How to Achieve Confidence in Complicated Measurements

Measurement Assurance For Regenerative Medicine and Advanced Therapies

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Challenges for Regenerative Medicine and Advanced Therapies Products

1. Characterization of product

Quality Attributes
- Identity
- Quantity
- Purity
- Sterility
- Potency

2. Control of the manufacturing process

assure consistency of product during Scale Up,
Change in personnel, process, location
Improved methods, Changes in raw materials
Ground Truth
Ground Truth
Hard to find in biology

...it is important to understand your measurements:

• What is the measurand?
• Is your assay measuring what you intend?
• Are there assay variables (personnel, reagents, unknown factors) that are influencing the assay result?
• Can other labs get the same result?
• Is the measurement biologically meaningful?

…..Compared to what?
Understand your measurements:

- **Qualify the assay**
  - ACCURACY: Orthogonal method
  - PRECISION: Reproducibility: same day replicates, day to day, different technicians
  - ROBUSTNESS: sensitivity to assay parameters
  - SPECIFICITY: sensitivity to matrix effects
  - DYNAMIC RANGE AND RESPONSE FUNCTION: Instrument benchmarking. +/- controls. Calibration curve. Limit of detection

Ground Truth
Hard to find in biology

.....Compared to what?
Measurement Qualification

- Are the results the same?
  - Can’t tell *precision* without sufficient replicates that demonstrate dispersion in the measurement.

- Are the results accurate?
  - Can’t tell *accuracy* without something to *compare* it to.

NIST, Salit/Deuwer
Comparability through reference materials

Easy to imagine for measurement of lead in water

Harder to envision for measuring complex biologicals and biological function
What is measurement assurance?

Knowing the level of confidence you have in the data that you are using to make a decision.

Having the data that provide credibility of the measurement result.

There are many strategies for achieving measurement assurance
Assuring comparability: Interlaboratory studies, Design of Experiment for Robust Protocols

Differences in absolute absorbance

Differences in measurement uncertainties

Differences in response functions

DoE

Result: robust protocol and comparable results
Identifying Sources of Uncertainty – Reportable Parameters?
Identifying sources of measurement uncertainty

Zook, et al., Nature Biotechnology 2014
Evaluating the performance of a cell counting method: Experimental design and statistical analysis
Assuring comparability in instrumentation: traceability to a reference material

NIST SRM 1934/ Calibrated fluorimeter
Fluorescein
Nile Red
Allophycocyanin (APC)
Coumarin 30

Equivalent Reference Fluorophore (ERF) Number

Different Manufacturers’ calibration beads

Not comparable to one another

Light obscuration flow instrument
For accurate bead concentration

Comparable to one another

Flow Cytometry Quantitation Consortium
81 Federal Register 136 (15 July 2016), pp. 46054-46055
ERF Value Assignment to Cytometer Calibration Microbeads Submitted by Consortium Members

Lili Wang
MIATA Reporting Framework

Minimal Information About:
- The Sample
- The Assay
- Data Acquisition
- Results
- Lab Environment

Manuscript: Materials and Methods

Hall of Fame
- Critical protocol variables
- Transparency
- Improved interpretation of results
- MIATA stamp
- Enhanced visibility of published work
- Increased citation rate
MIATA: What is it NOT?

MIATA is NOT

Any standard to apply to any assay or lab immune monitoring setup.

Does not touch upon when to run what assay and how to run it.

Is without prejudice on how immune monitoring is performed and results are obtained.
Is the assay sufficiently sensitive to detect a problem?

If you detect a problem, are you sure the assay results are correct?
The measurement process

Sample Collection
- Trypsin/collagenase/EDTA treatment

Sample Preparation
- Disaggregation of cell clumps

Sampling
- Dilutions

Counting Preparation
- Viability or Ab stain

Count remaining cells

Light scattering or microscopy to assess extent of disaggregation

Check accuracy of volumes by weight

Load hemocytometer
- Collect data via microscope
- Flow cytometer

Ishikawa (Cause-and-Effect) Diagram

Process Controls

Reference Materials

Performance Specifications

Orthogonal Measurements

Measurement Process Flow Diagram
Measurement Assurance is Necessary for Confident Decisions

Addressing these challenges will be a community effort:

- Tool/methods development
- Interlaboratory comparisons
- Data sharing

Some tools for achieving measurement assurance:*  
- Ishikawa (cause/effect) diagram to identify sources of variability  
- Design of Experiment  
- Process Controls  
- Charting  
- Validation specifications  
- Interlaboratory comparisons  
- Reference Materials for traceability  

* There are many different ways of realizing confidence in measurements.
Thank You