# NIST at ICCVAM: Tools to improve confidence in alternative test methods

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# Some key focuses at NIST

- **Measurements**
  - Develop new measurement methods
  - Improve accuracy/precision of measurements
- **Reference Materials**
  - Well-defined materials for use as a reference when making measurements
  - Enables inter-lab comparability
  - Physical artifacts for calibrating instruments
- **Standards**
  - Documentary standards, ASTM, ISO
  - Reference data (chemical spectra)
  - Technical Notes: “Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results” (GUM)
- **Biology/biotechnology**
  - **Cell-related measurements and technology (~1990)**
  - **Cytotox measurements, organism measurements (~2005)**


**Food-matrix reference materials to facilitate nutritional labeling**

**NIST Synthetic RNA controls (ERCCs) used in sequencing of Ebola virus genomes to characterize patterns of viral transmission**

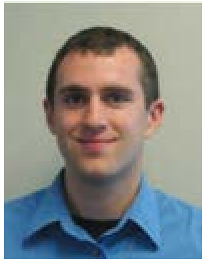

# Measurement Assurance in Biological Assays

**Cause and Effect Analysis: A new approach for developing robust nano-bio assays**
Workshop hosted by EMPA (Switzerland) on June 18 & 19, 2015
16 participants in attendance from 3 countries

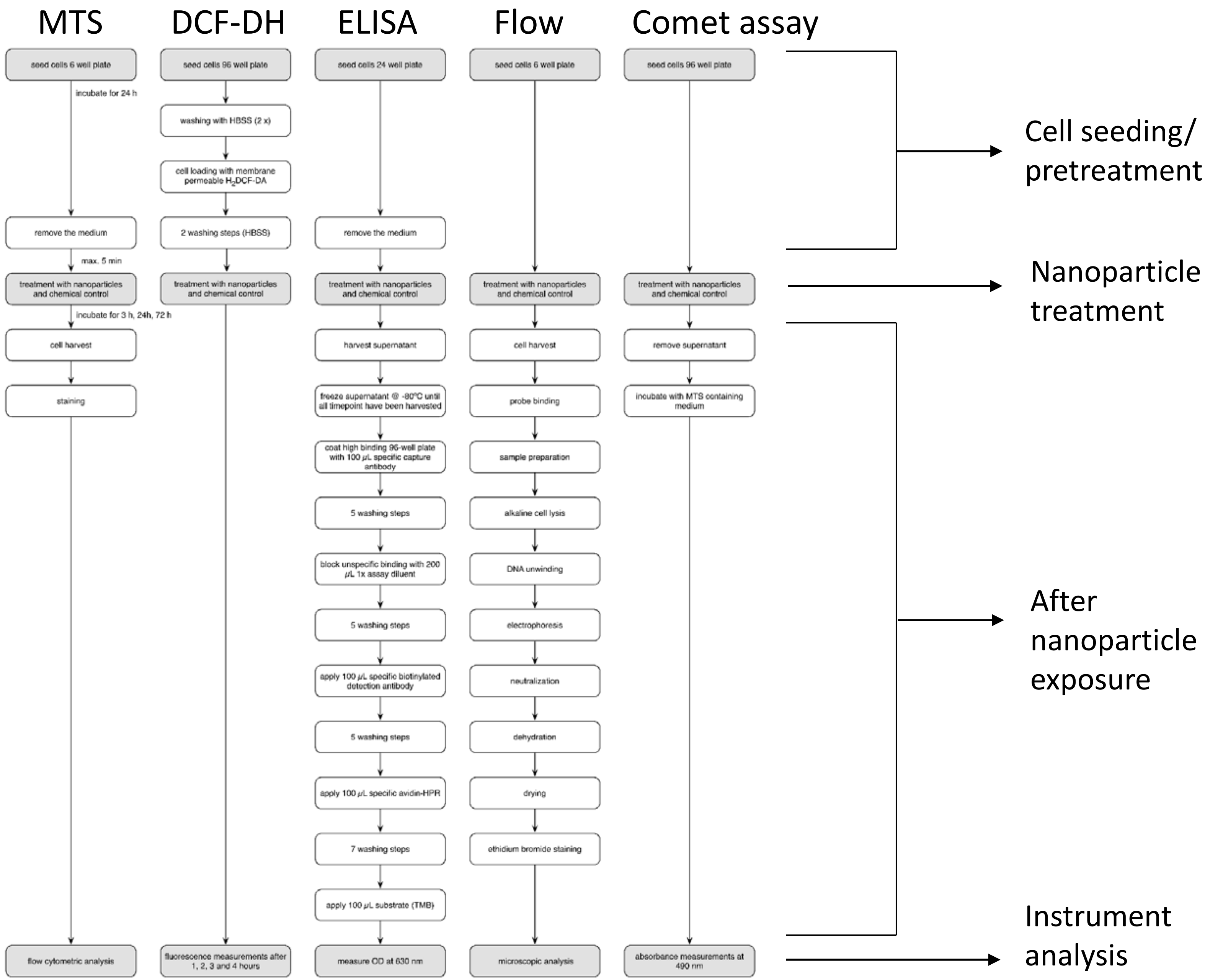
Evaluated five *in vitro* assays for use with nanoparticles:
MTS assay (cell viability)
DCF-DH assay (ROS generation)
Flow cytometry assay (quantification of viable, necrotic, or apoptotic cells)
Comet assay (genotoxicity)
ELISA assay for IL-8 (inflammation response)

For each assay, we developed a flow chart, cause-and-effect analysis, and control experiments

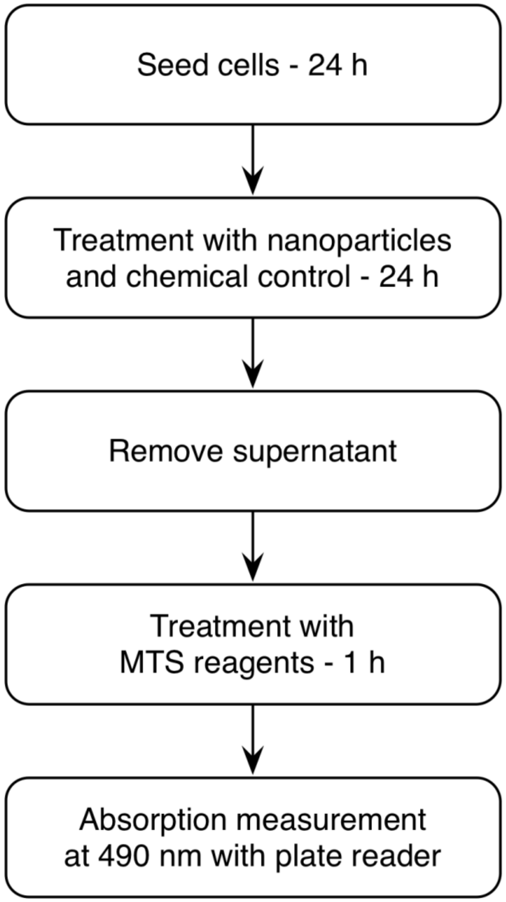




# Flow charting



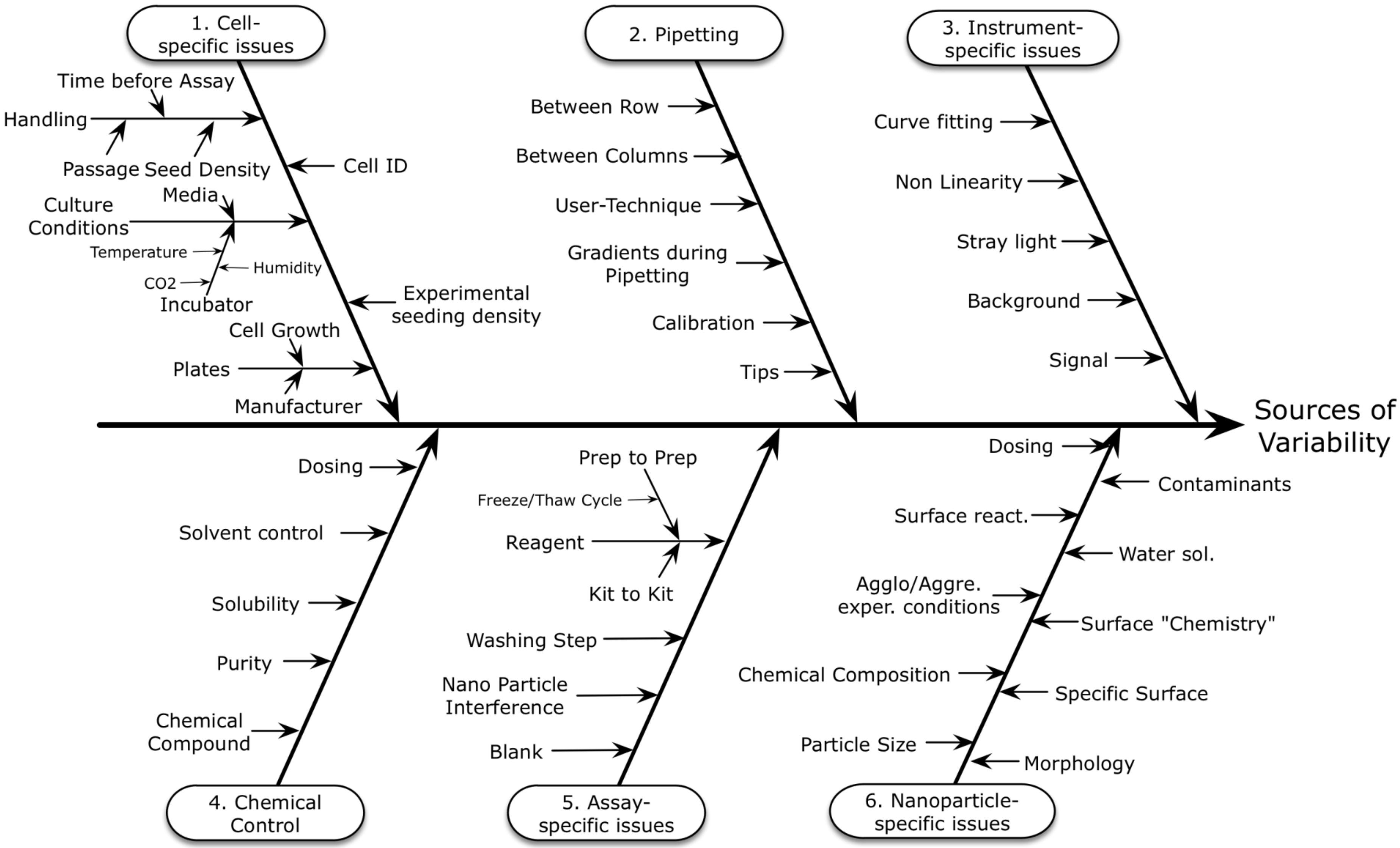
# MTS cell viability assay




Summary Instructions:

1. Receive NP, serum, cells, chemical control
2. Negative control- no treatment
3. Positive control- 100 uM CdCl2
4. Manufacturer’s protocol
5. Cell proliferation rate-  21h
6. Normalize treatment to no-treatment well

# Identify sources of variability using cause & effect analysis




Rosslein, M., Elliott, J. T., Salit, M., **Petersen, E. J.**, Hirsch, C., Krug, H. F., Wick, P. Chemical Research in Toxicology, **2015,** 28(1), 21-30.

# What is the purpose of cause and effect analysis?

1. Method to lay out implicit knowledge

2. Systematic approach to identify potential sources of variability in an assay and to highlight key sources of variability

3. Can be used to help design process control experiments, improve plate layout, and with writing a protocol

4. Can be used iteratively to improve assay quality by decreasing variability in key assay steps which decreases the total variability in the assay

# Design a new plate format with process control measurements

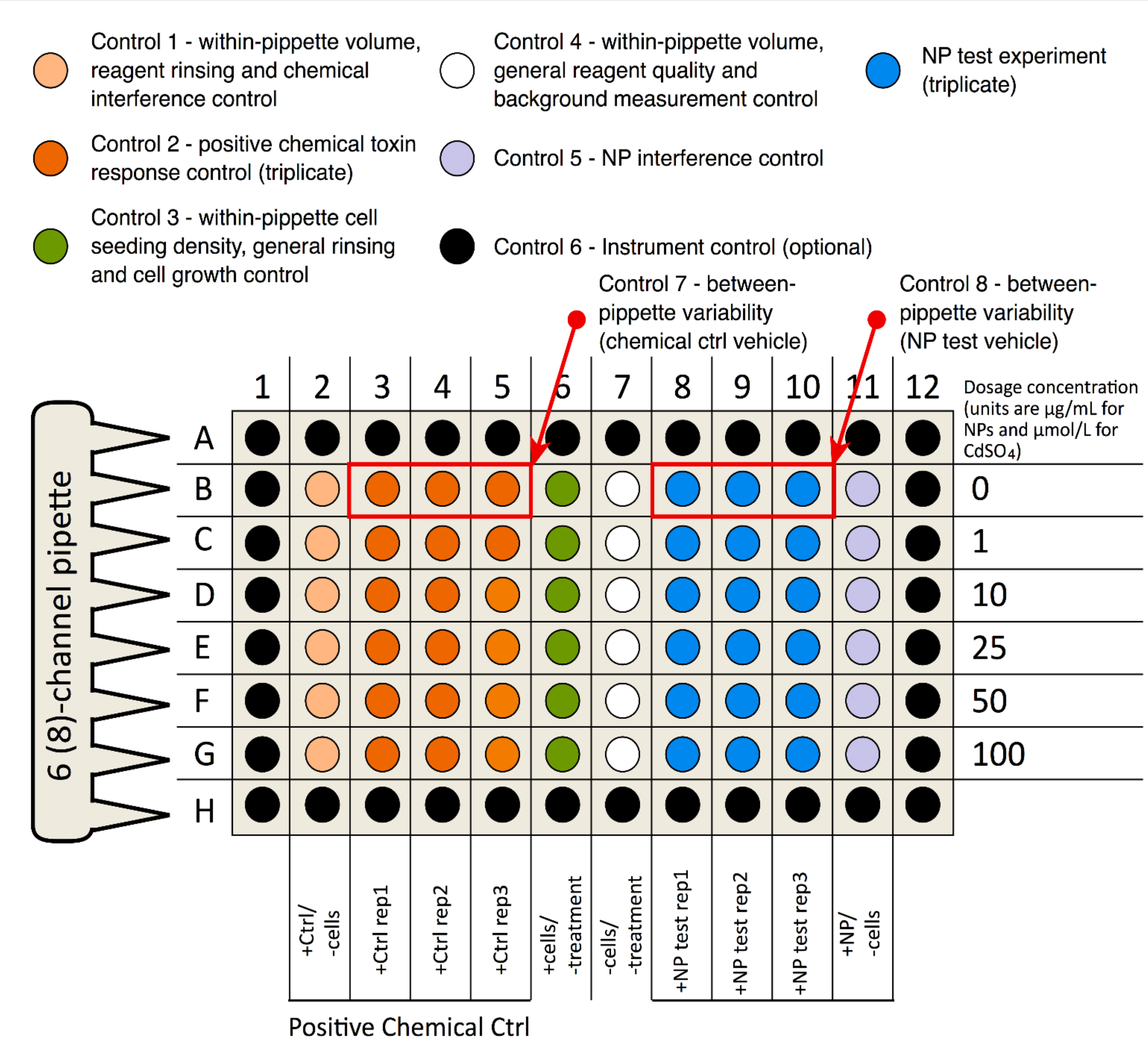
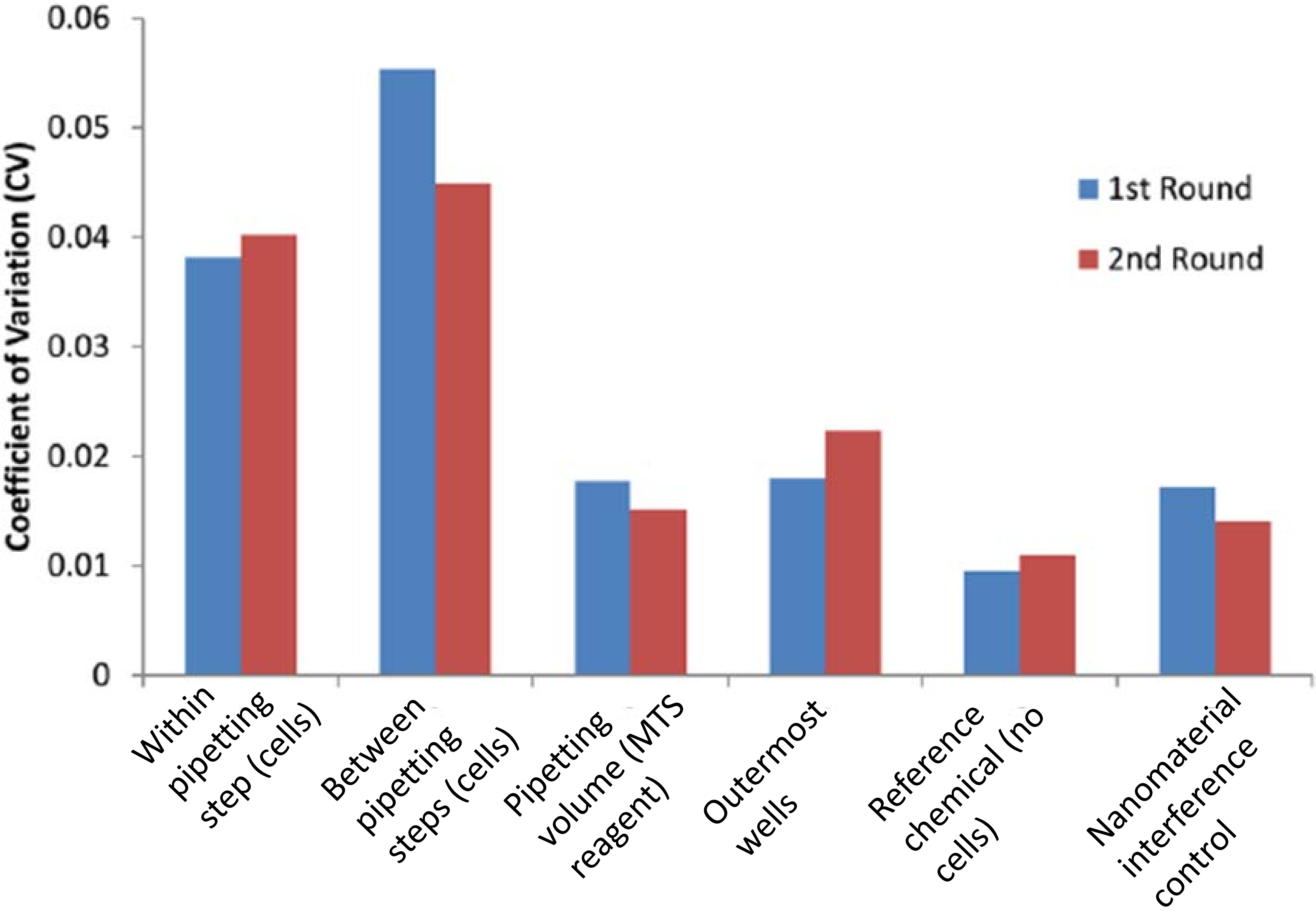



Plate design includes 8 control measurements in addition to the NP measurement

# Single-laboratory results

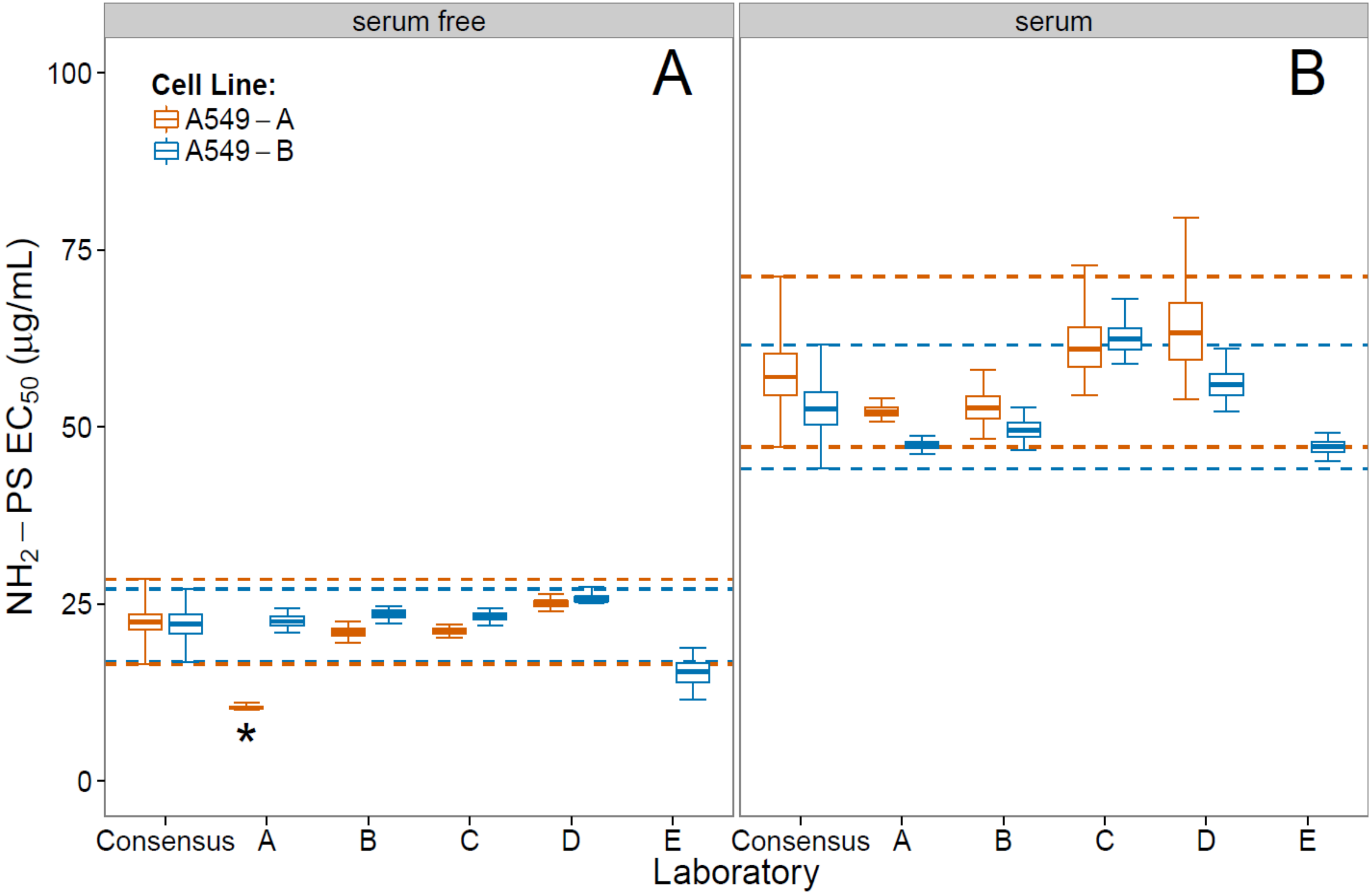



Cell pipetting caused highest amount of variability among controls

# Interlaboratory comparison with MTS assay

- 5 national metrology institutes were involved in the interlaboratory comparison
- Experimental design:
  - Share two A549 cell lines from ATCC and EMPA
  - Serum from local provider
  - Reagents from local provider
  - Serum and serum-free tests
  - Multiple replicates
  - Share nanoparticles (+ve PS) and chemical control ($CdCl_2$)

# NP EC50 values



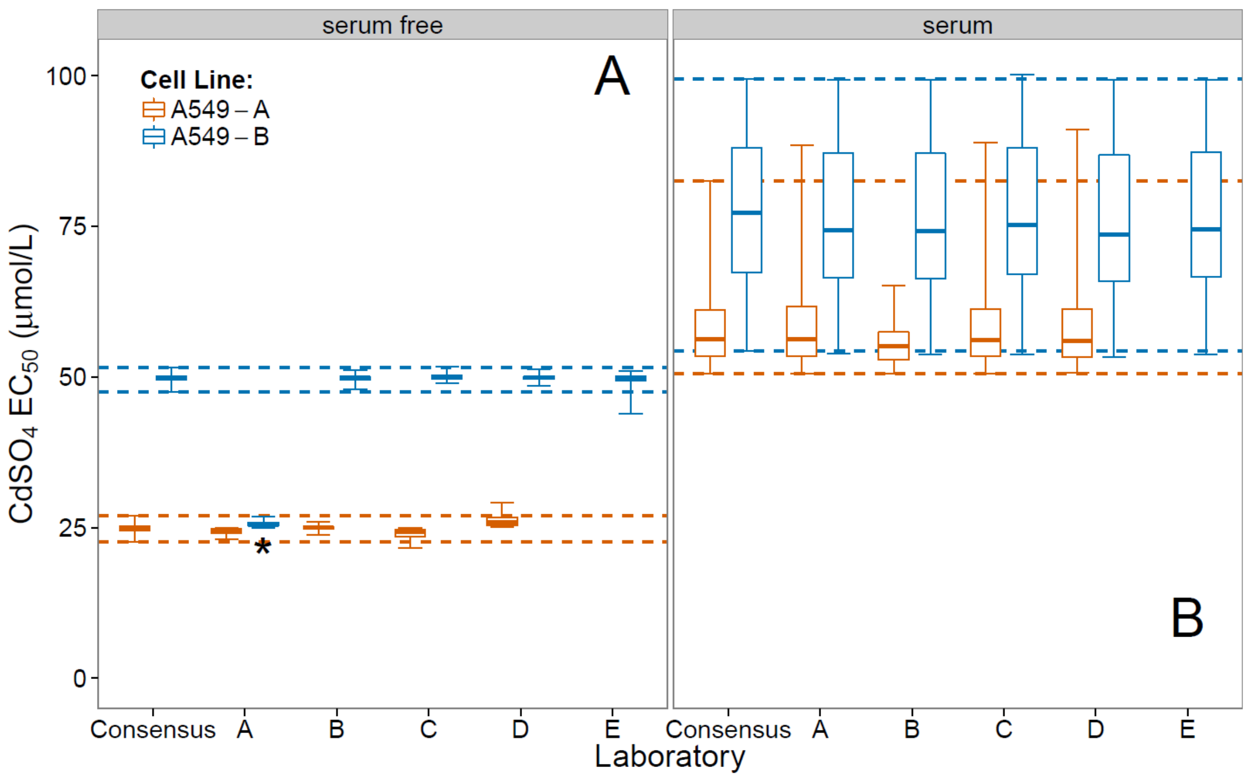

-Looks like harmonization between the laboratories

-No cell line differences

-The serum conditions increases variability

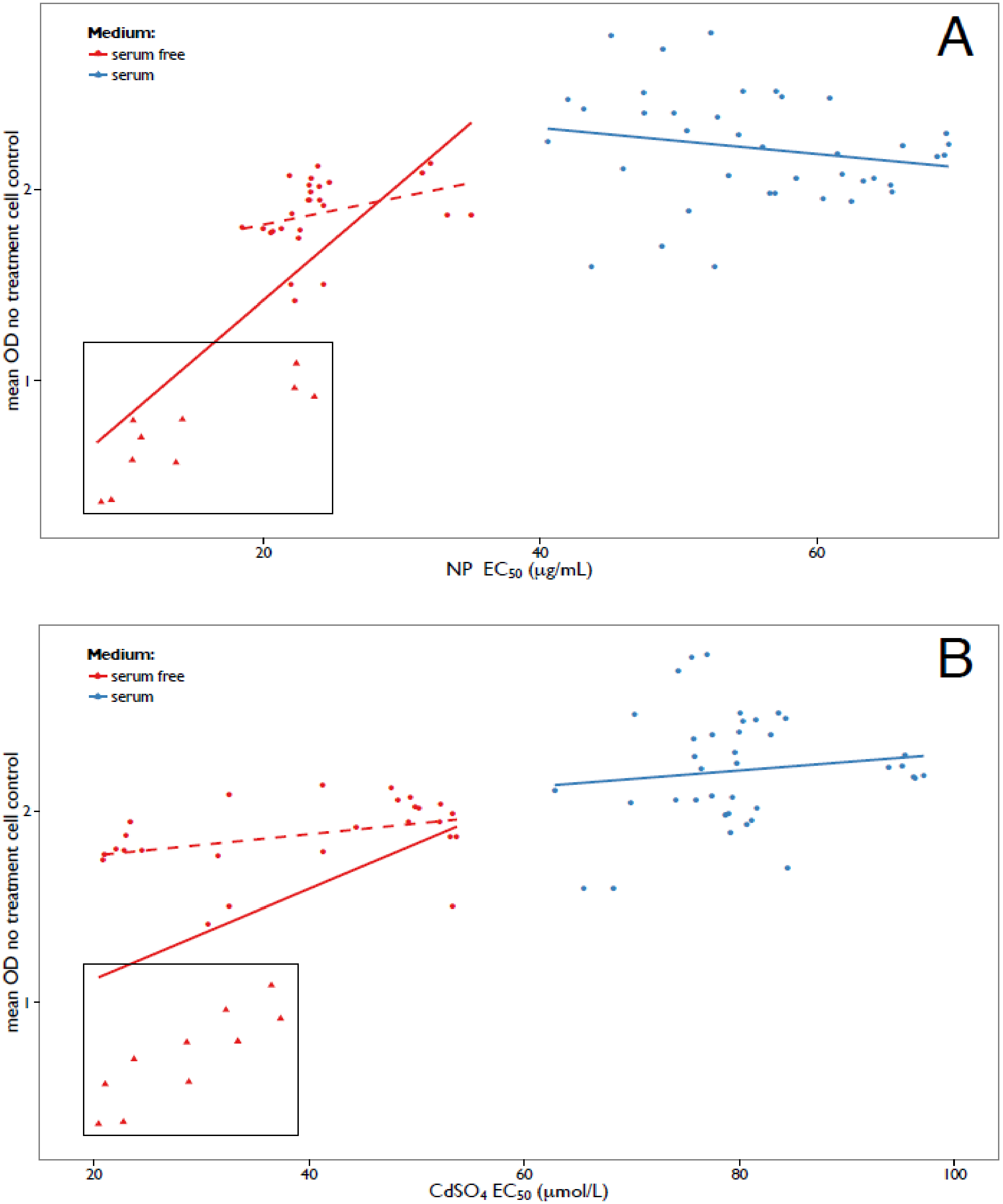
# Can the system control measurements identify the cause of the outlier?

- Chemical Process Control- tests overall measurement system



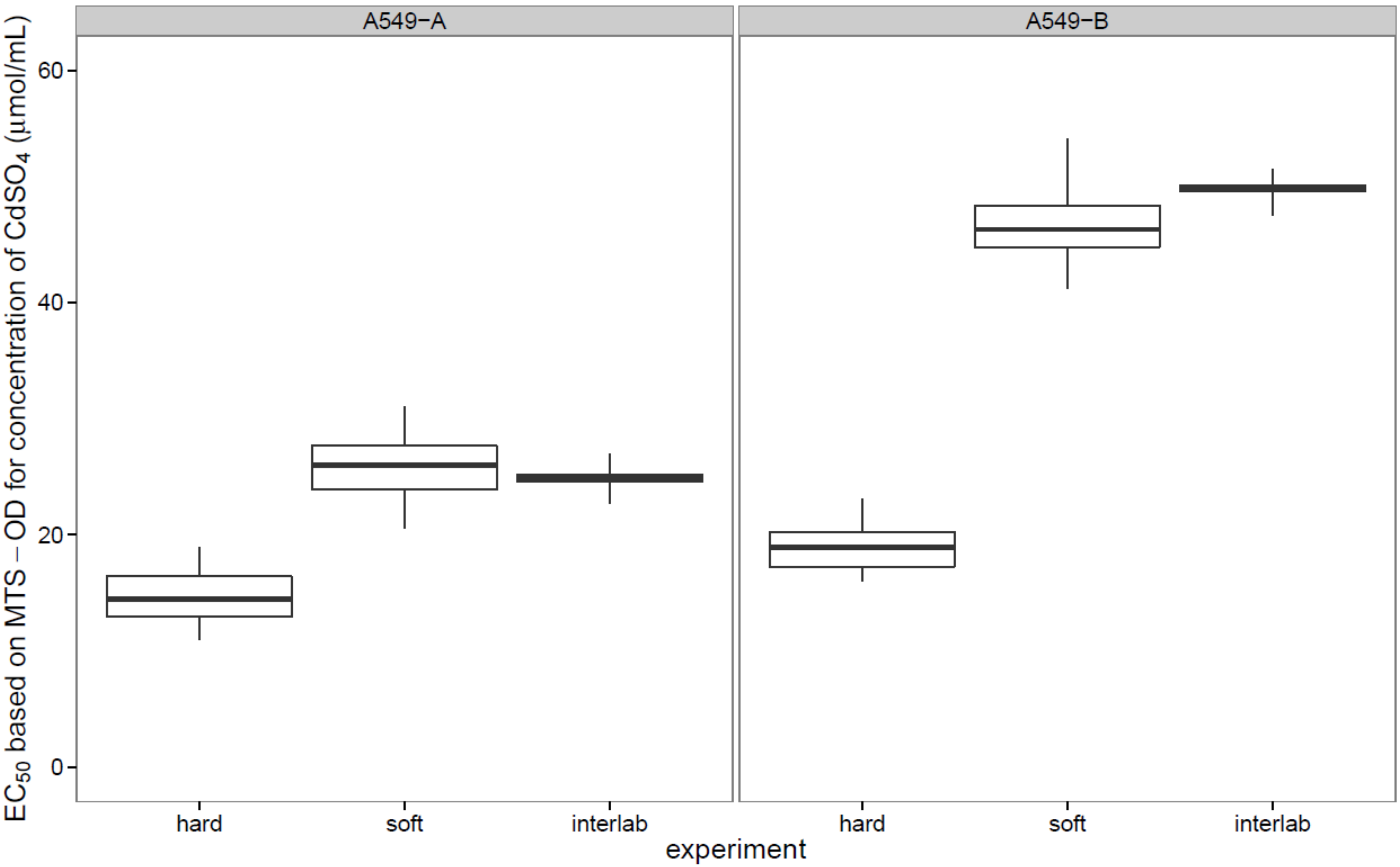

Serum free conditions, variability less than with NP
Differences between cell lines

# How sensitive are we to cell seeding variability




- Correlation between no-treatment cells and NP EC50
- If outliers are removed, no strong correlation
- Suggests that within this range of cell seeding variability (OD=1.5-2.5) no big effect on EC50

# Impact of cell rinsing for lab A




Changing the rinsing procedure brought lab A results to the interlab consensus values

# What is the purpose of process control measurements?

1. Provide evidence that that the measurement process occurred as expected.

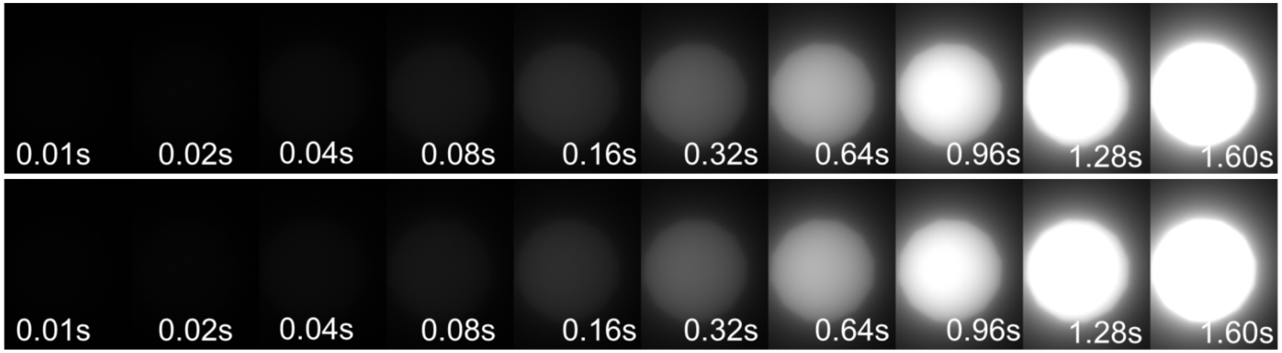
2. Should meet specifications before acceptance of the test result.

3. Can be used to identify relative contributions to total variability in assay result.  Protocol modifications?

4. Ideal for designing protocols for an interlaboratory comparison

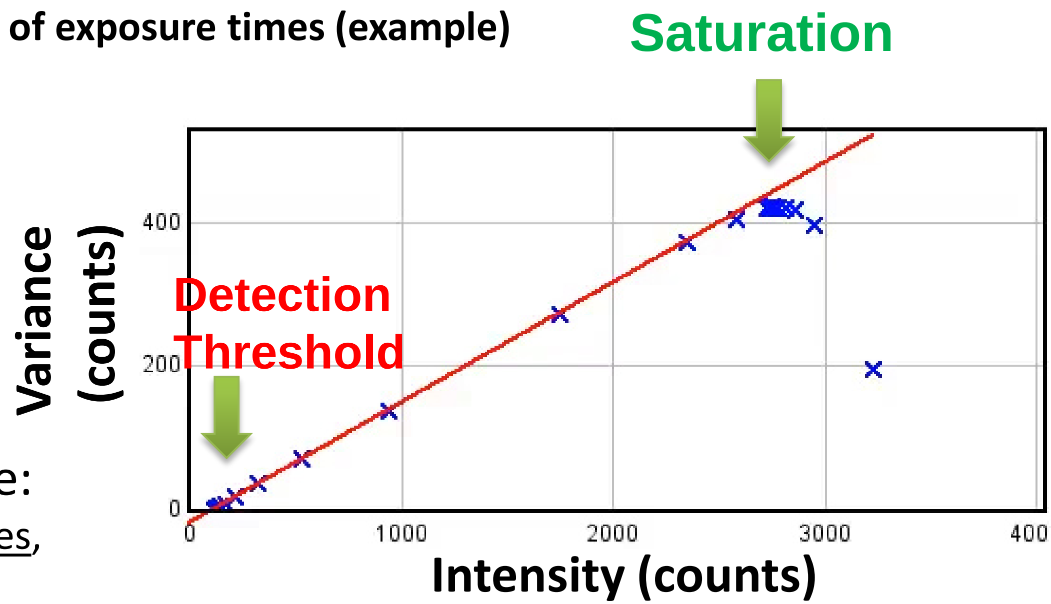
5. Can be used to assess the functioning of different components in a complex assay

# Instrument calibration: Process for Determining Analytical Performance of a Widefield Fluorescence Microscope




**Series of image pairs acquired over a range of exposure times (example)**

The difference between images is used to compute pixel variance ('noise')




- Inspired by Photon Transfer Curve: Janesick, <u>Scientific Charge-Coupled Devices</u>, SPIE Press, (2001)
- $\text{Saturation} = \underset{t>0}{\operatorname{argmax}}(\sigma^2{}_{glass}(t))$

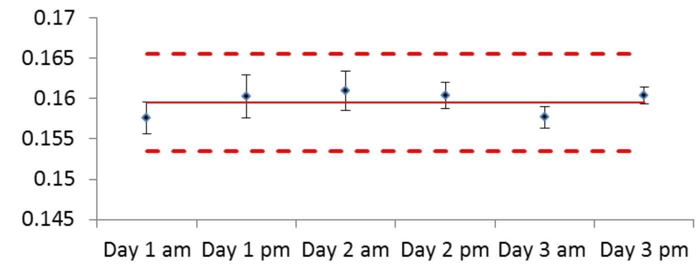
Halter M et al. *Cytometry Pt A*, **2014.**

# Instrument calibration: Establishing Instrument Specifications

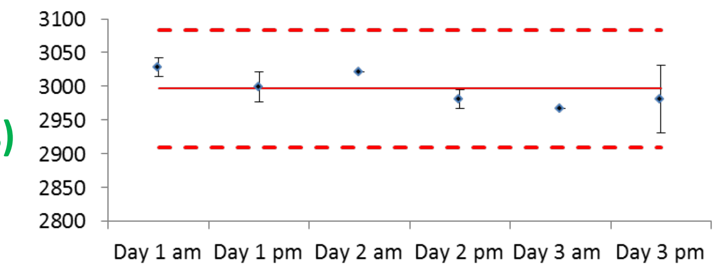
By *<u>charting</u>* the **Detection Threshold**, **Saturation**, and **Intensity Response*** over time, you can:

- Demonstrate comparability between fluorescence intensity measurements
- Identify changes in the analytical performance of your widefield microscope

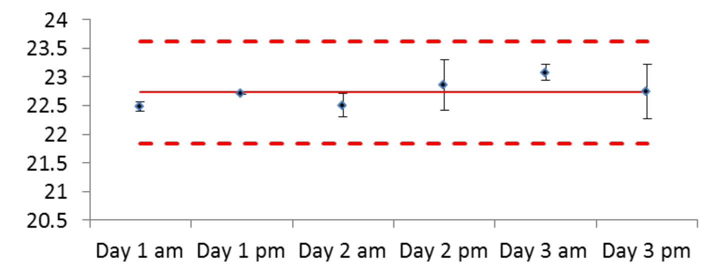
**Detection threshold (ms)**




**Saturation (ms)**




**Intensity response* (counts/(μm$^2$×ms))**




*can be used to normalize for day-to-day intensity variations

# Potential NIST-ICCVAM interactions

- Current- Protocol evaluation for electrophilic allergen screen assay
  - Cause-and-effect analysis
  - Process control considerations
  - Discussion
  - Potential involvement in interlaboratory comparison

# Collaborators

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