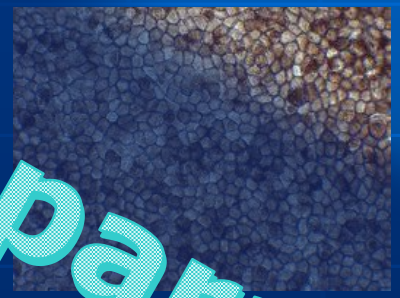
How to Achieve Confidence in Complicated Measurements

Measurement Assurance For Regenerative Medicine and Advanced Therapies

Anne Plant
Biosystems and Biomaterials Division
National Institute of Standards and Technology
Gaithersburg MD
anne.plant@nist.gov

Challenges for Regenerative Medicine and Advanced Therapies Products

1. Characterization of product



Comparability

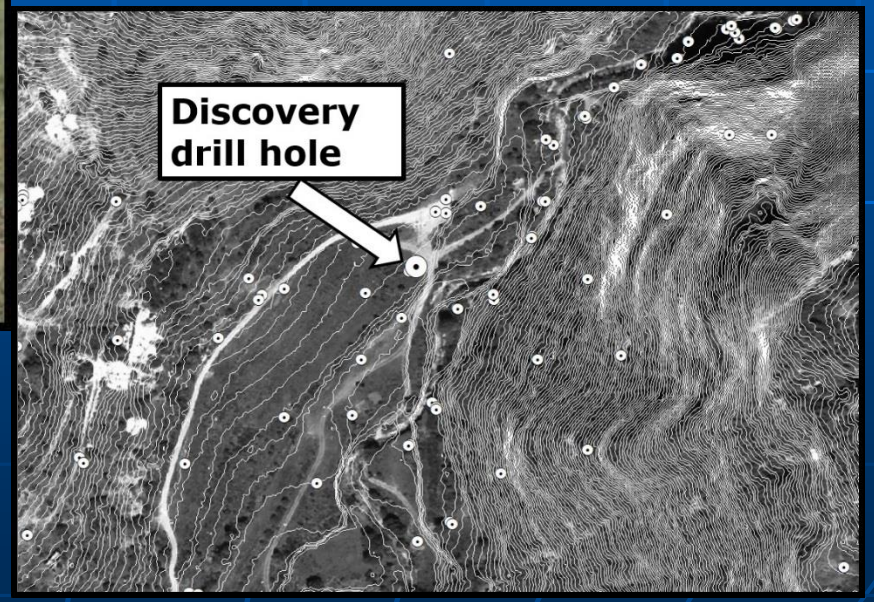
Quality Attributes

- 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

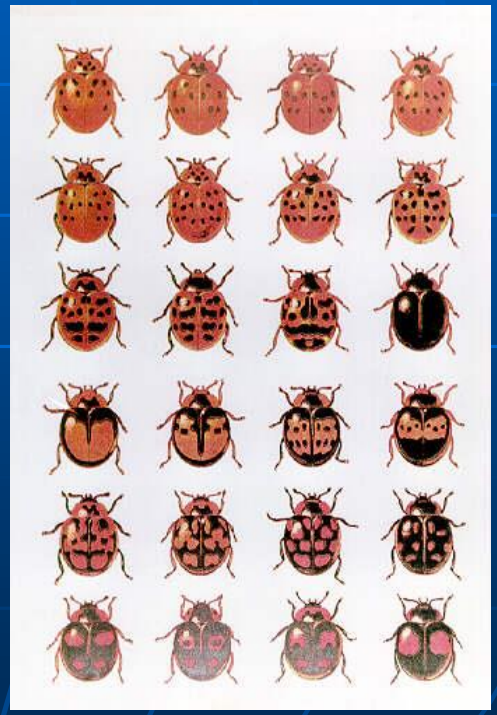


Ground Truth
Hard to find in biology

…it is important to understand your measurements:

…..Compared to what?

- 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?



Ground Truth
Hard to find in biology

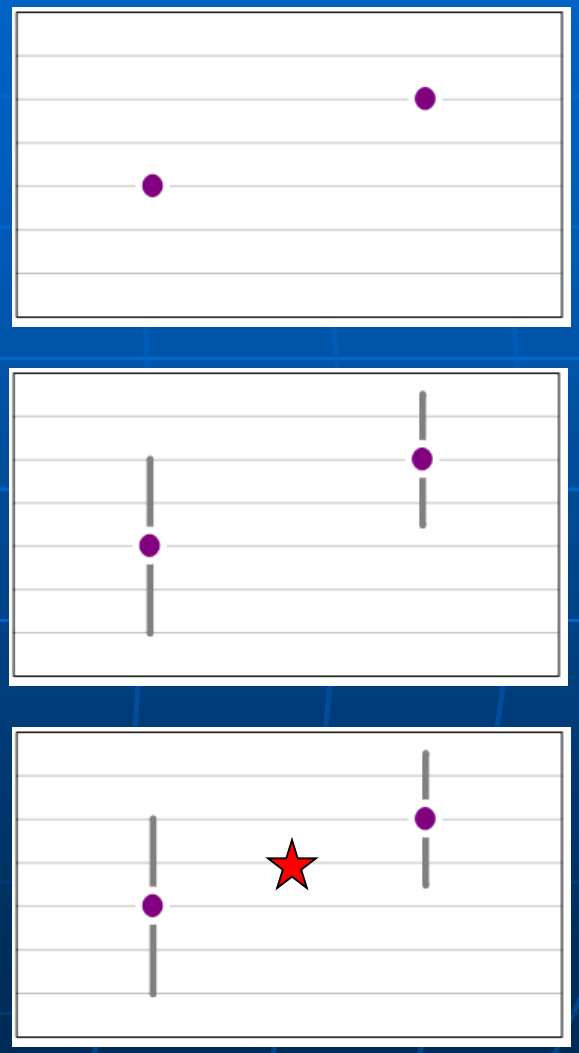
Understand your measurements:

…..Compared to what?

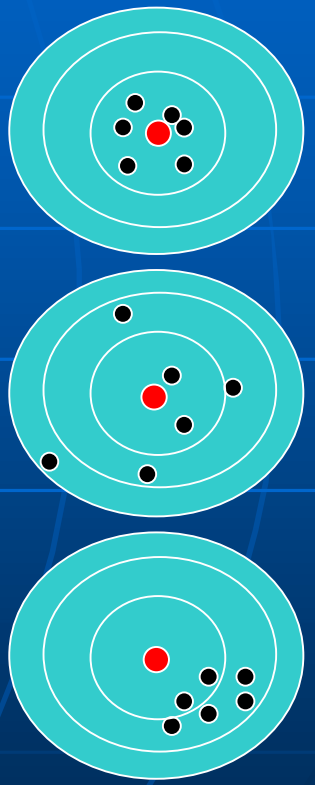
- **Qualify the assay**
  - ACCURACY: Orthogonal method
  - PRECISION: Reproducibility: same day replicates, day to day, different technicians
  - ROBUSTNESS: sensitivity to assay parameters
  - SPECIFICITY: sensitivity to matrix effects
  - DYNAMIC RANGE AND RESPONSE FUNCTION: Instrument benchmarking.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

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.



.....Compared to what?



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?

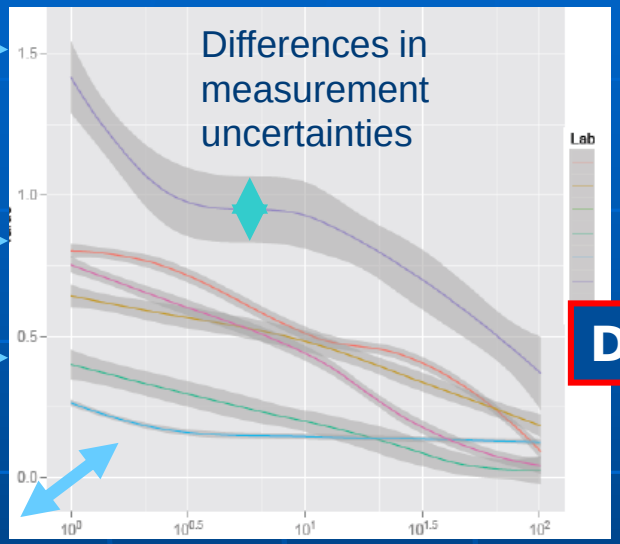
Knowing the level of confidence you have in the data that you are using to make a decision.

Having the data that provide credibility of the measurement result.

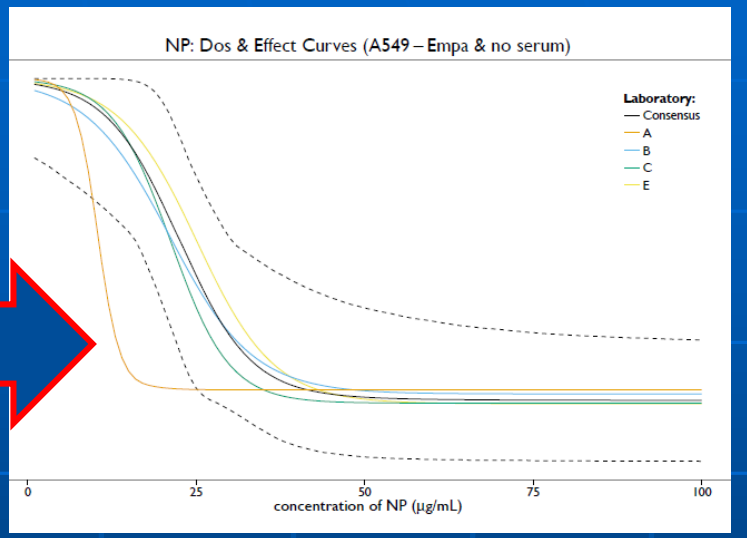
There are many strategies for achieving measurement assurance

Assuring comparability: Interlaboratory studies, Design of Experiment for Robust Protocols

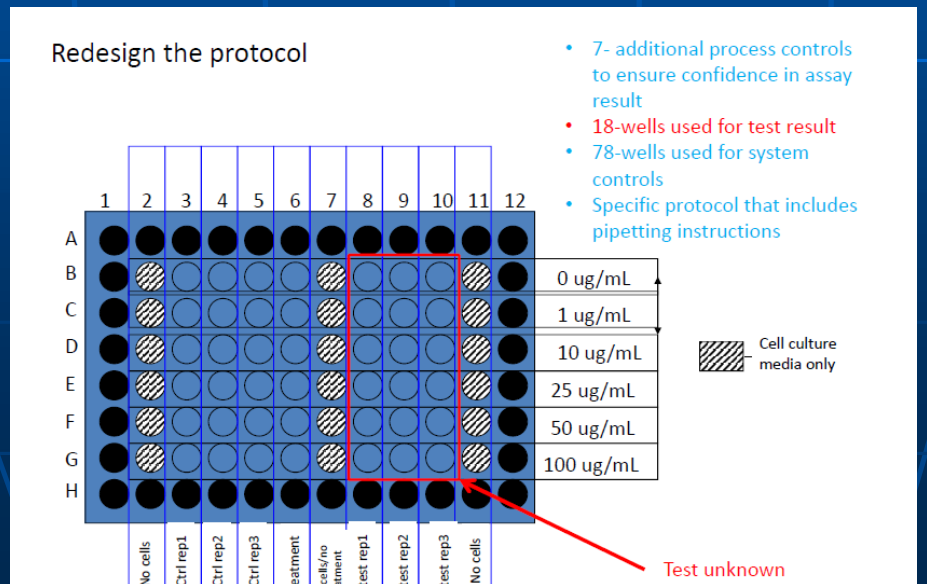
Differences in absolute absorbance



DoE



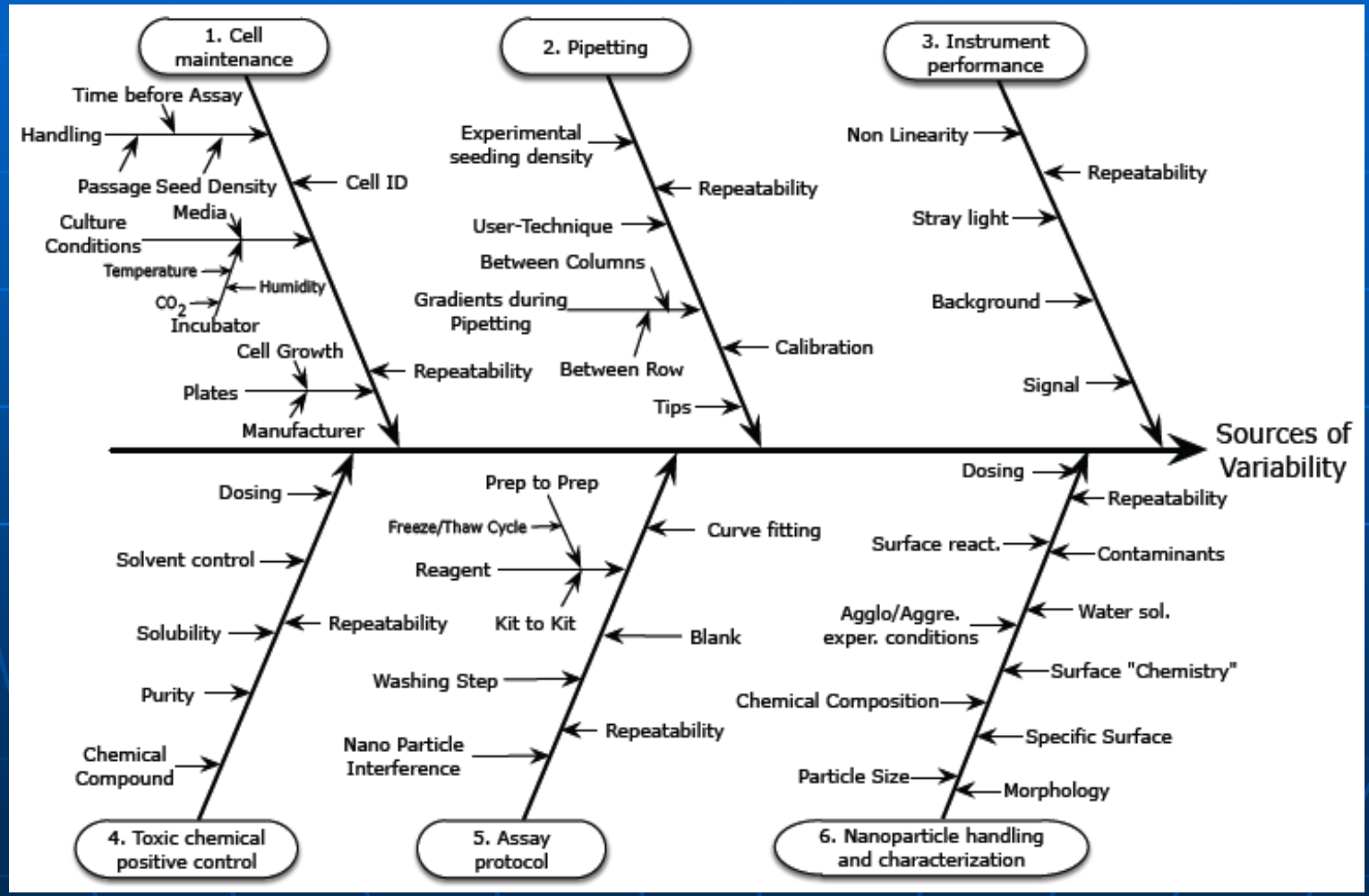
Differences in response functions



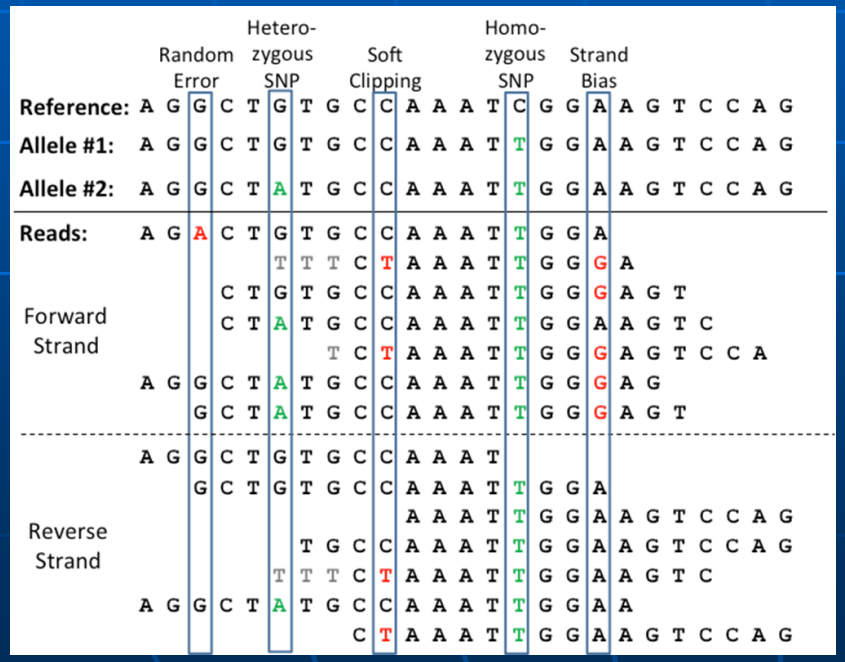
Result: robust protocol and comparable results

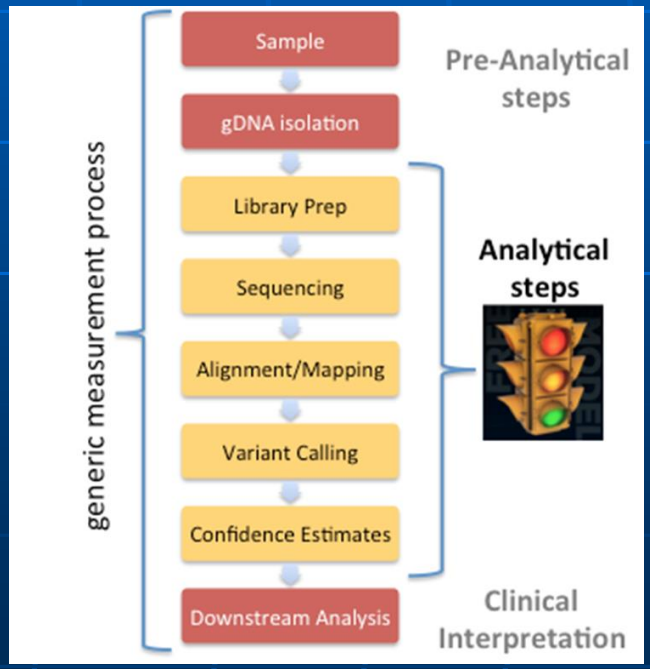
John Elliott

Identifying Sources of Uncertainty – Reportable Parameters?



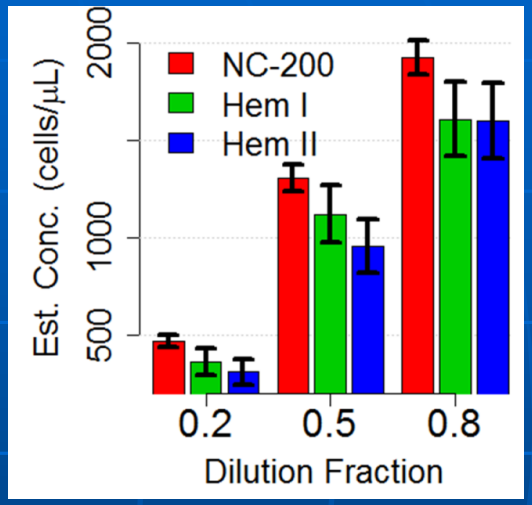
Identifying sources of measurement uncertainty

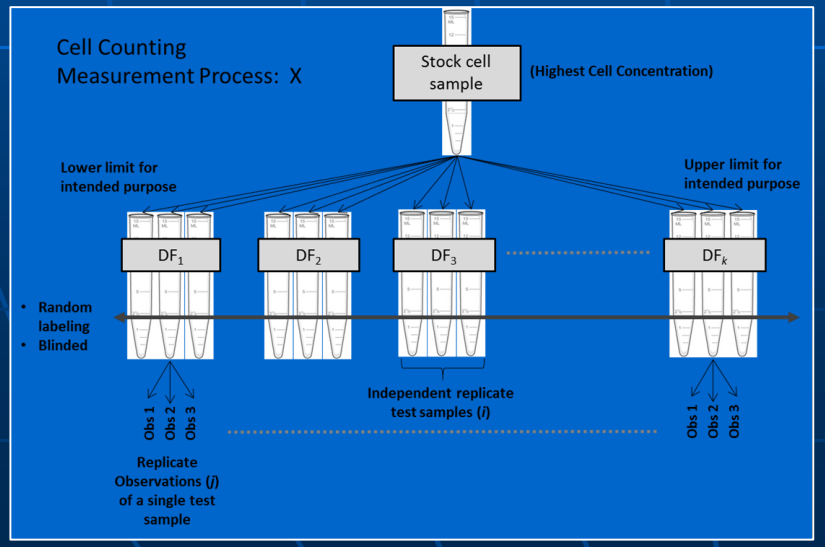


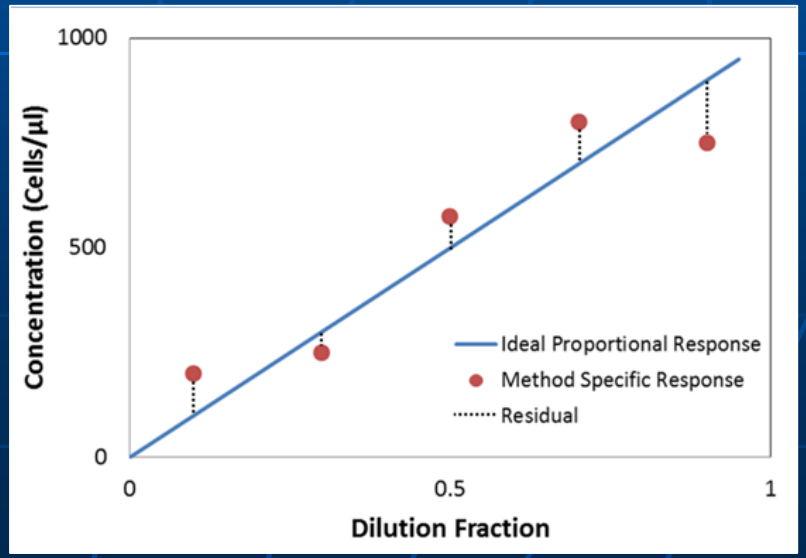


Zook, et al., Nature Biotechnology 2014

Evaluating the performance of a cell counting method: Experimental design and statistical analysis







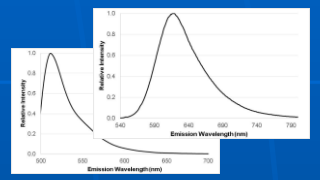
ISO

S. Sarkar et al.

Assuring comparability in instrumentation: traceability to a reference material

NIST SRM 1934/ Calibrated fluorimeter

Fluorescein
Nile Red
Allophycocyanin (APC)
Coumarin 30



Different Manufacturers' calibration beads

Not comparable to one another

Equivalent Reference Fluorophore (ERF) Number

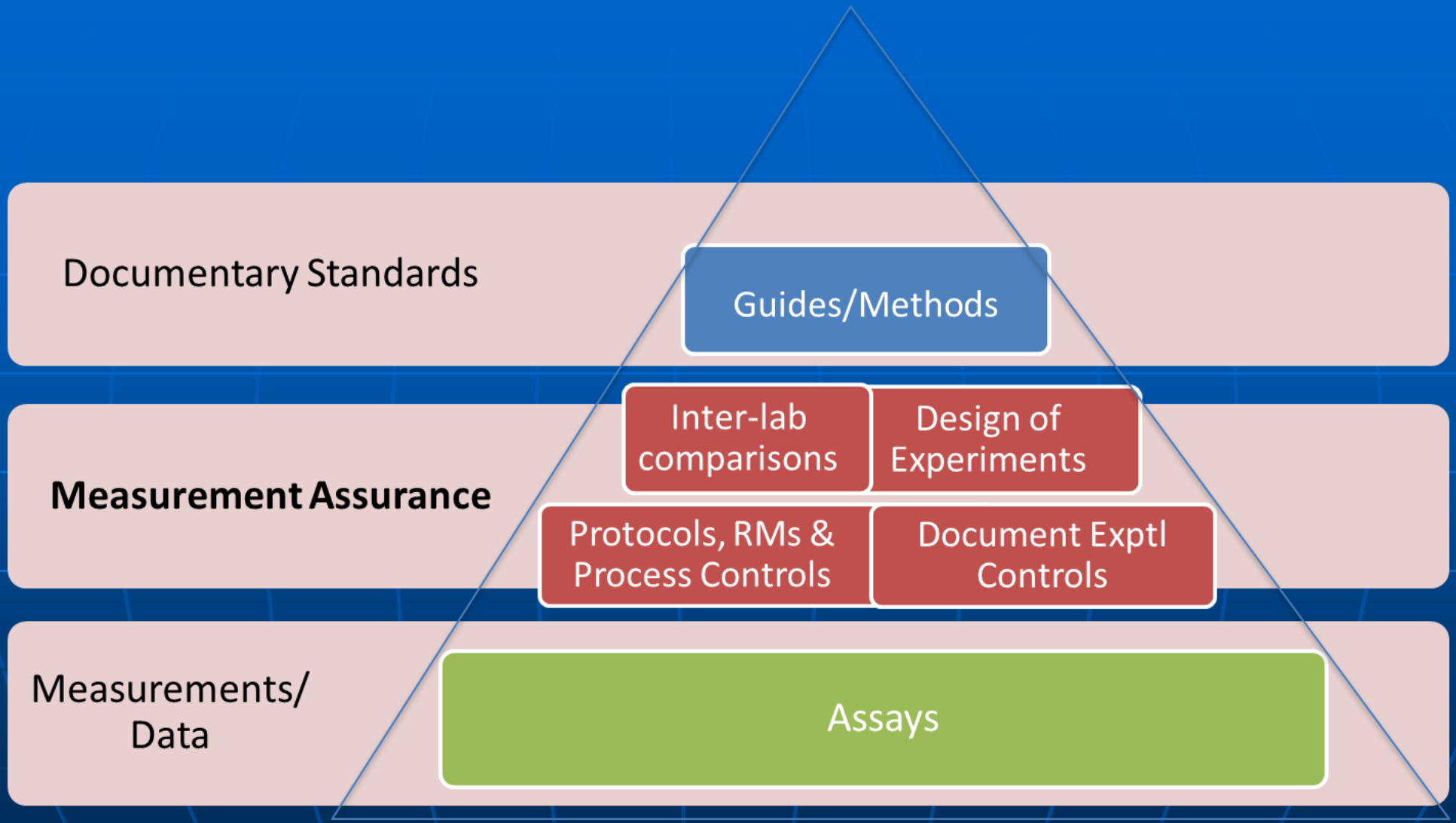
Comparable to one another

Light obscuration flow instrument
For accurate bead concentration

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


Documentary Standards
Guides/Methods
Measurement Assurance
Inter-lab comparisons
Design of Experiments
Protocols, RMs & Process Controls
Document Exptl Controls
Measurements/ Data
Assays

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 Reporting Framework

MIATA is NOT

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

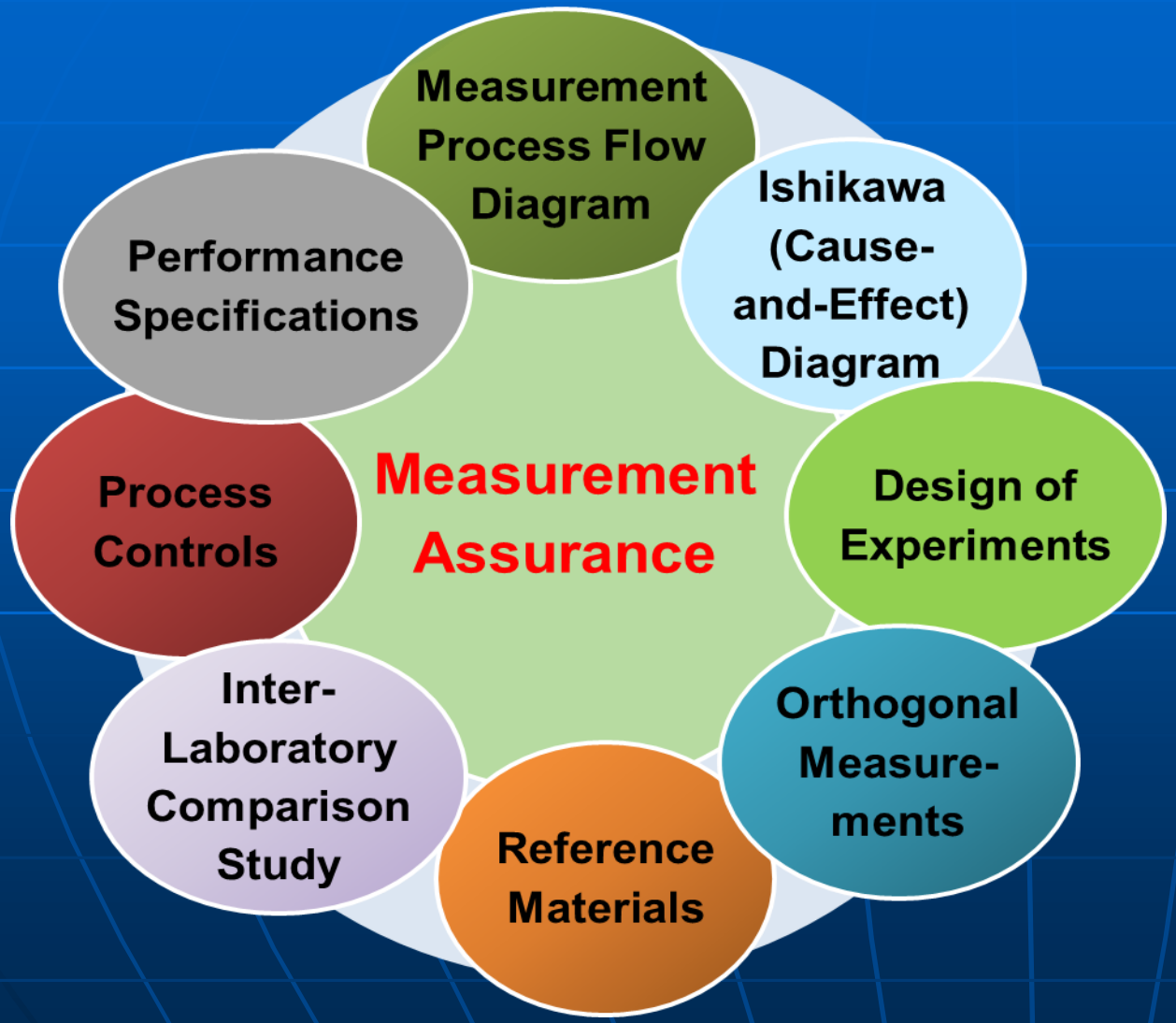
↓

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.

How well do we have to measure?



- 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

Measurement Process Flow Diagram

Sample Collection
- Trypsin/ collagenase / EDTA treatment

Count remaining cells

Sample Preparation
- Disaggregation of cell clumps

Light scattering or microscopy to assess extent of disaggregation

Sampling
- Dilutions

Check accuracy of volumes by weight

Counting Preparation
- Viability or Ab stain

/Data Collection
- Load hemocytometer
- Collect data via microscope
- Flow cytometer

Ishikawa (Cause-and-Effect) Diagram

Process Controls

Performance Specifications

Reference Materials

Orthogonal Measure-ments

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