Measurement Assurance for Cell-based Assays: Cell Count

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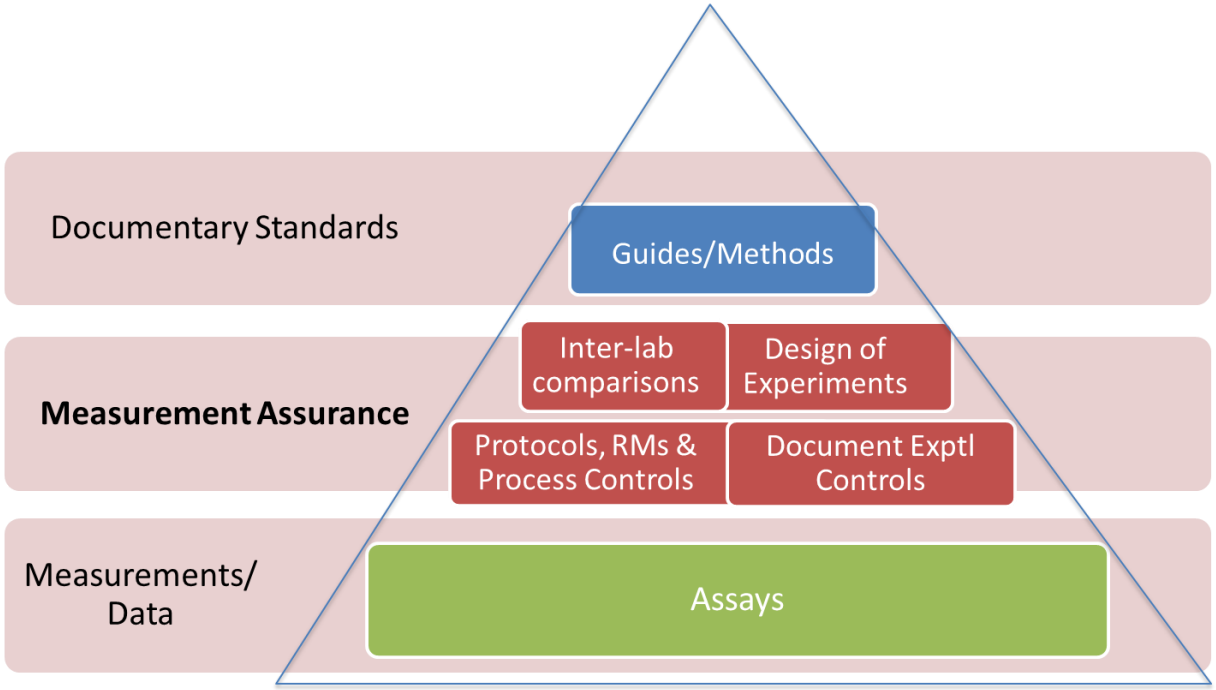
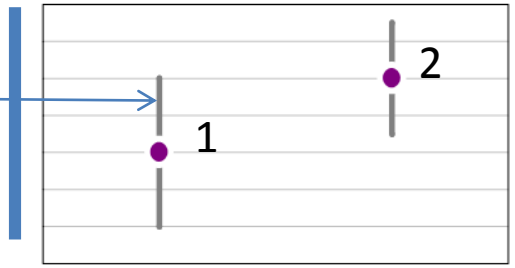


What is Measurement Science?

- A systematic approach that informs on the comparability and trust in a measurement result
  - Data-based decision making
- Components of a measurement:
  - Value- it is on a scale; enables compared to other measurements
  - Uncertainty- variability in the measurement; enables statistics
  - Evidence- evaluation of the measurement system; confidence

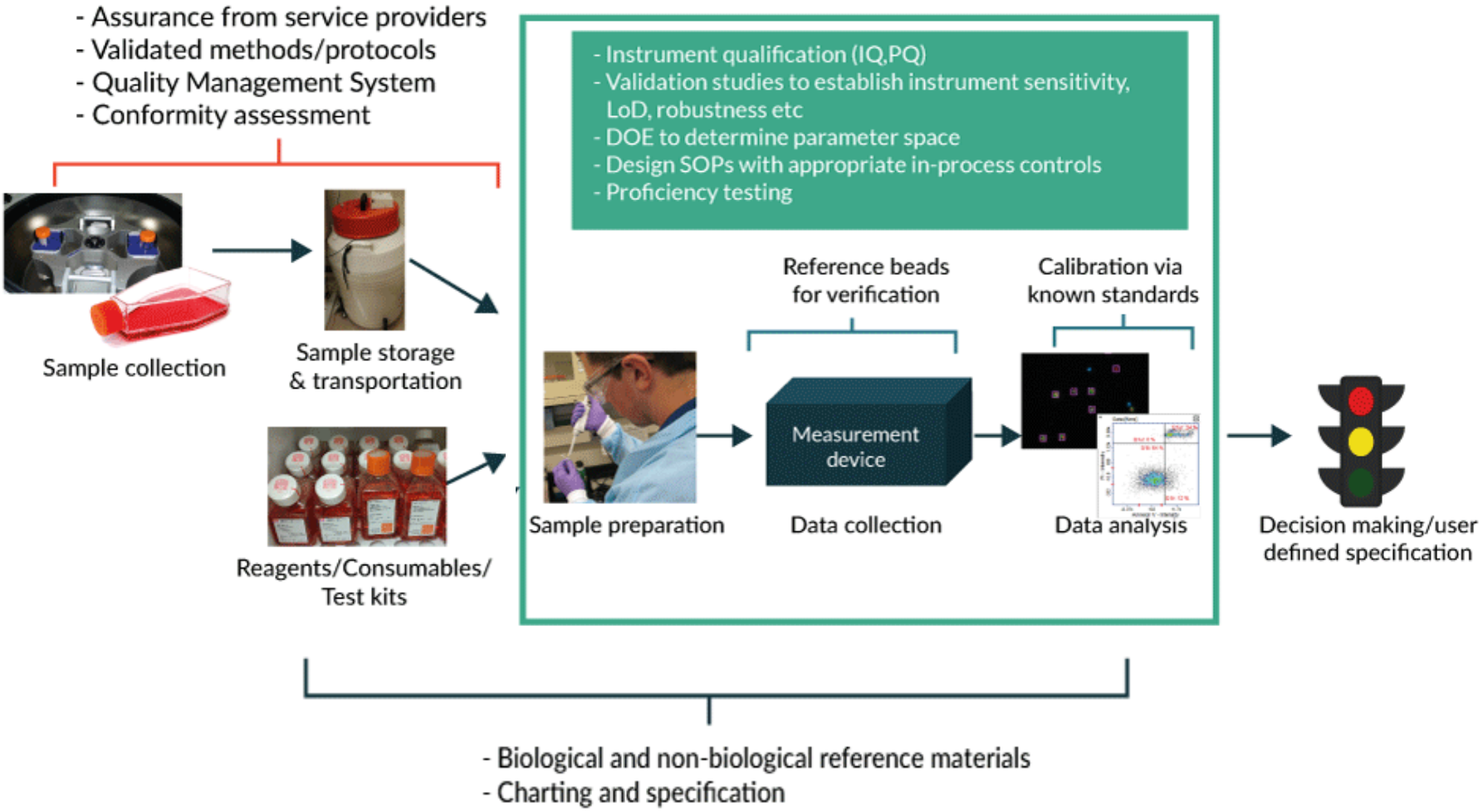


✓ Controls meet specification

What is Measurement Science?

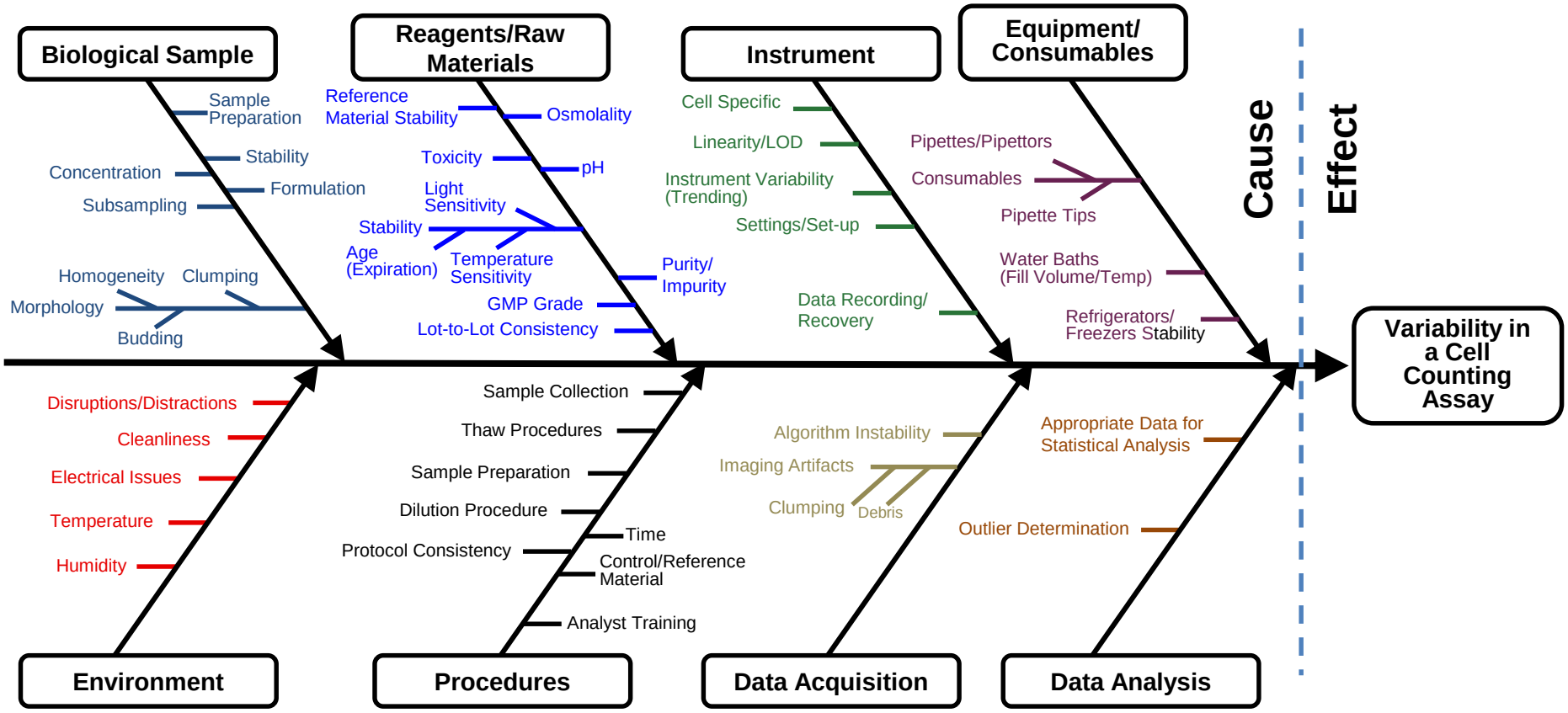
- Treat the assay as a measurement process
- Control experiments provide evidence that the measurement process is proceeding as expected
- Adapt the process quality tools to cell assays
  - Cause and effect diagram
  - In-line controls
  - Control charting
  - Sensitivity analysis
  - Experimental design
  - Interlaboratory testing
  - Acceptance specifications
- Increases confidence in the measurement

A Cell Count Measurement 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.

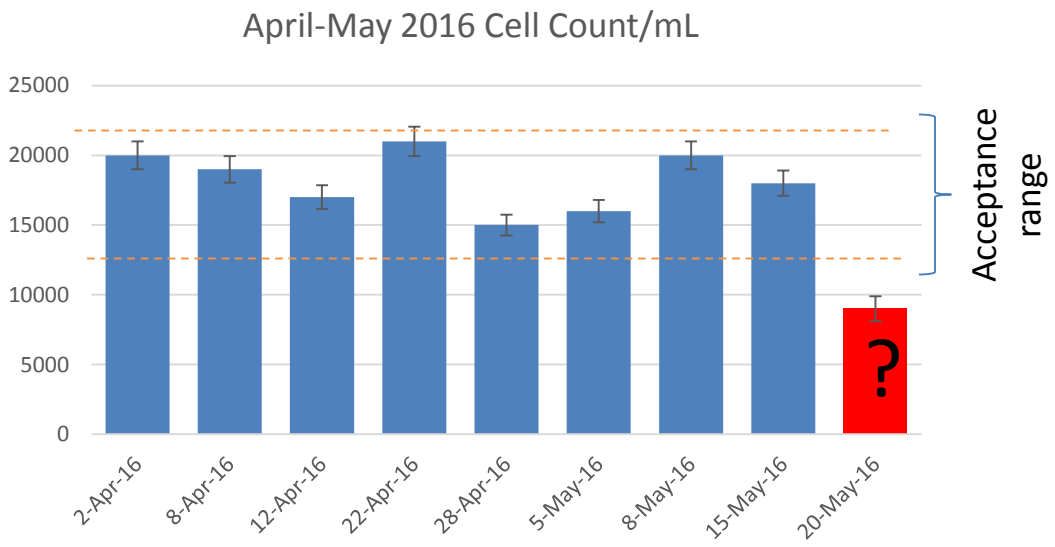
Cause and Effect Diagram for Generalized Cell Count Measurement



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.

Use Case: Expanding a Stock Cell Line

- Thaw a stock vial of cells, expand cells for 4 days, count cell concentration



- Is this right?
- What evidence do I have that provides measurement assurance (i.e. confidence)?

Dissecting the Cell Count Measurement

Measurement= $\frac{\#cells}{volume}$

Assumptions:

1. Single cell suspension
2. Count all cells
3. Known volume tested
4. Reagents/Instrument work as expected
5. Linear range, above LOD, etc

- Measurement science approach (i.e. what is evidence that these assumptions are true?)
- Are there control experiments that can detect the following?

Reagents bad
Cells in aggregates clog in fluidics
Change in instrument detection

Not in linear range
Volume detection bad
Fluidics malfunction

What does the evidence look like?

- Data from control experiments provide the evidence for measurement assurance.
- They are method dependent and inform on parts of the measurement process.
- Can be in-frequent measurements (i.e. linear range, LOD, instrument settings, matrix effects)
- Can be in-line measurements that provide confidence in the measurement system/process

Example: Cell Count via Imaging

TABLE 1

Examples of in process controls for an automated imaging cell counting measurement process.

Aspect to be validated	Specific concerns	In-process control	Specifications
Reagent quality	Reagents are free of contaminants and precipitants	Running a reagent only control	Object count and object size distribution are within specification for a no-cell control experiment
Image background intensity and distribution	Imaging chamber is clean and illumination is even across image to ensure proper image analysis	Review of raw image data	Raw image background intensity is within specification
Object spatial distribution	Flow into the imaging chamber is unobstructed	Review of processed image data	Objects are distributed randomly and within specification
Object size distribution	No large aggregates or small debris	Review of processed image data	Object sizes are within specification
Camera focus/ magnification	Image is in focus and magnification is correctly set	Running a control material with known size	A control material of known size is within specification

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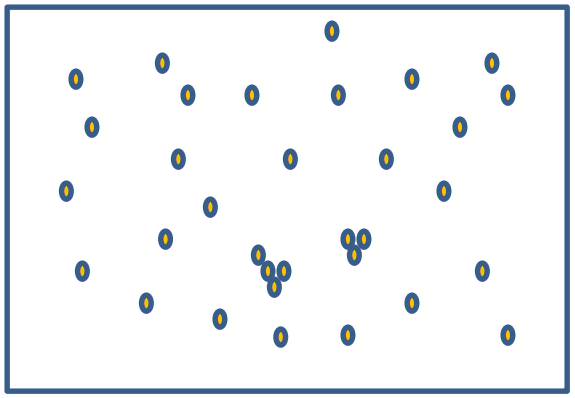
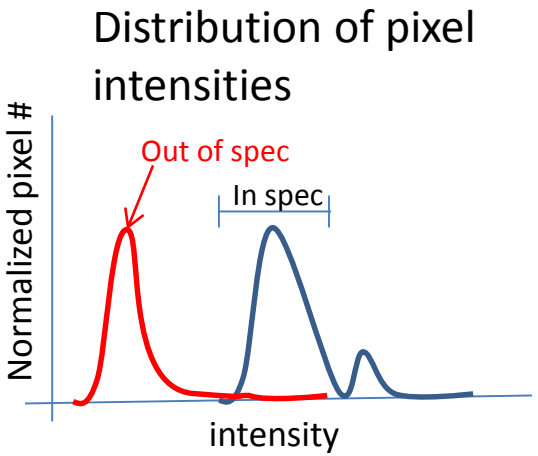
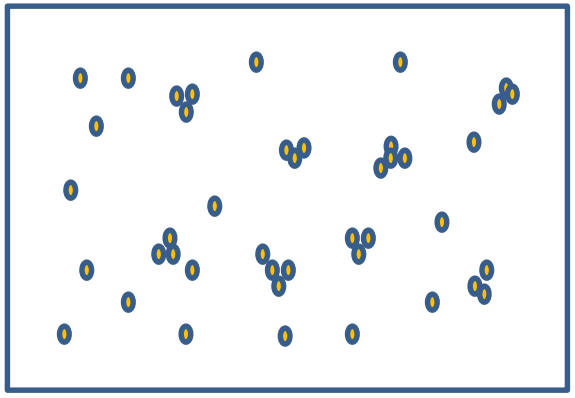
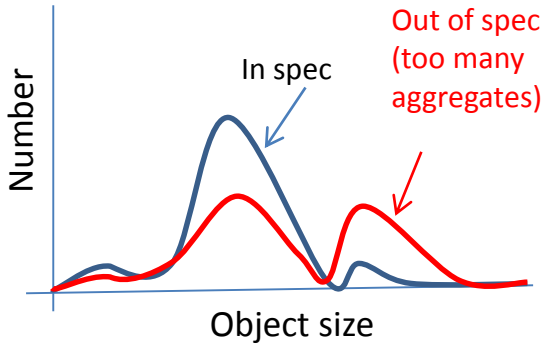
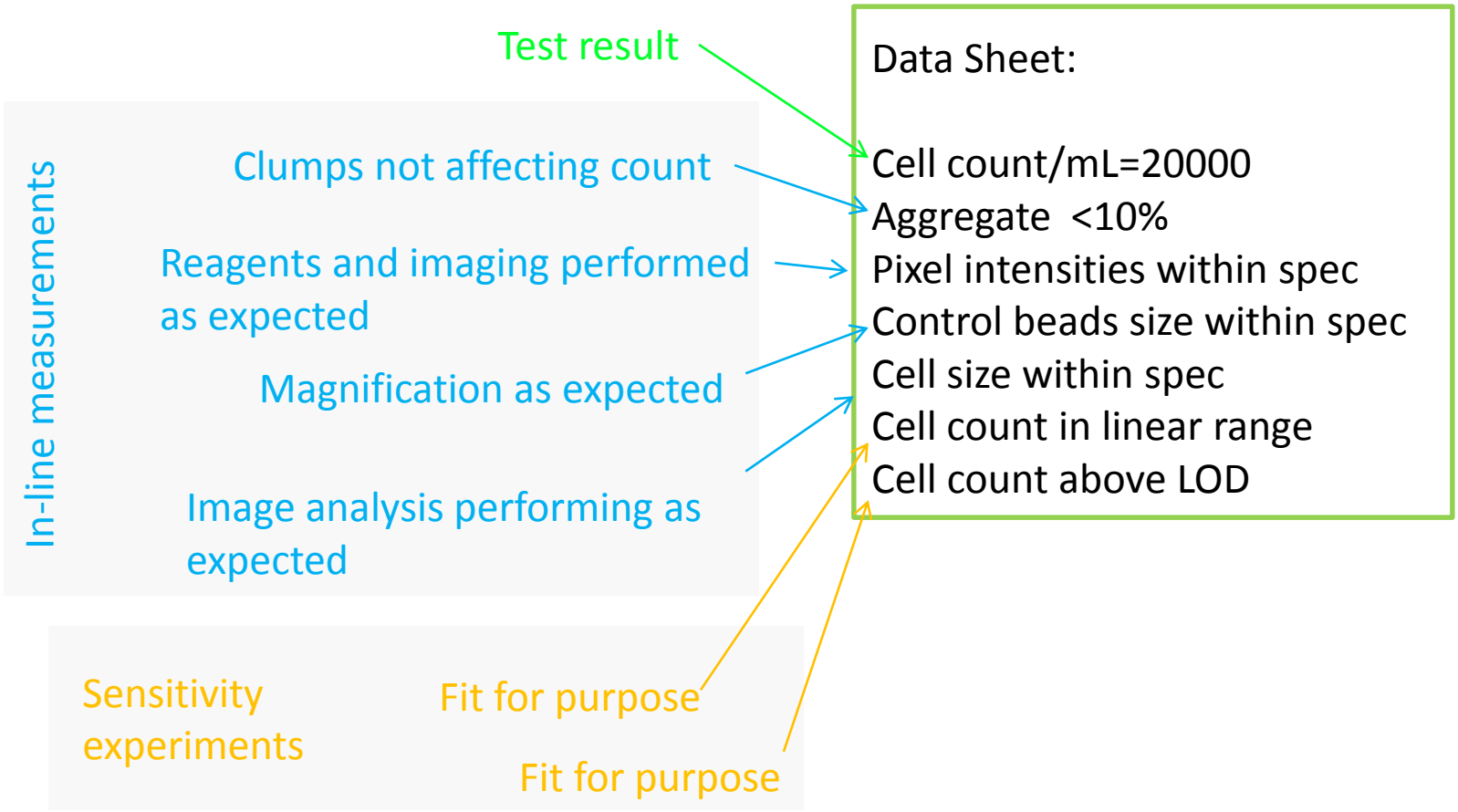
What might the data look like?

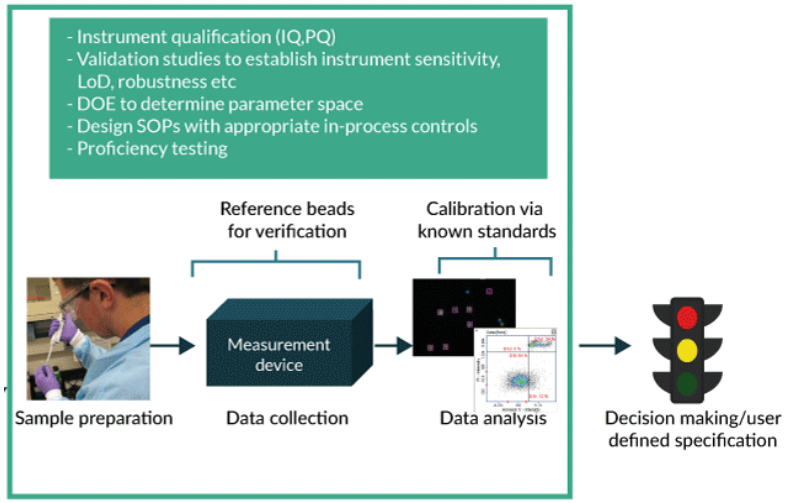
Evidence for:	Count Image	Measurement
Reagent quality, illumination and detection system		
Cell clump, sample prep		

Evidence provides Confidence in the Measurement!



Summary

- Treat the assay as a measurement process.
- What are the sources of variability?
- Dissect the primary measurement and think about assumptions.



- Design sensitivity experiments and in-line measurements to validate assumptions. Evidence!
- Method dependent, cell dependent, sample prep dependent, but identification of generalized methods used for measurement assurance in cell counting would be applicable to many use cases.
- Research in these measurement assurance strategies could lead to standards in the future.