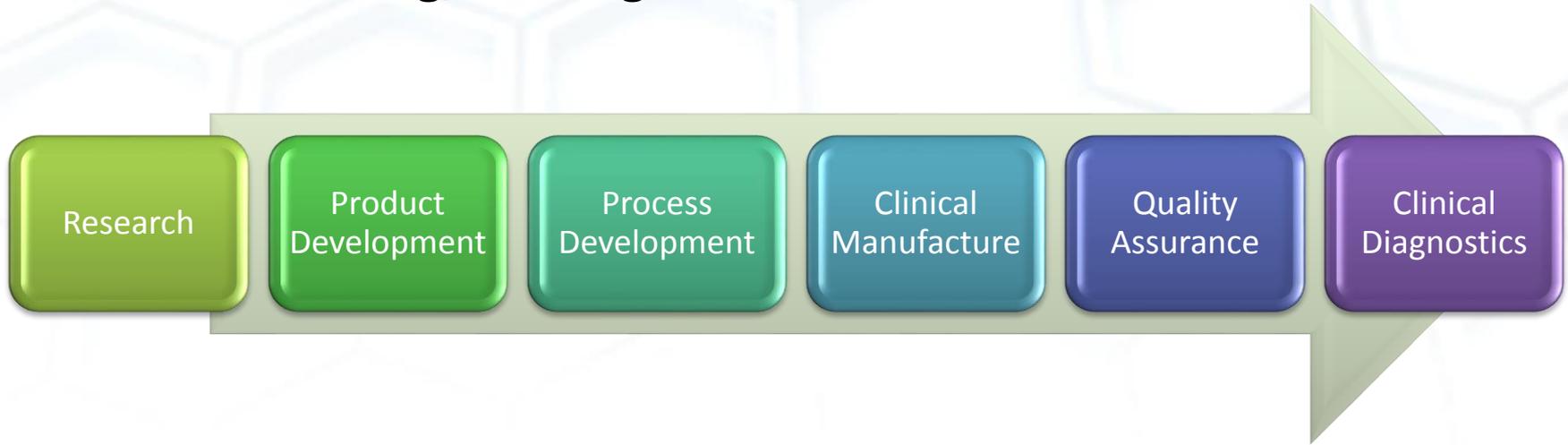
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# Cell Count

Cell count is a fundamental measurement in biotechnology, biomanufacturing, and regenerative medicine.

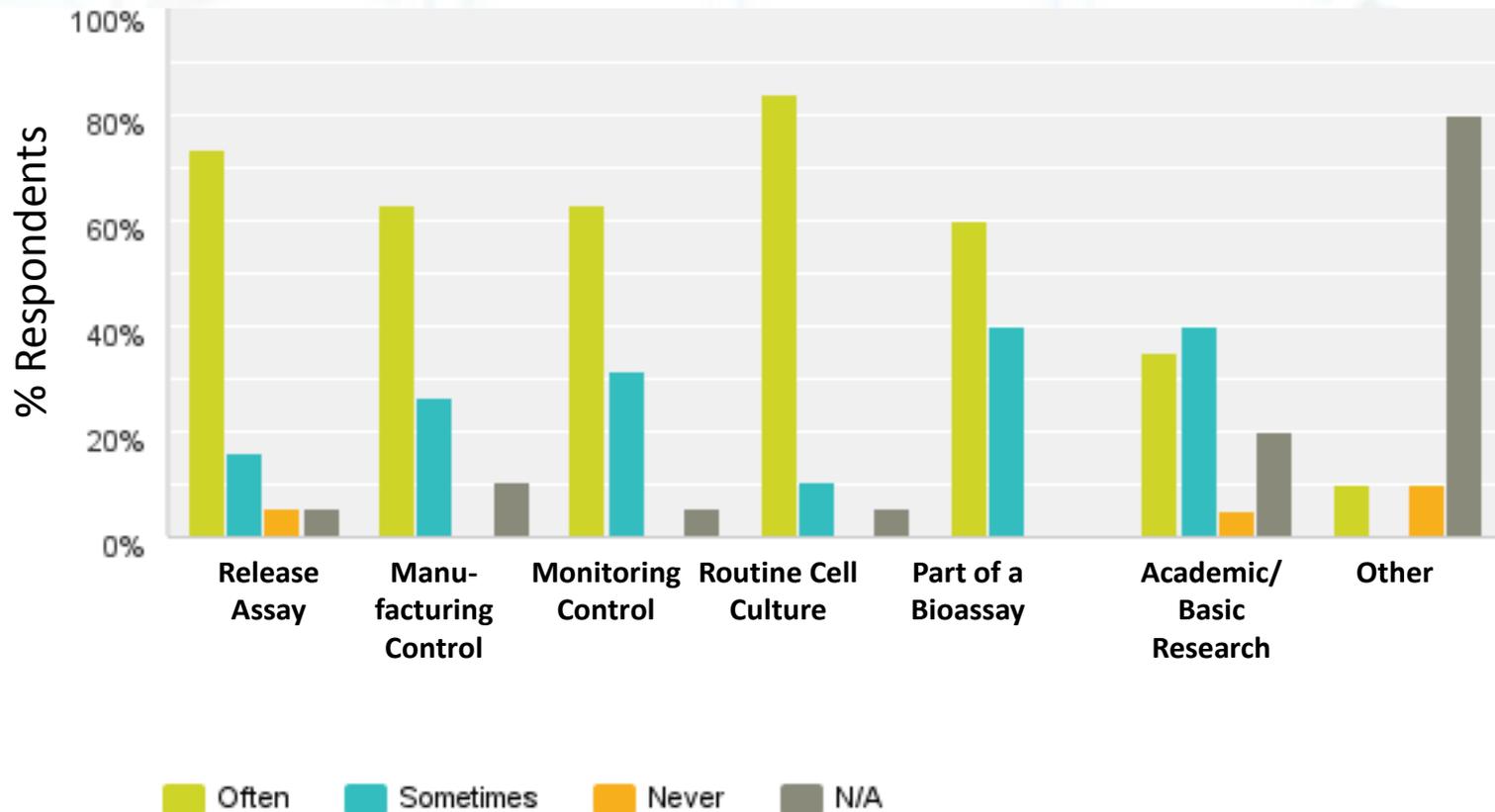


## Cell number can effect:

- Potency and efficacy of a cell therapy treatment (e.g. dosing)
- Manufacturing process for cells and biopharmaceutical products
- Rate of growth of regenerated tissue in a biomaterial scaffold
- Bioassays that are normalized by activity per cell
- Functional assays based on cell count (e.g. proliferation)

# Workshop Participants

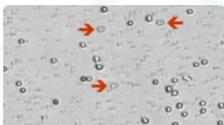
How often do you use cell counting for the following purposes?



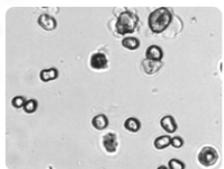
# Wide range of cell counting needs and methods

## Range of Sample Properties

Cell Suspensions with Debris (PBMCs)



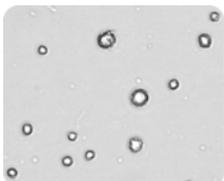
Irregularly Shaped Cells (Primary Hepatocytes)



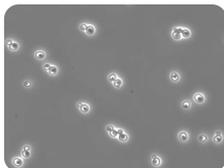
Clumpy Cells (MCF-7 breast cancer cell line)



Cells of varying size (MSCs)



Budding Cells (Yeast Cells)



## Range of Intended Purposes

In-process count estimate for feed rate

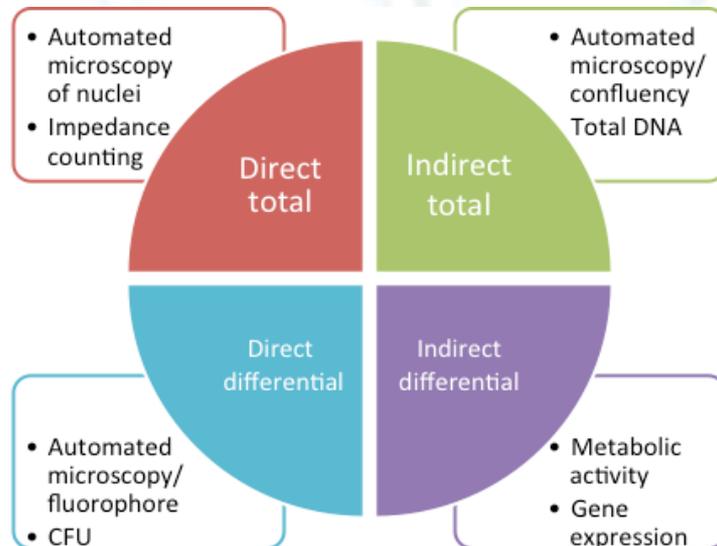
Count estimates for passaging and culture

Monitoring cell response to stimuli

Count and Viability as an indicator of potency

Accurate counts for cell therapy dosing

## Range of Counting Methods

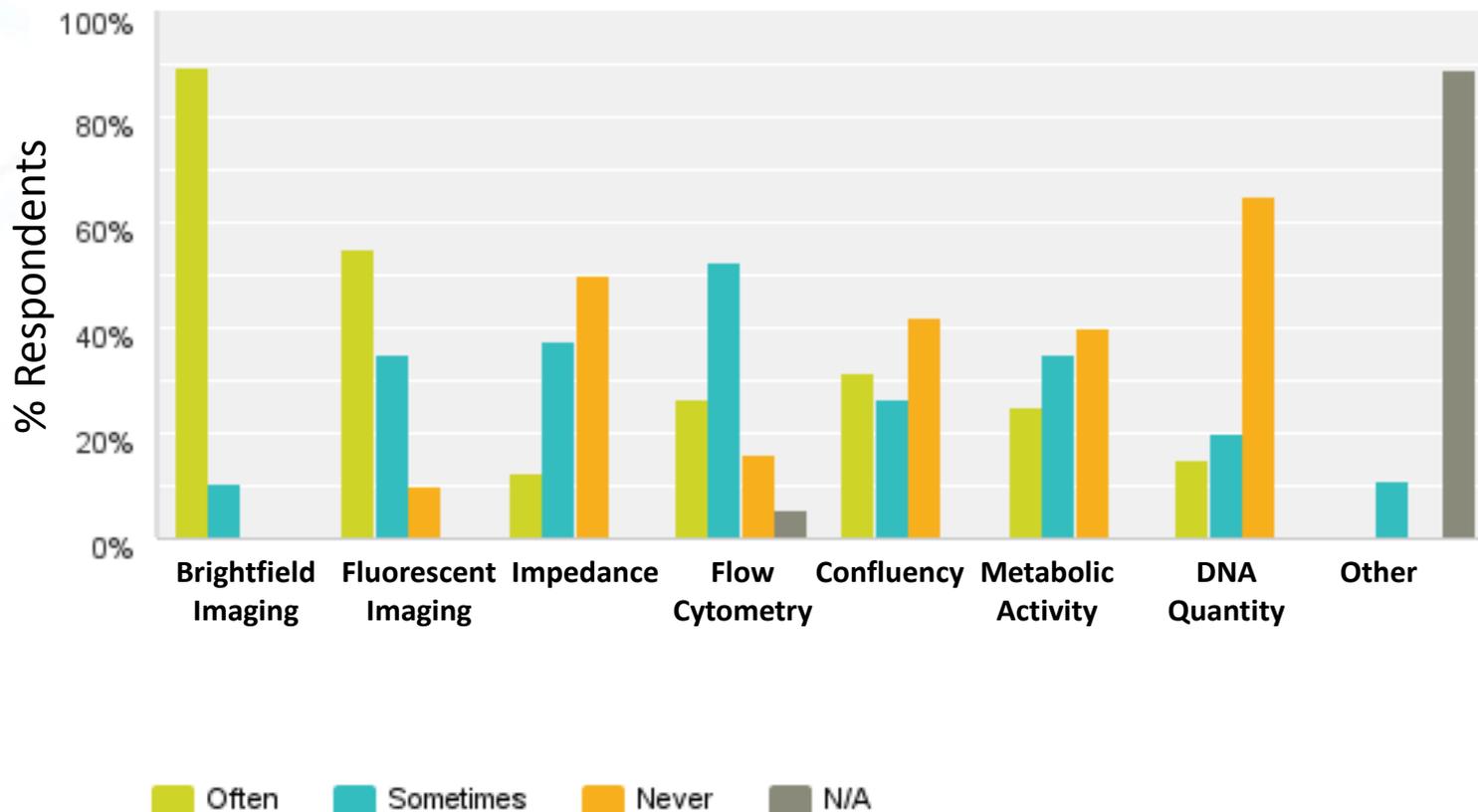


What strategies can we implement to assure confidence in cell counts across these broad needs?

Images: nexcelom.com and NIST

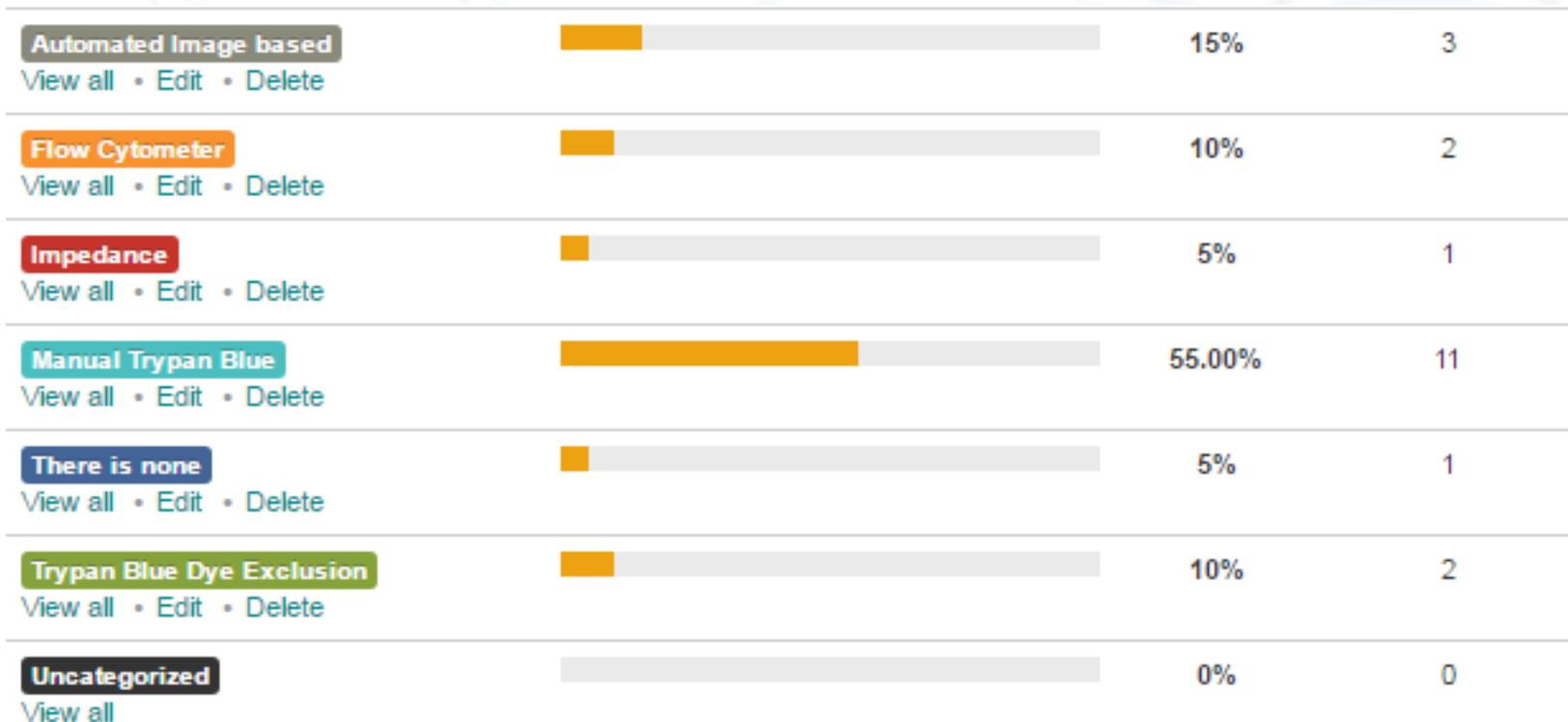
# Workshop Participants

How often do you use the following cell count methods?



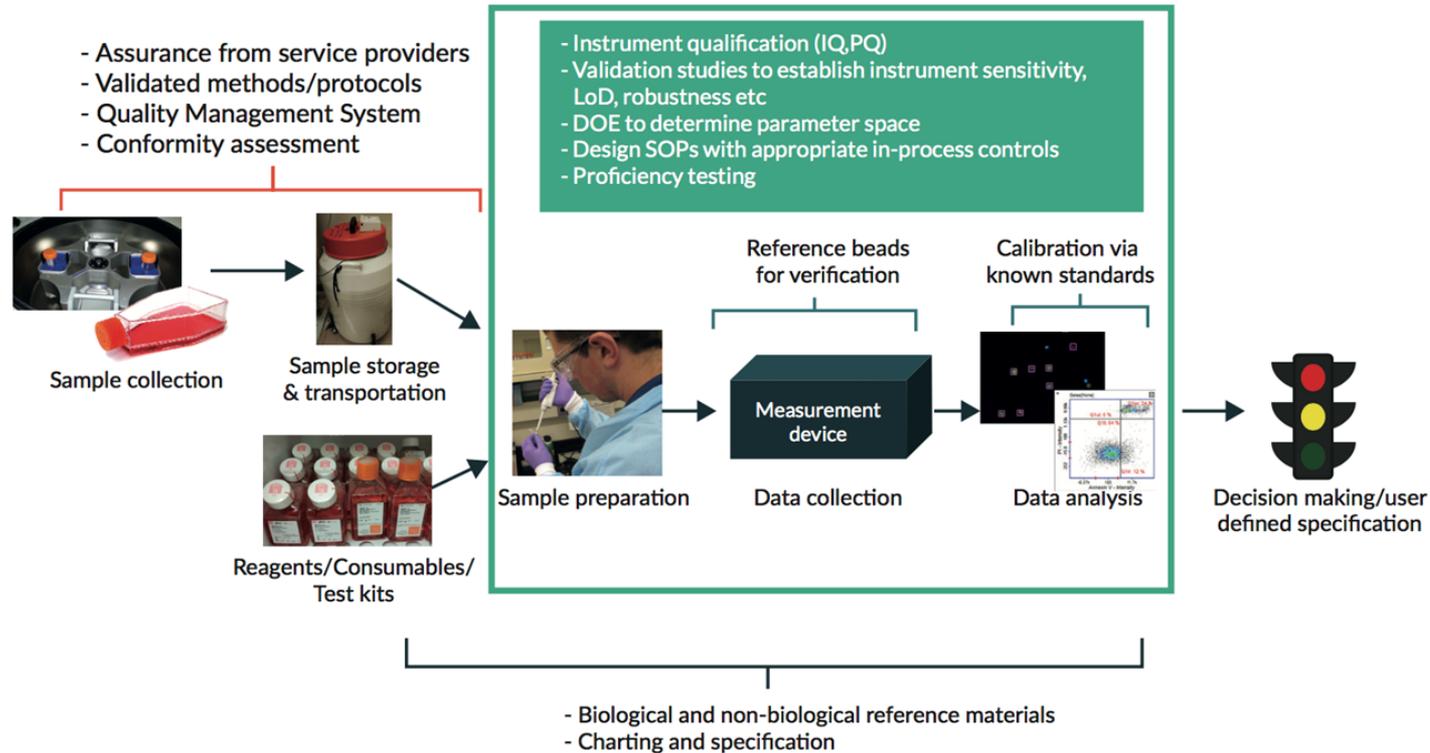
# Workshop Participants

What do you consider to be the gold standard (or reference method) for cell counting?



# Measurement Process for Cell Counting

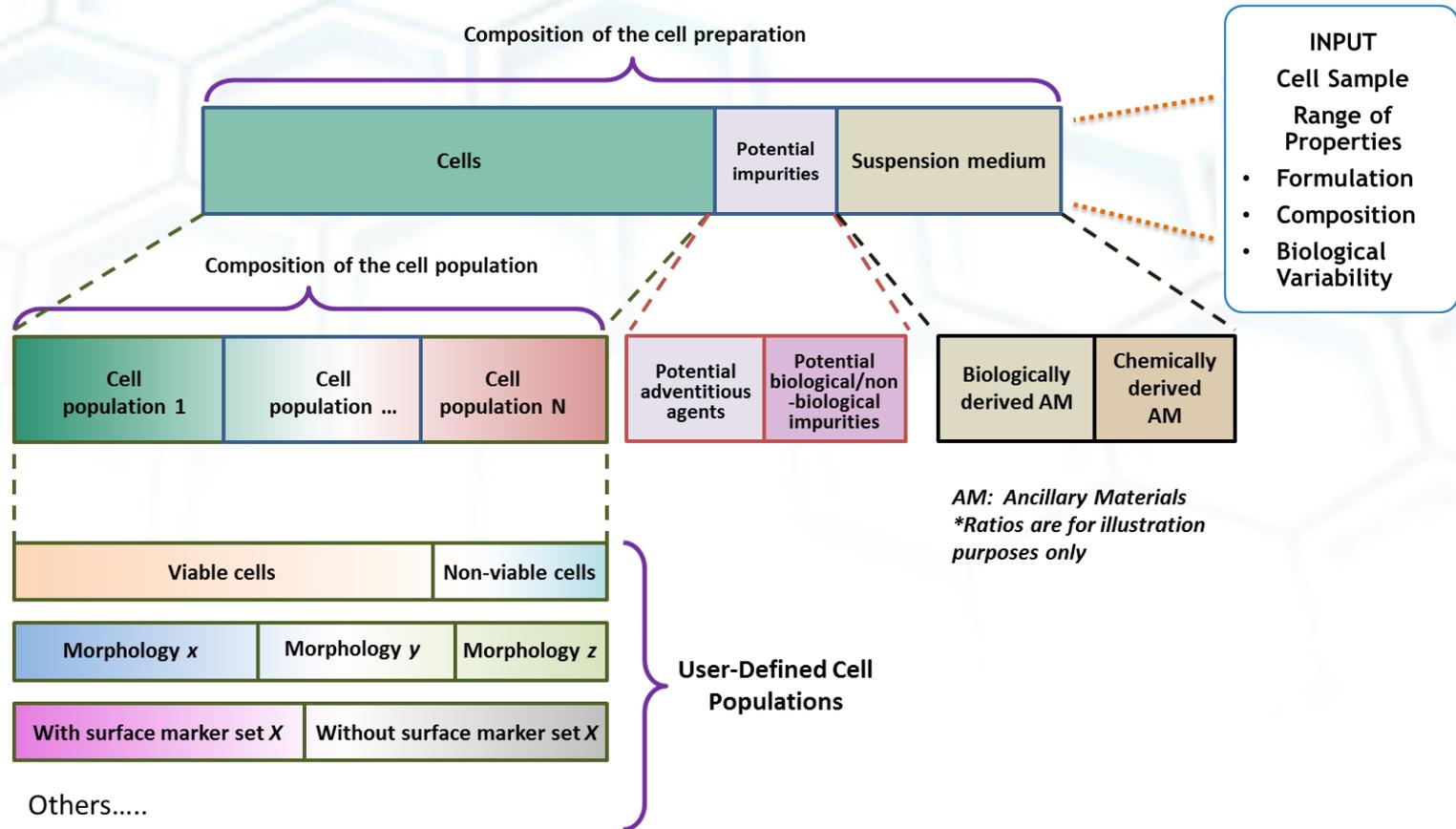
An example of a generalized cell counting process that involves an automated imaging device, and where potential controls and standards for managing and minimizing sources of variability could be used.



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

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.

# Cell Sample Composition Can Affect Cell Counting

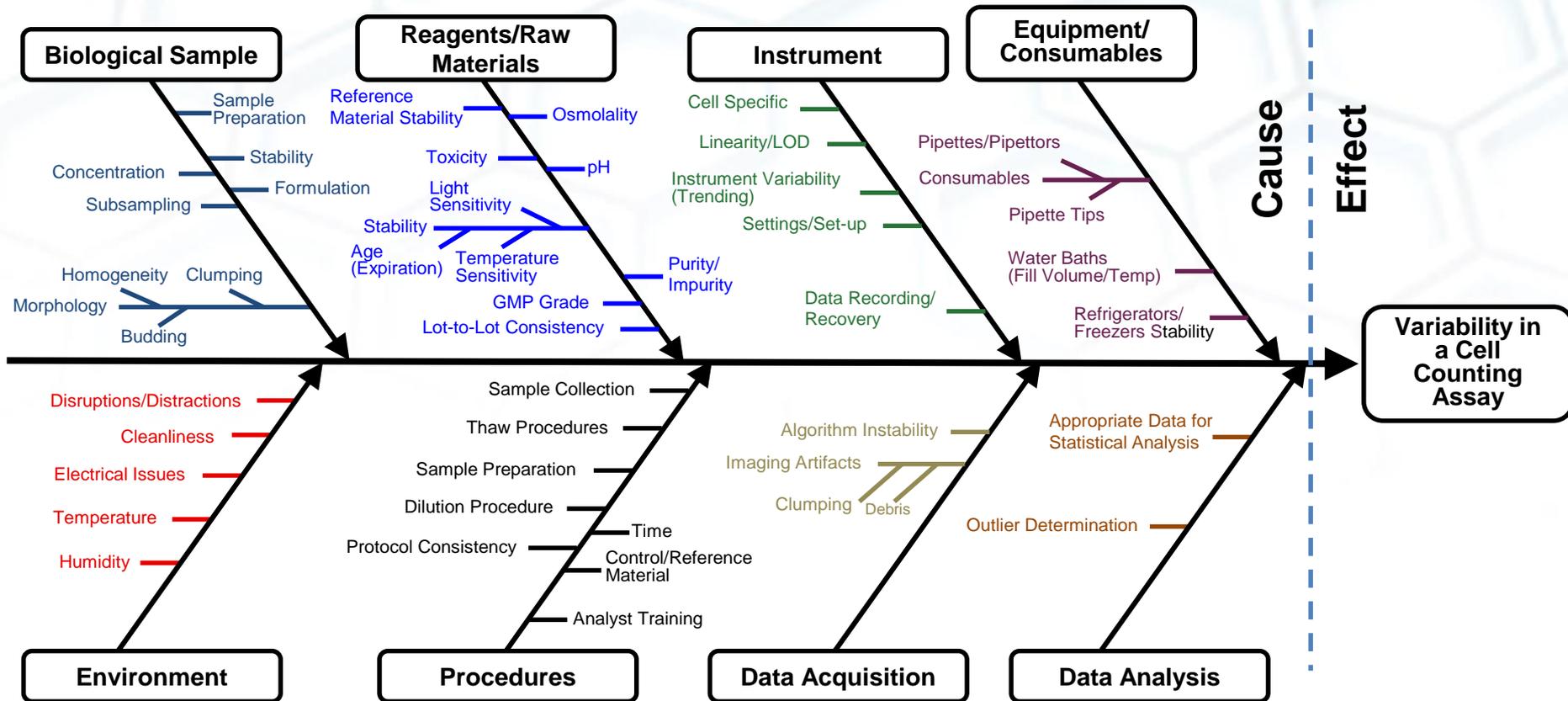


Many components of the cell sample can influence an analytical method

What are the samples properties to considered when conducting a cell counting method?

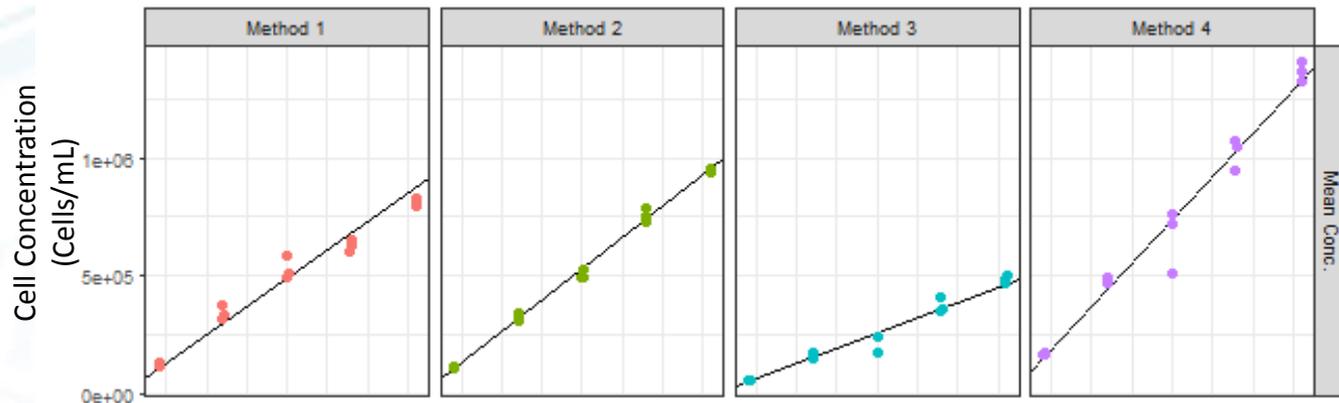
Lin-Gibson, S., Sarkar, S., & Ito, Y. (2016). Defining quality attributes to enable measurement assurance for cell therapy products. *Cytotherapy*, 18(10), 1241-1244.

# Sources of Variability in a Cell Counting Measurement Process



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.

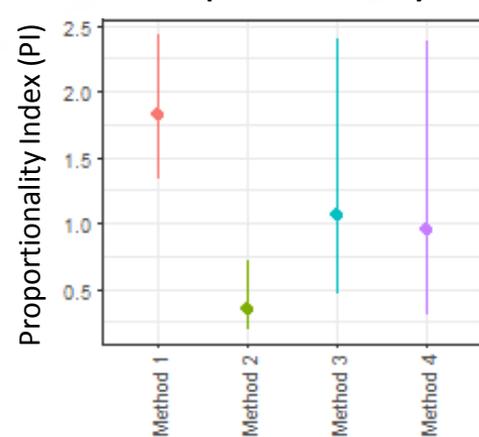
# Evaluating Cell Counting Methods



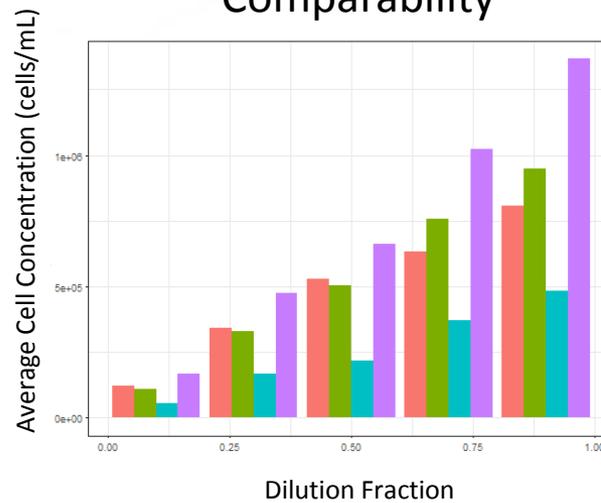
- Comparability between methods
- Accuracy
- Precision
- Linearity (proportionality)
- Reproducibility

## Experimental Designs to Evaluate the Quality of a Cell Counting Method

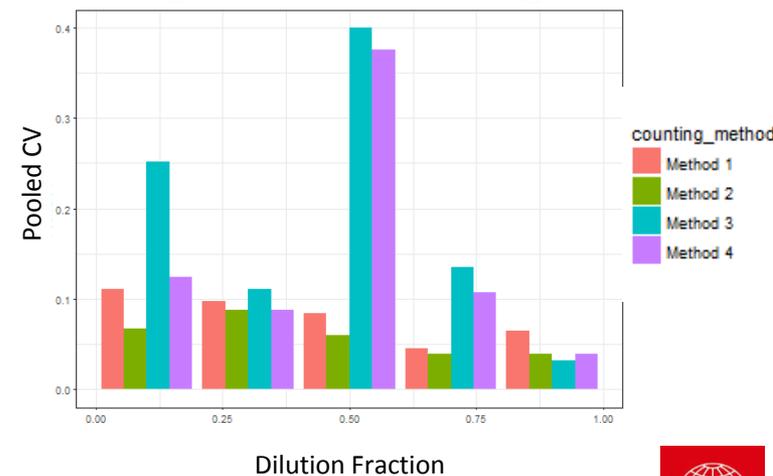
### Proportionality



### Comparability



### Precision



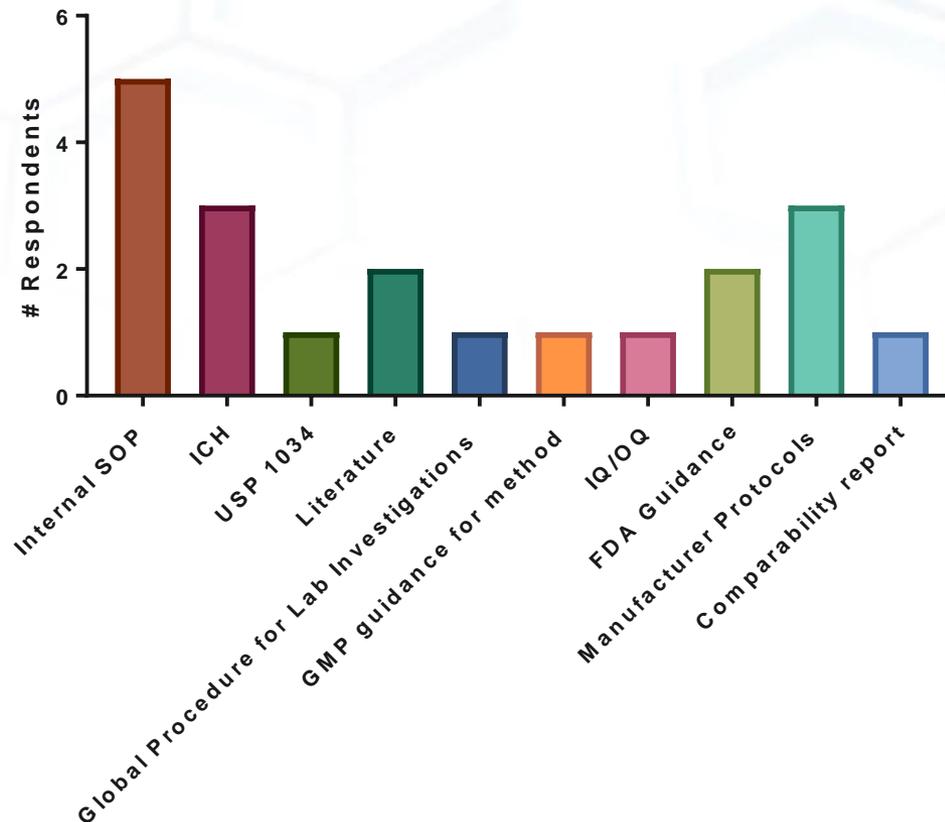
[ISO/WD 20391-2](#) [Under development: 20.60]

Biotechnology -- Cell Counting -- Part 2: Experimental design and statistical analysis to quantify counting method performance



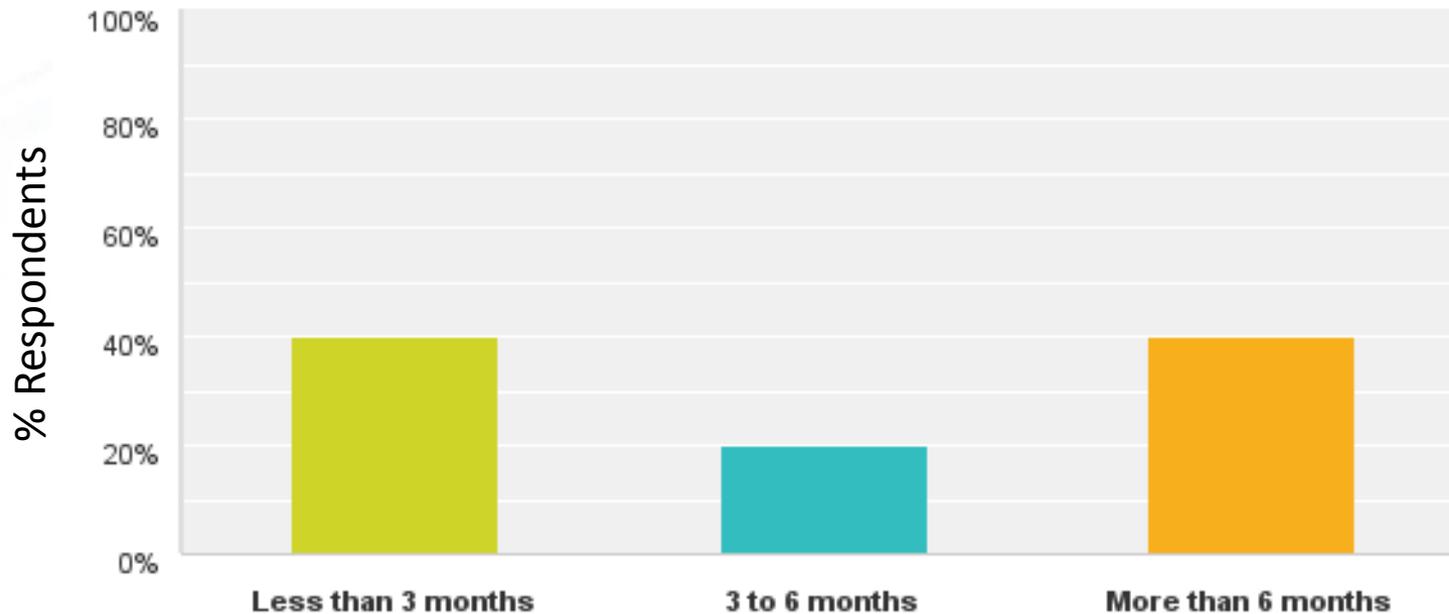
# Workshop Participants

List guides, documentary standards, best practices etc that you used when establishing your cell counting measurement

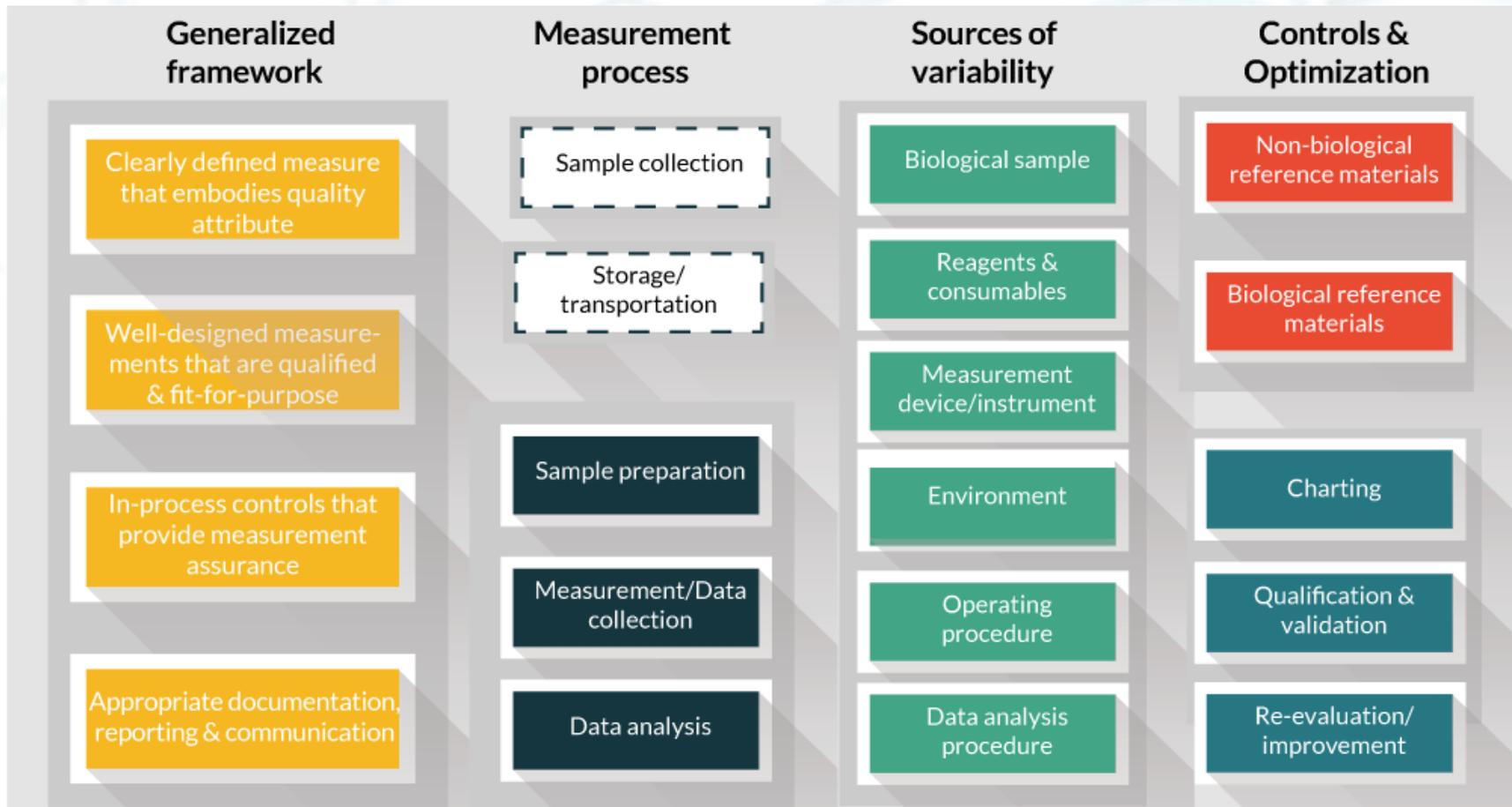


# Workshop Participants

How much time was spent developing a validated cell counting measurement?

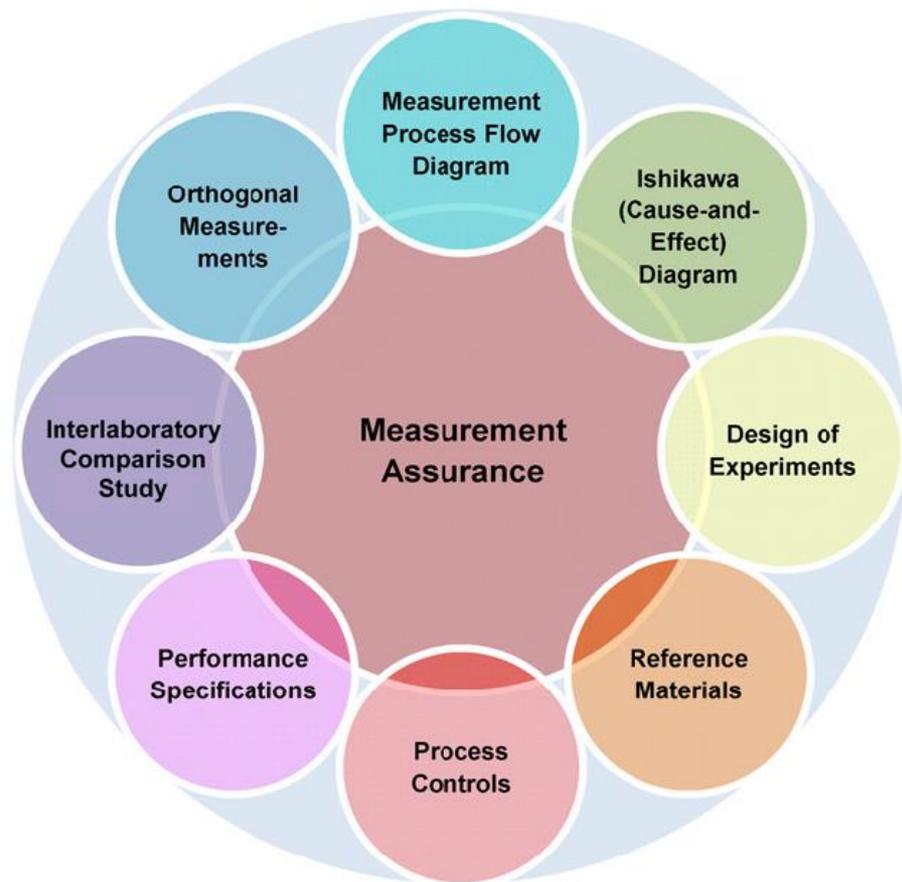


# Designing a Cell Counting Measurement Process that is Fit-for-purpose



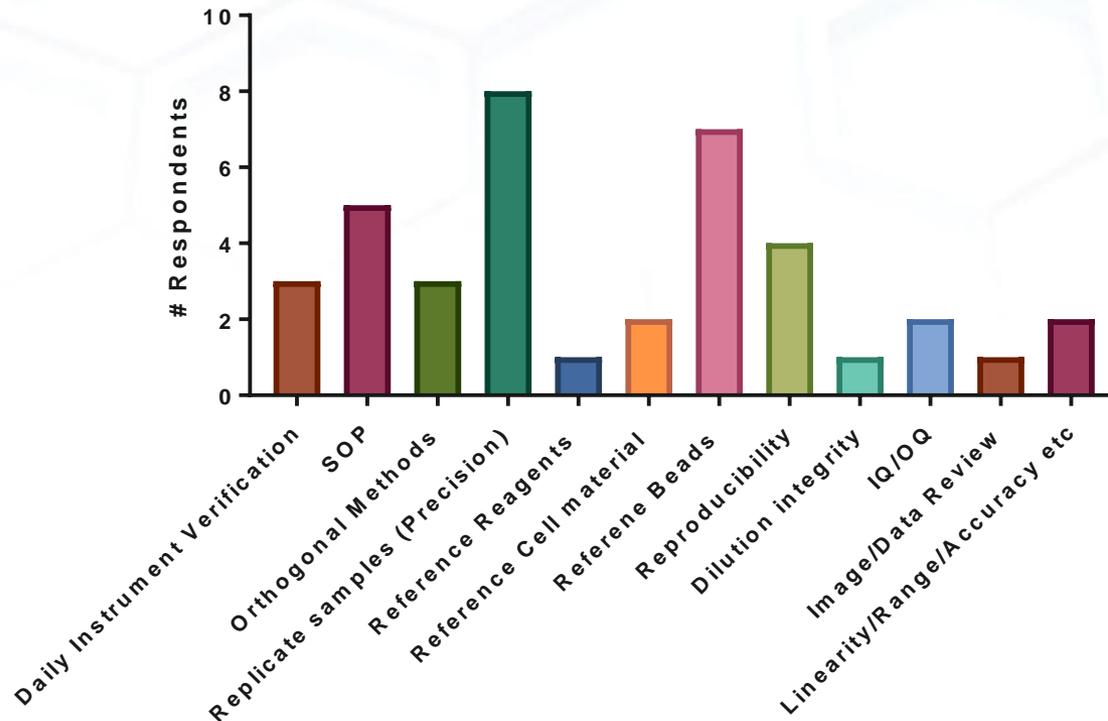
Lin-Gibson, S., Sarkar, S., Elliott, J. T., & Plant, A. L. (2016). Understanding and managing sources of variability in cell measurements. *Cell Gene Therapy Insights* 2016;2(6),663-673.

# Measurement Assurance Strategies to Keep in Mind

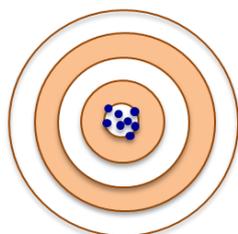
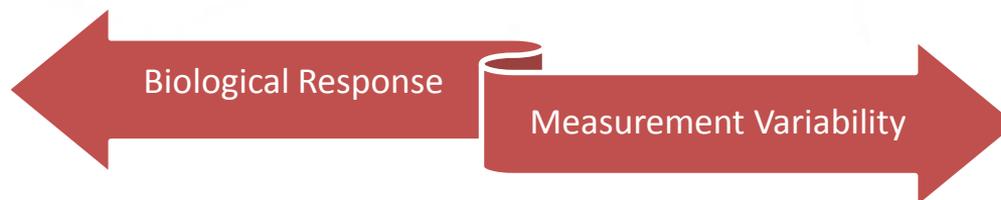
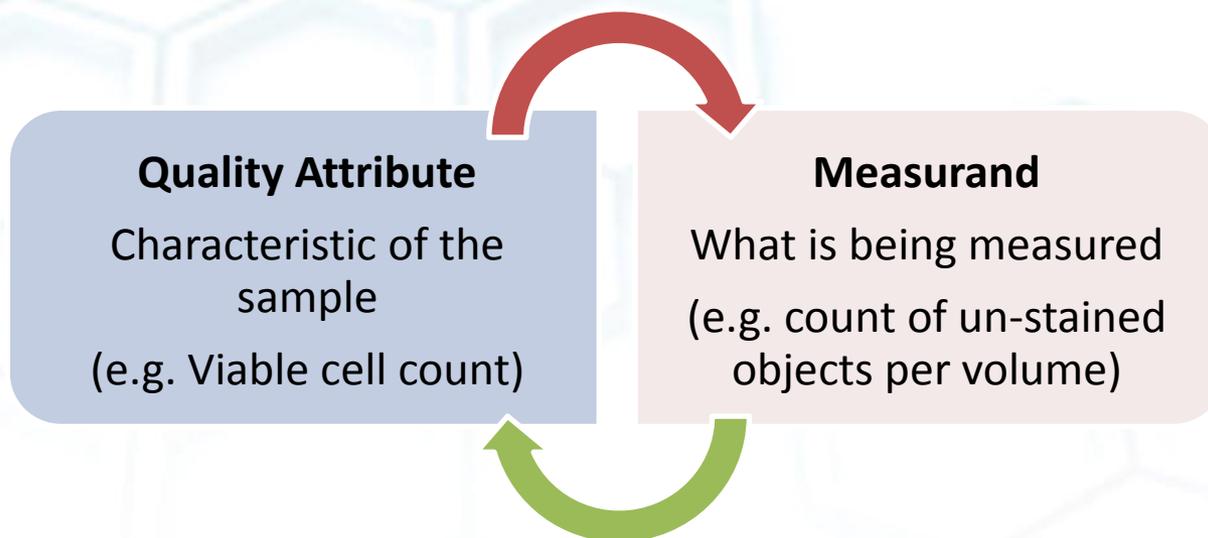


# Workshop Participants

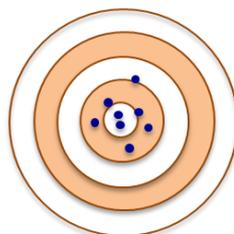
List measurement assurance strategies for cell counting that you currently apply



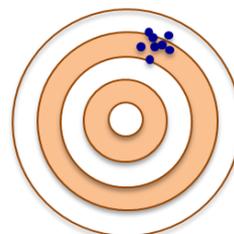
# Concepts to Keep in Mind



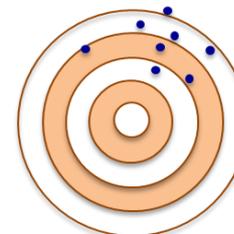
✓ Accuracy  
✓ Precision



✓ Accuracy  
✗ Precision



✗ Accuracy  
✓ Precision



✗ Accuracy  
✗ Precision

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