Measurement Assurance in Cell-based Assays

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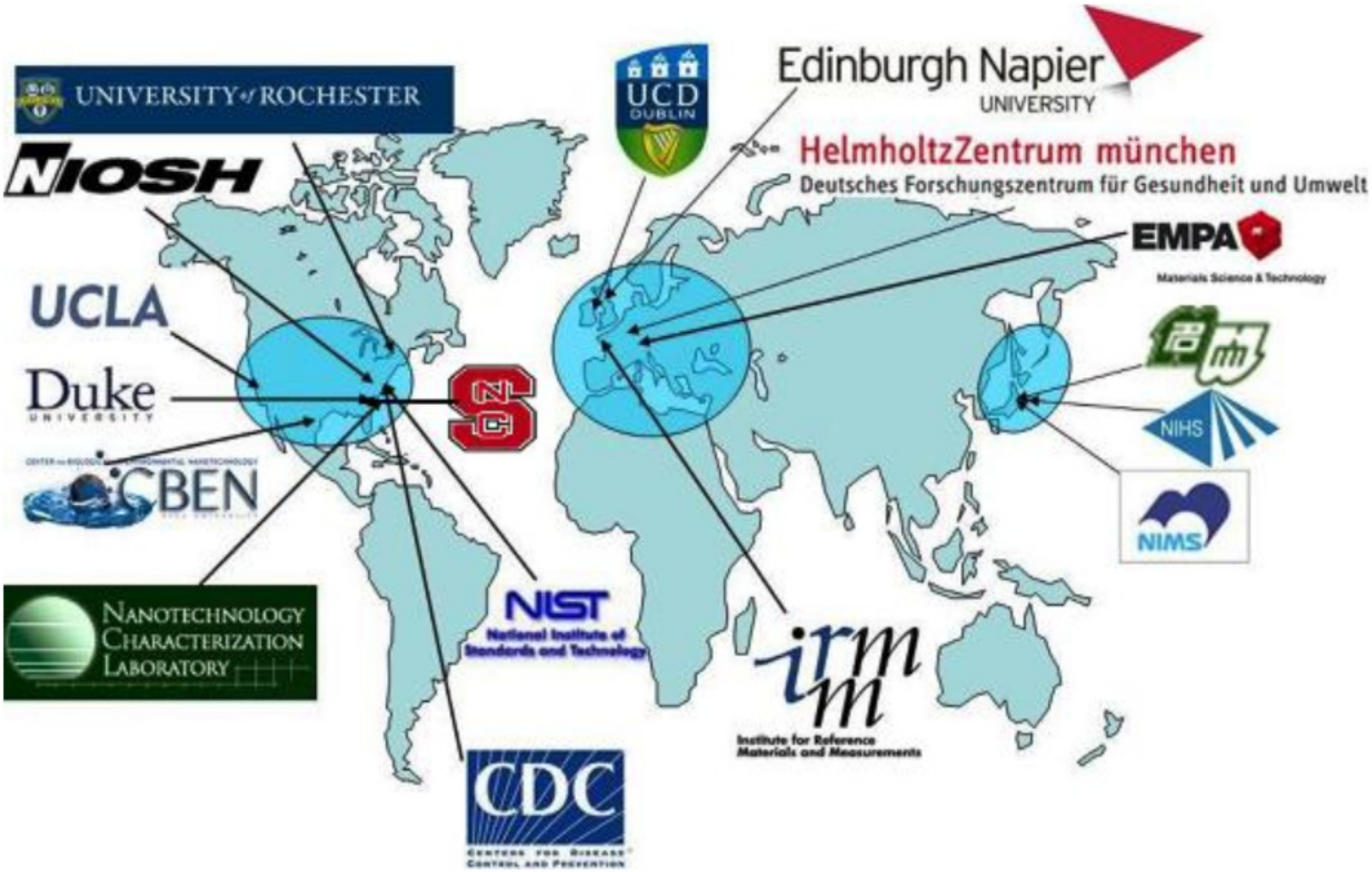
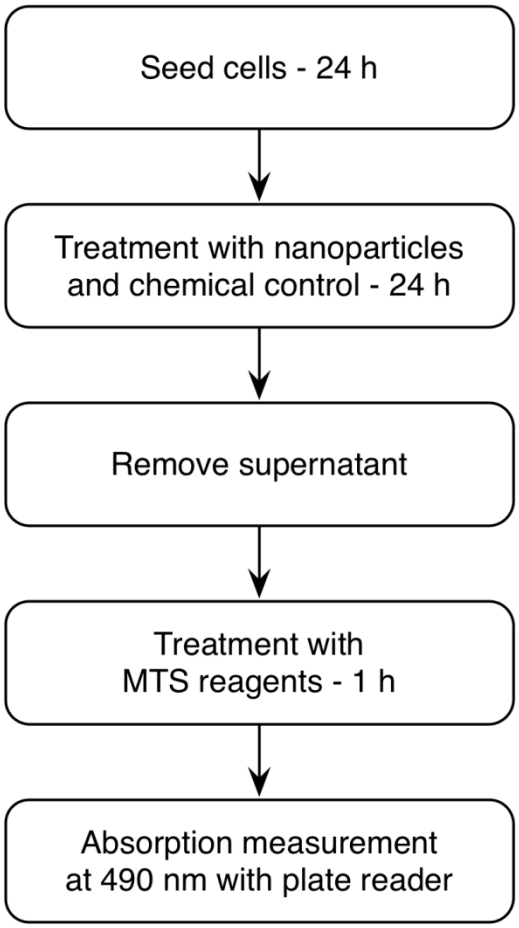
What is the problem?

- How do we improve confidence in a biological measurement?
- Reduce uncertainty in answer?
- Cellular measurements are complicated
  - Cell culture, extended periods, manual
  - Manual steps in setting up experiments
  - Multiple reagents
  - Complex Instrumentation
- How do you demonstrate confidence?

Example: IANH International Alliance for NanoEHS Harmonization



MTS cell viability assay- Nano

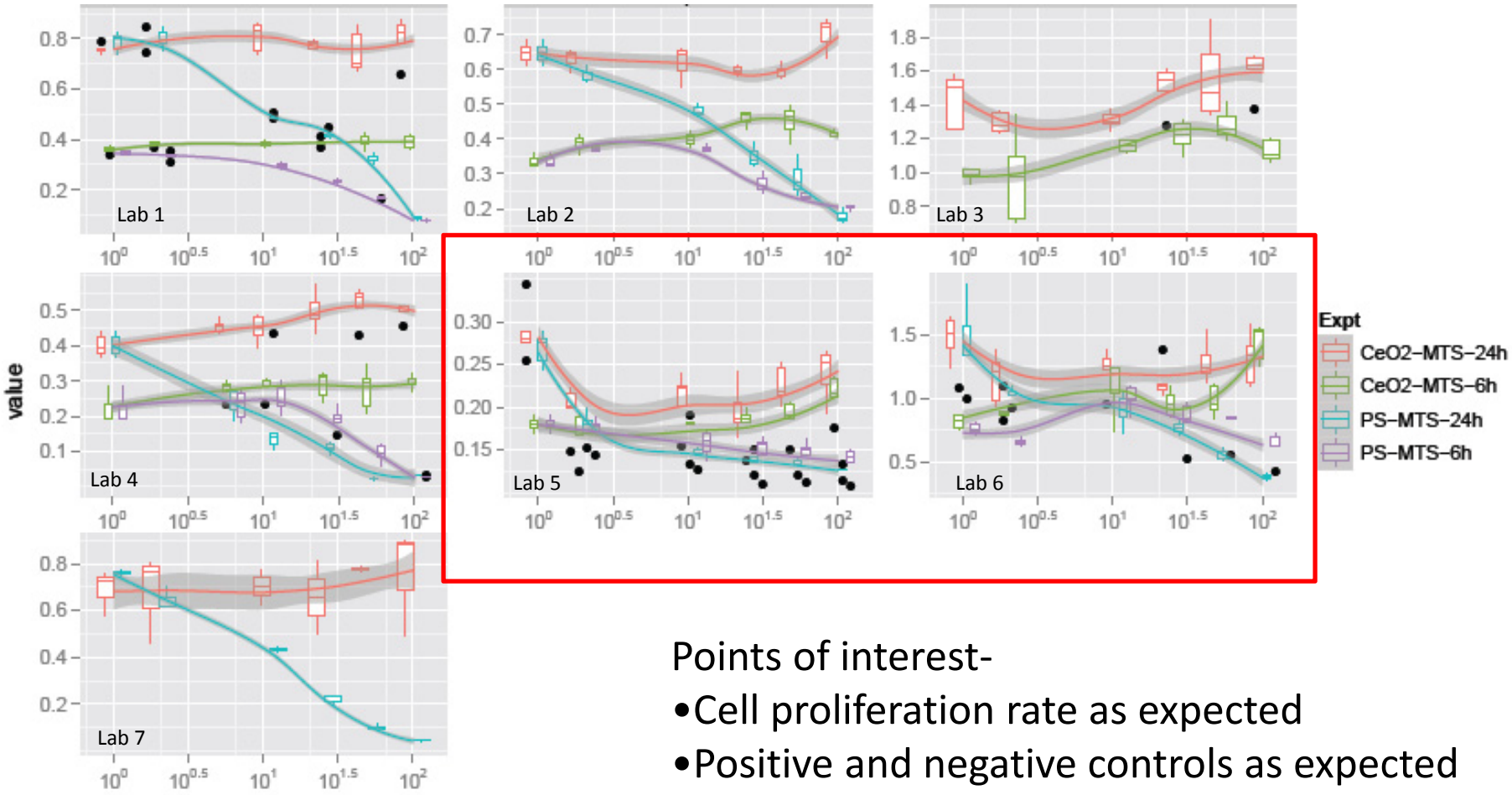


- How to know all factors of assay are correct?

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
7. Do 5 replicates

Raw Data-absolute absorbance, individual scale, all MTS experiments by different labs



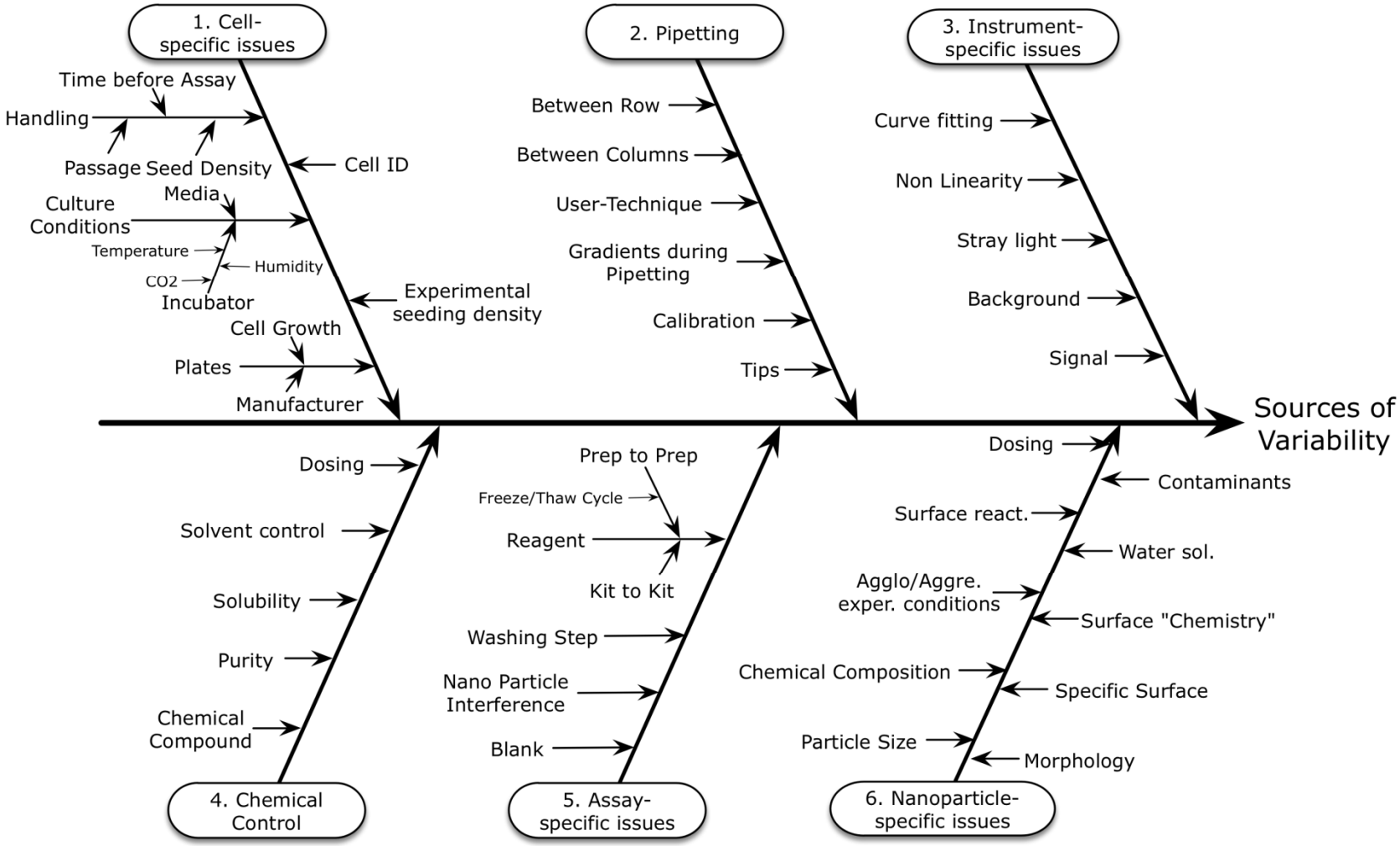
Points of interest-

- Cell proliferation rate as expected
- Positive and negative controls as expected (kind of)

What can we do to increase confidence in the measurement

- Treat the assay as a measurement **process**
- Add **process** controls as evidence that the measurement process is proceeding as expected
- Adapt the “seven basic tools for quality” to cell assays
  - Cause and effect diagram
  - Check sheet
  - Control charts
  - Histogram
  - Pareto chart
  - Scatter diagram
  - Flow chart

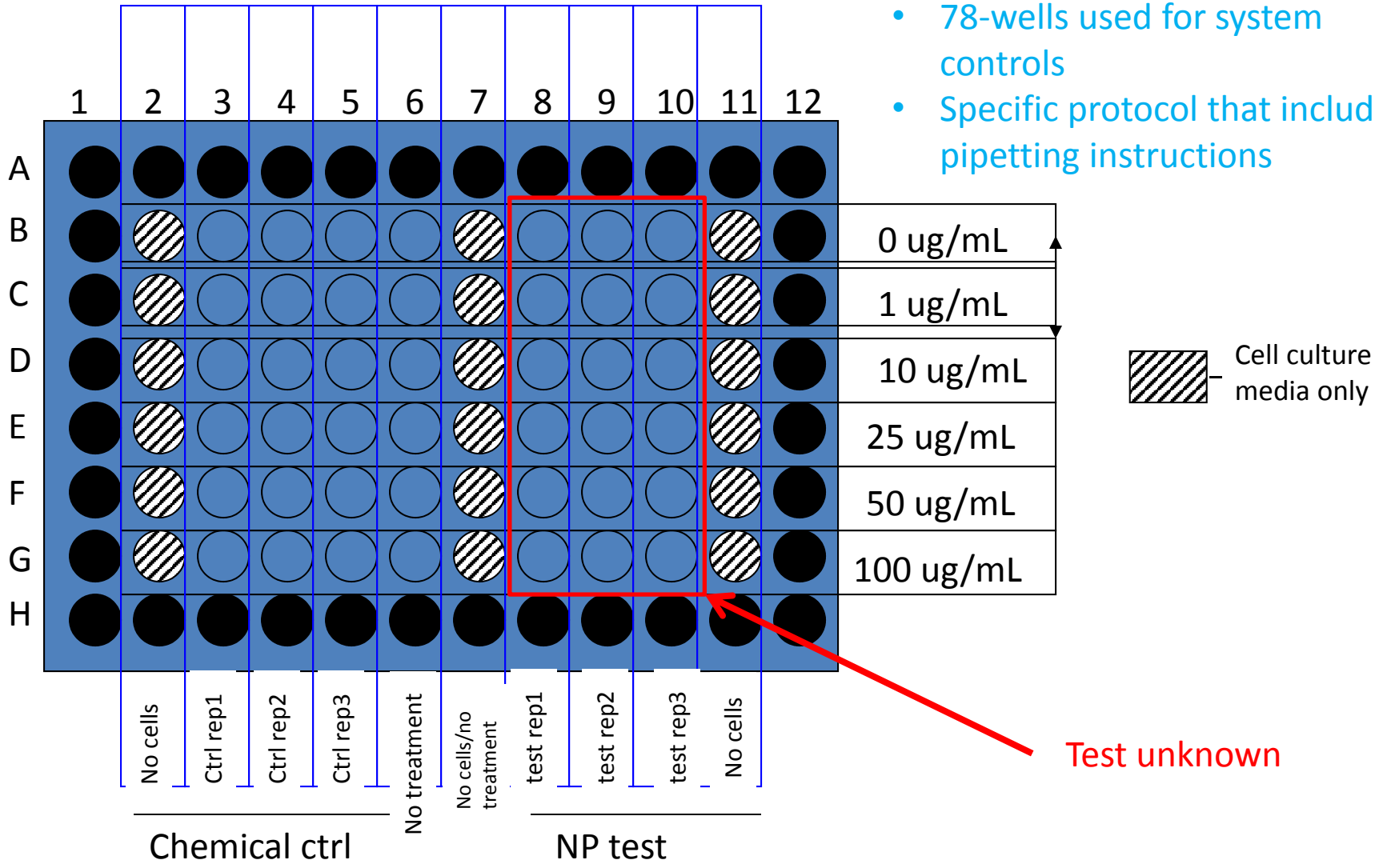
Find sources of variability in assay



Cause and effect diagram for MTS assay

Redesign the protocol

- 7- additional process controls to ensure confidence in assay result
- 18-wells used for test result
- 78-wells used for system controls
- Specific protocol that includes pipetting instructions



New Interlaboratory comparison- What did we find?

- 5 NMIs 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 ($CdCl_2$)

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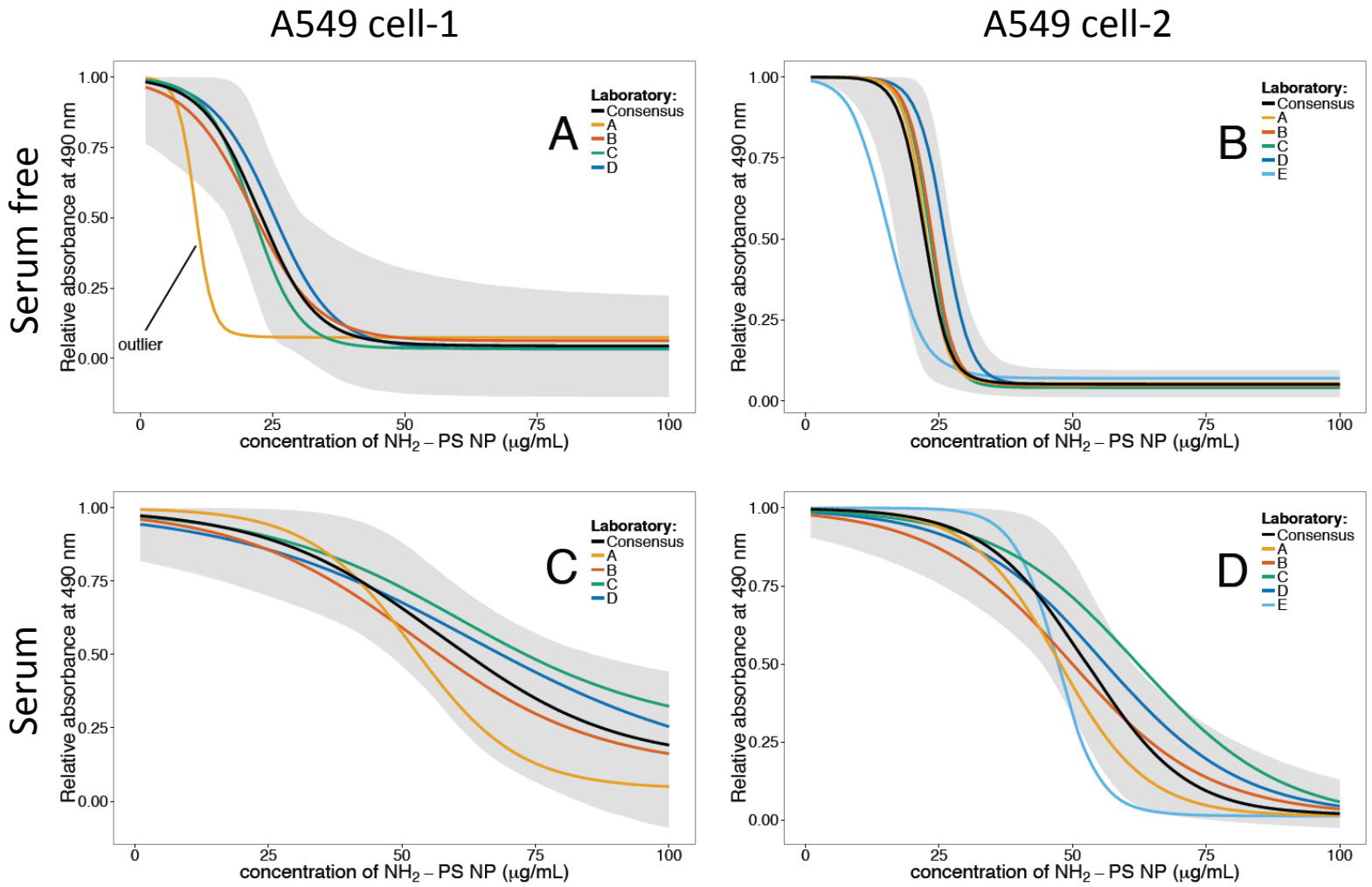
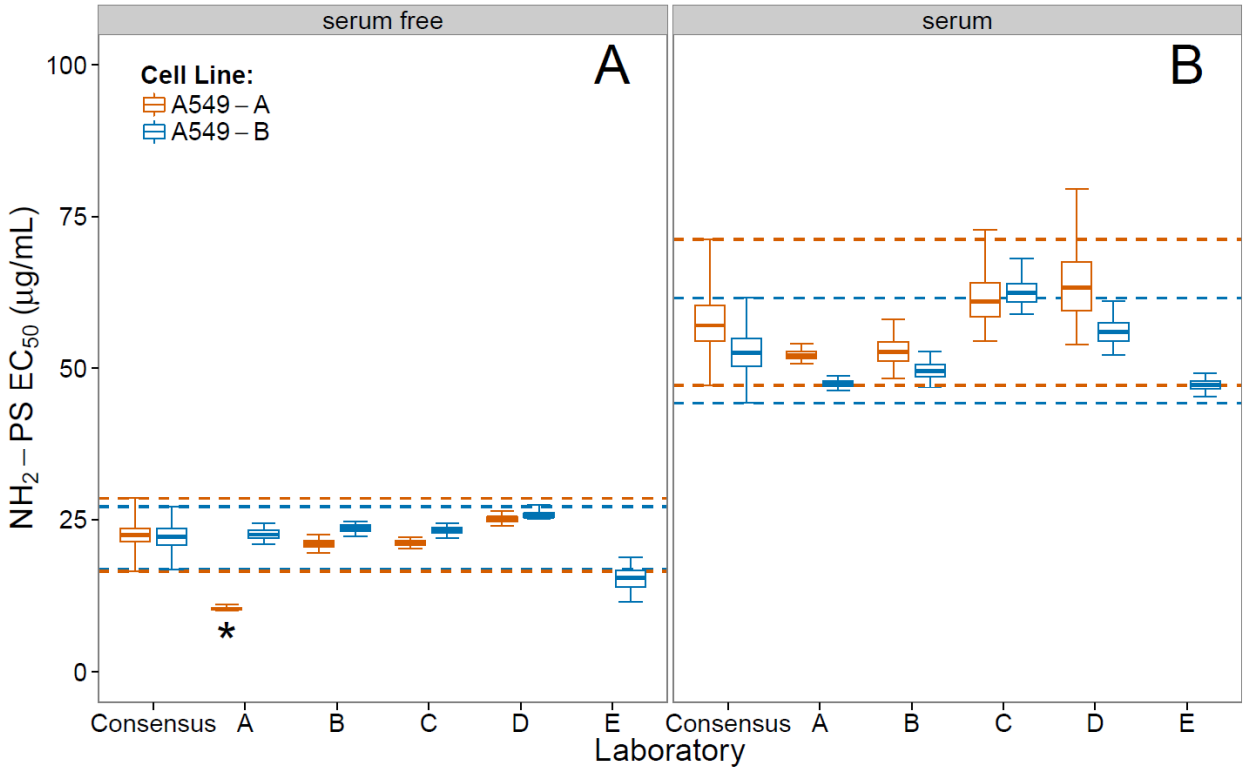


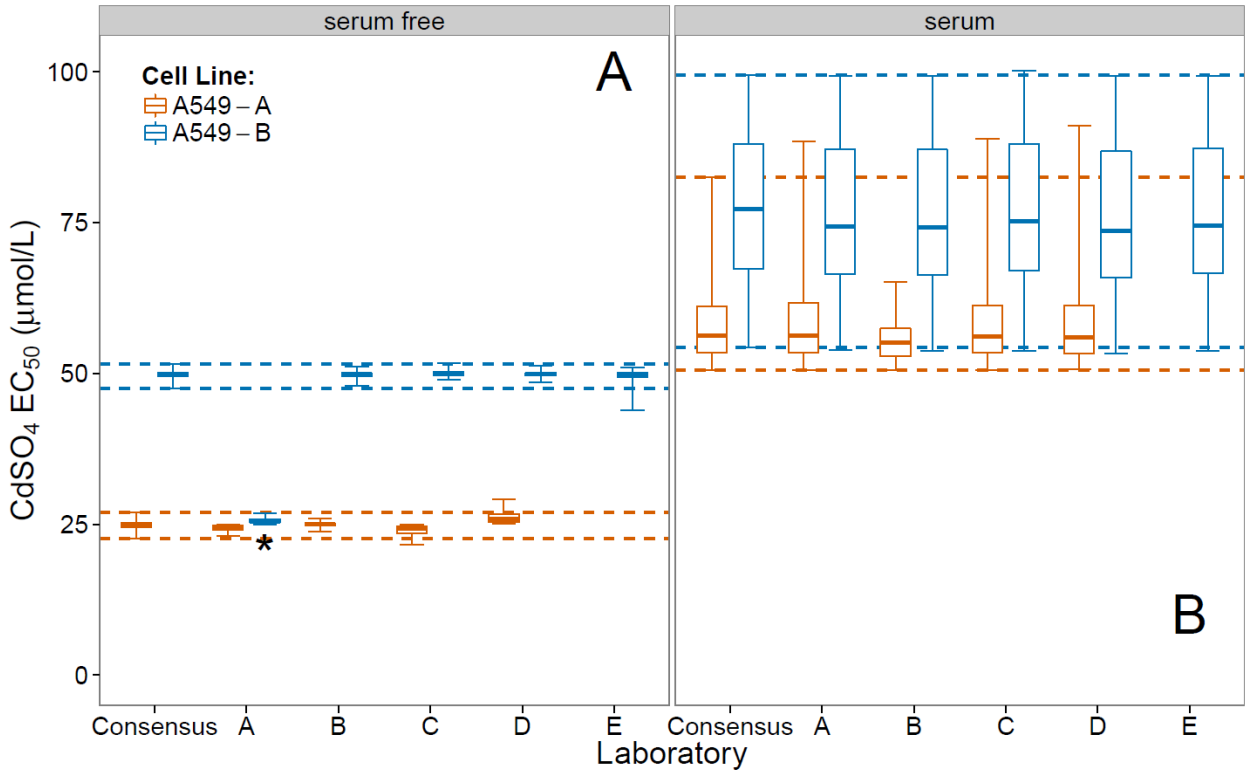
EC50 values- NP



-Looks like harmonization between the laboratories
-No cell line differences
-Nhe serum conditions increases variability

Lets look at the controls

- Chemical Process Control- tests overall measurement system



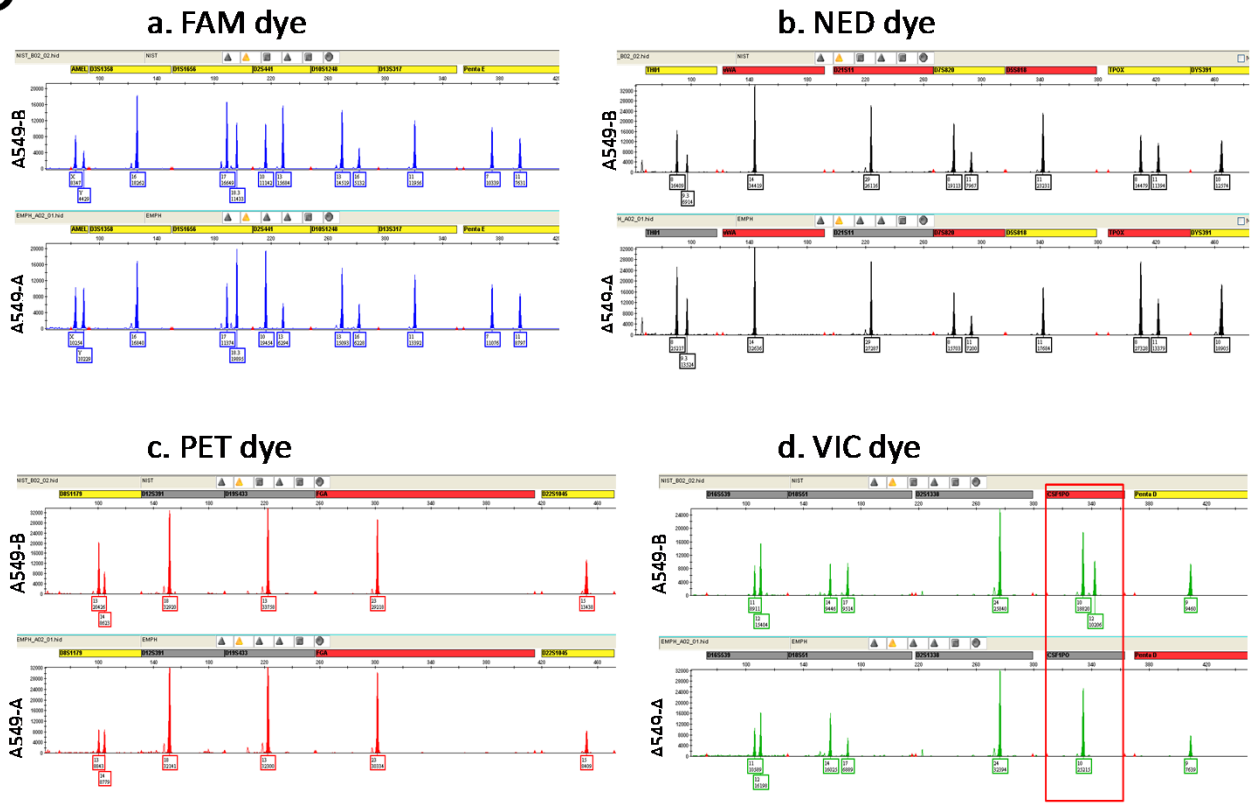
Serum free conditions, variability less than with NP

Differences between cell lines

Cell line differences?

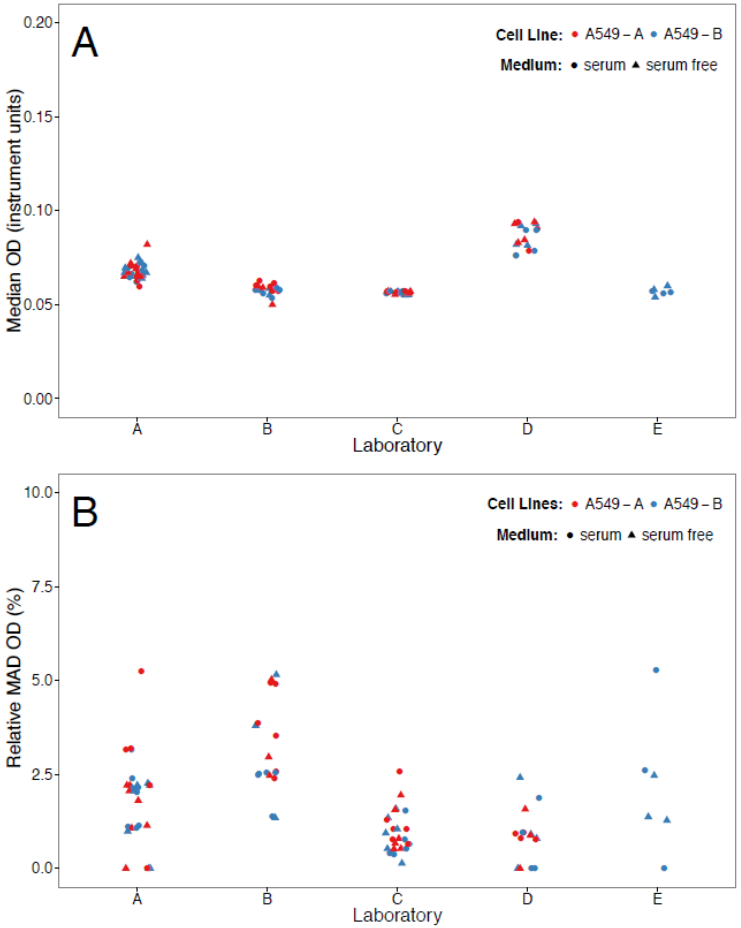
Cell line	Cell cycle time (h)	Medium volume (μm³)[1]	Short tandem repeat (STR) analysis[2]
A549-A	22.6±2.2[3]	2327±94	Missing allele 12 (CSF1PO)
A549-B	22.5±2.4[3]	2047±90	In agreement with ATCC

- Cell ID

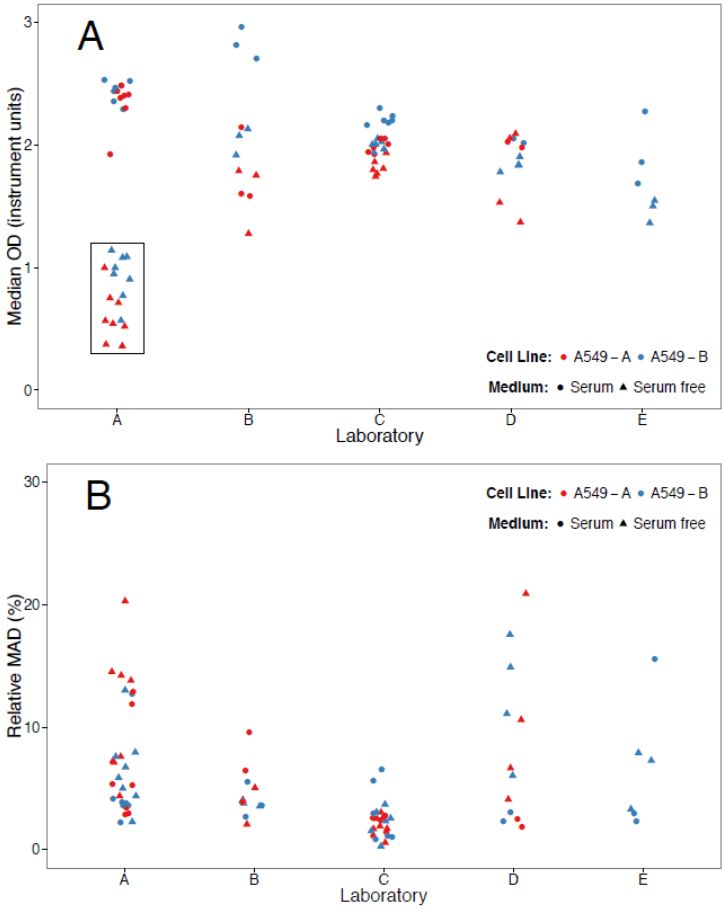


Pipetting volumes and cells

Within pipette volume control

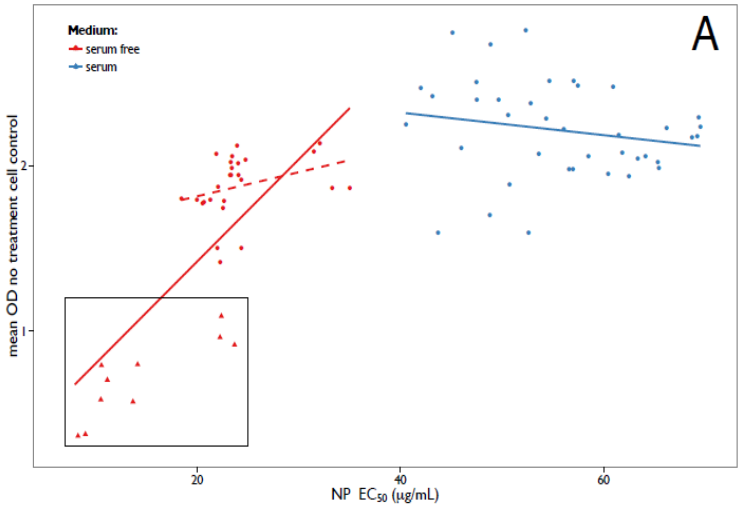


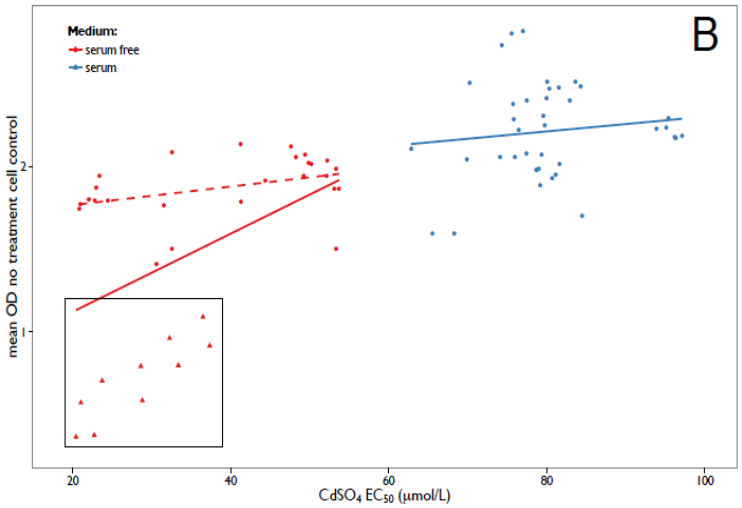
Within pipette cell control



Variability in pipetting volumes<< variability in pipetting cells

How sensitive are we to cell 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

Specification of process controls:

4 laboratories
>4 day-to-day replicates/lab
2 cell sources
4 serum sources

Control	Serum free			Serum		
	target value	range	variability	target value	range	variability
Control 1 (within) B6 – G6	1.8 OD	1.5-2.0 OD	<10%	2.0 OD	1.8-2.3	<7%
Control 2 (between) B3-B6 B8-B10	1.5 OD	1.3-1.8 OD	<12%	2.2 OD	1.8-2.8	<7%
Control 3A Background B7-G7	0.06 OD	0.05-0.09 OD	< 6%	see serum free		
Control 3B [1] Background Chemical Control B2-G2	0.06	0.05-0.09	<6%	see serum free		
Control 3C [2] Background NP B11-G11						
Control 4 [3] Chemical reaction control	49.9	47.5-51.5		77.2	54.3-99.4	

specifications

1) If no additional background from the chemical reaction control is observed
2) No values given, because some of the laboratories observed a background signal under serum condition due to NP agglomerates sedimentation
3) Values of the NIST cell line are given. They are fresh out of storage from ATTC and

Conclusions:

- Interlab data with process controls presents a powerful view of a biological assay
- Variability in volume<cells<cells+NP<cells+NP+serum
- Process controls allow troubleshooting of protocol
  - Resuspension of cells
  - Dispersion of NP
  - Rinsing cells
- Check cell line ID. May affect controls and not test result
- Interlab with process controls can allow generation of specifications
- Meeting specifications provides evidence that the test procedure is as expected. “Accept test result”
- Adds Measurement Assurance to a Cell Assay.