Measurement Assurance in a Nanocytotoxicity Assay

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How do we improve confidence in alternative model measurements?

- Cellular measurements are complicated
  - Cell culture, extended periods, manual
  - Manual steps in setting up experiments
  - Multiple reagents
  - Instrumentation
- How do you prove measurement quality?

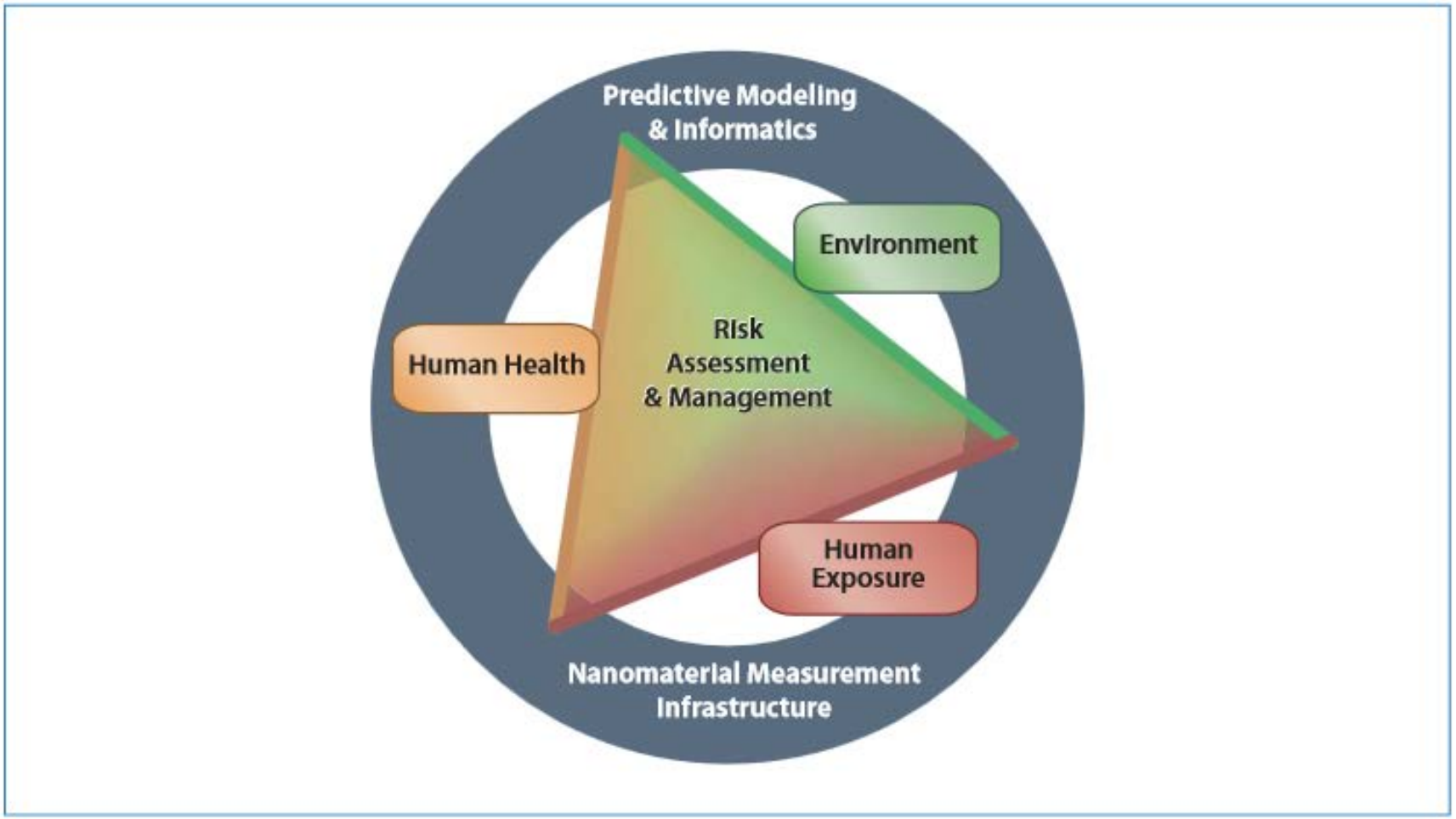
What can we do to increase confidence in the measurement

- 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

The importance and challenge of nanotechnology risk assessment

- Nanotechnology is expected to have a massive commercial impact
- However, measuring their potential toxicological effects is challenging
  - Many of the standard methods for dissolved chemicals require nanoparticle-specific modifications
  - Nanoparticles may cause artifacts with many assays
  - There is a huge range of nanoparticles (different sizes, coatings, chemical compositions, etc.) to test
  - Prioritization is needed for screening the potential effects and *in vitro* methods have been suggested for this purpose
  - But, there are disagreements among laboratories on the cytotoxic effects of many nanoparticles

NIST Role in Nano-Environmental Health & Safety



National Nanotechnology Initiative 2011 Environmental Health and Safety Research Strategy

ENVIRONMENTAL Science & Technology

Critical Review

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Identification and Avoidance of Potential Artifacts and Misinterpretations in Nanomaterial Ecotoxicity Measurements

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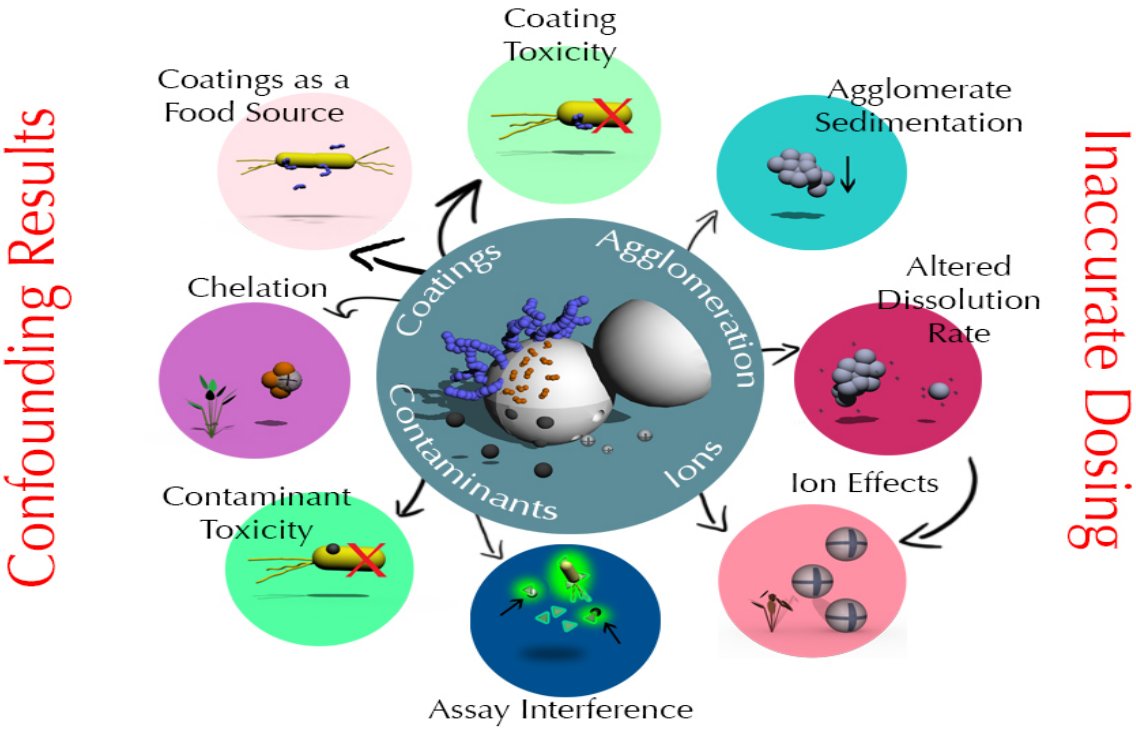

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Chemical Research in Toxicology

Perspective

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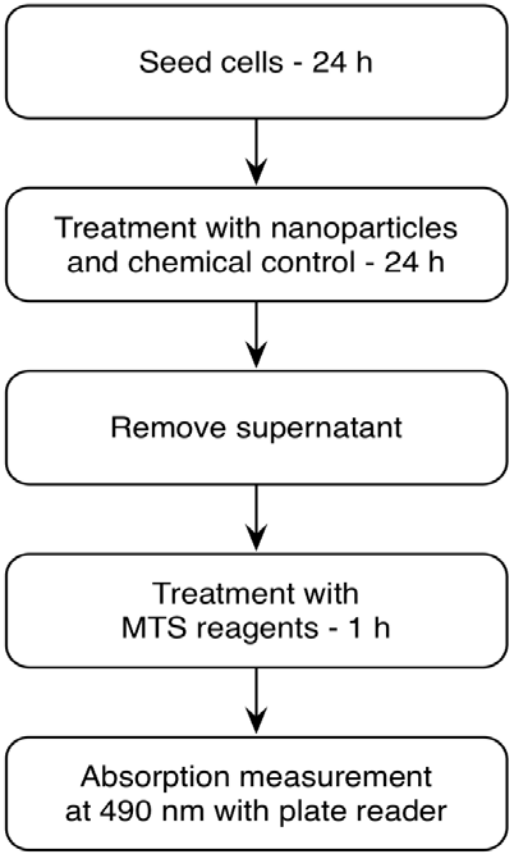
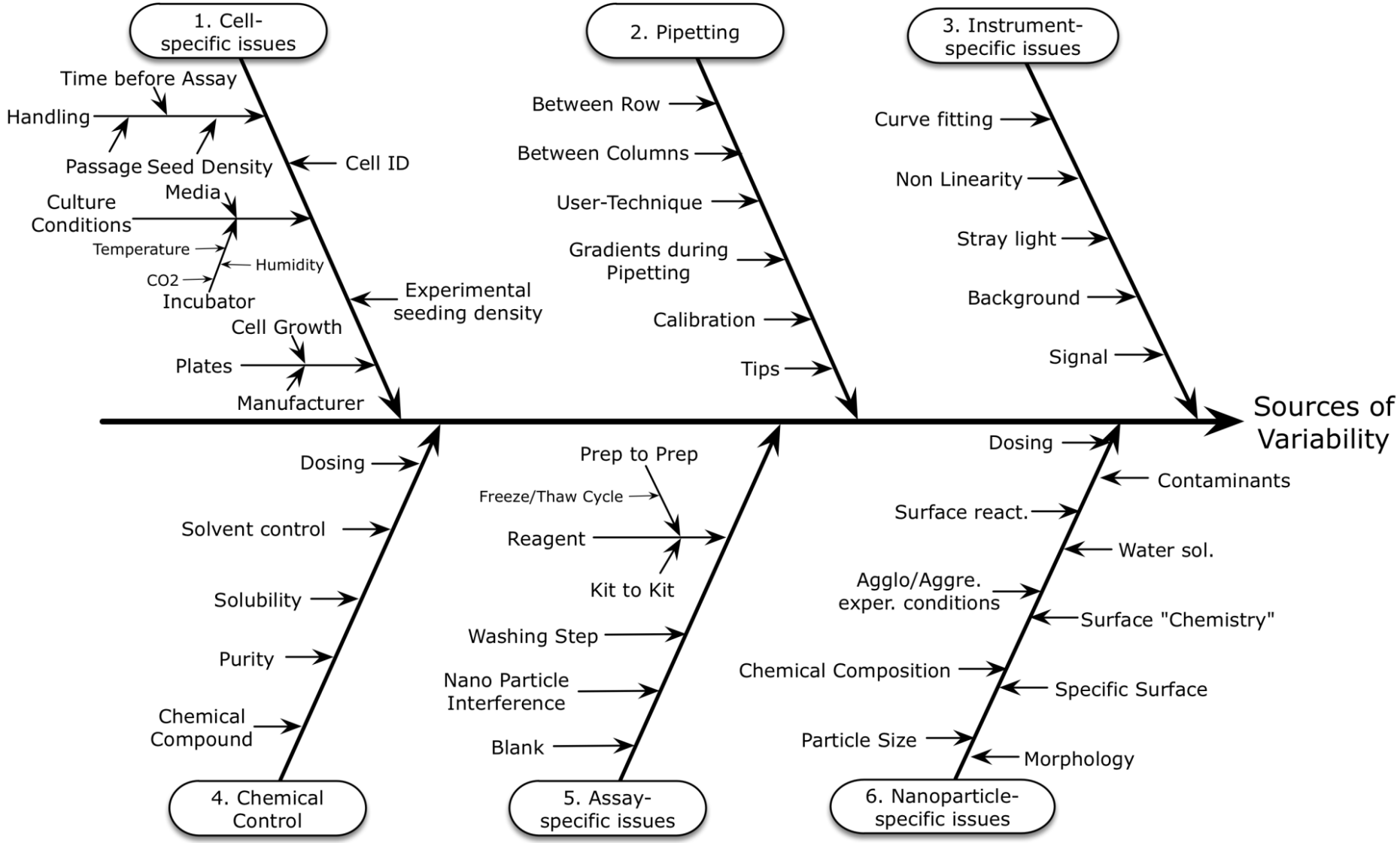
Use of Cause-and-Effect Analysis to Design a High-Quality Nanocytotoxicology Assay

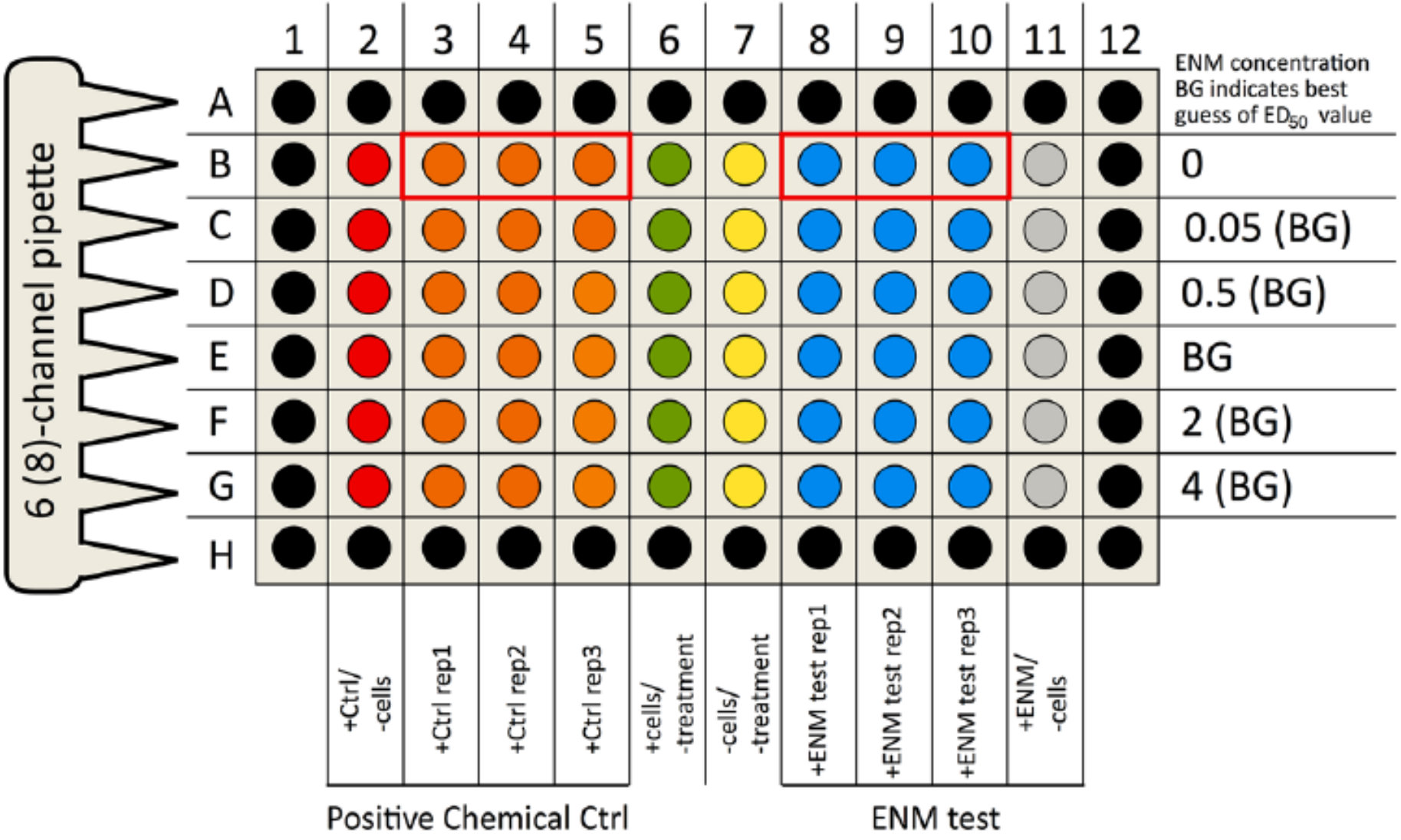


Find sources of variability in assay

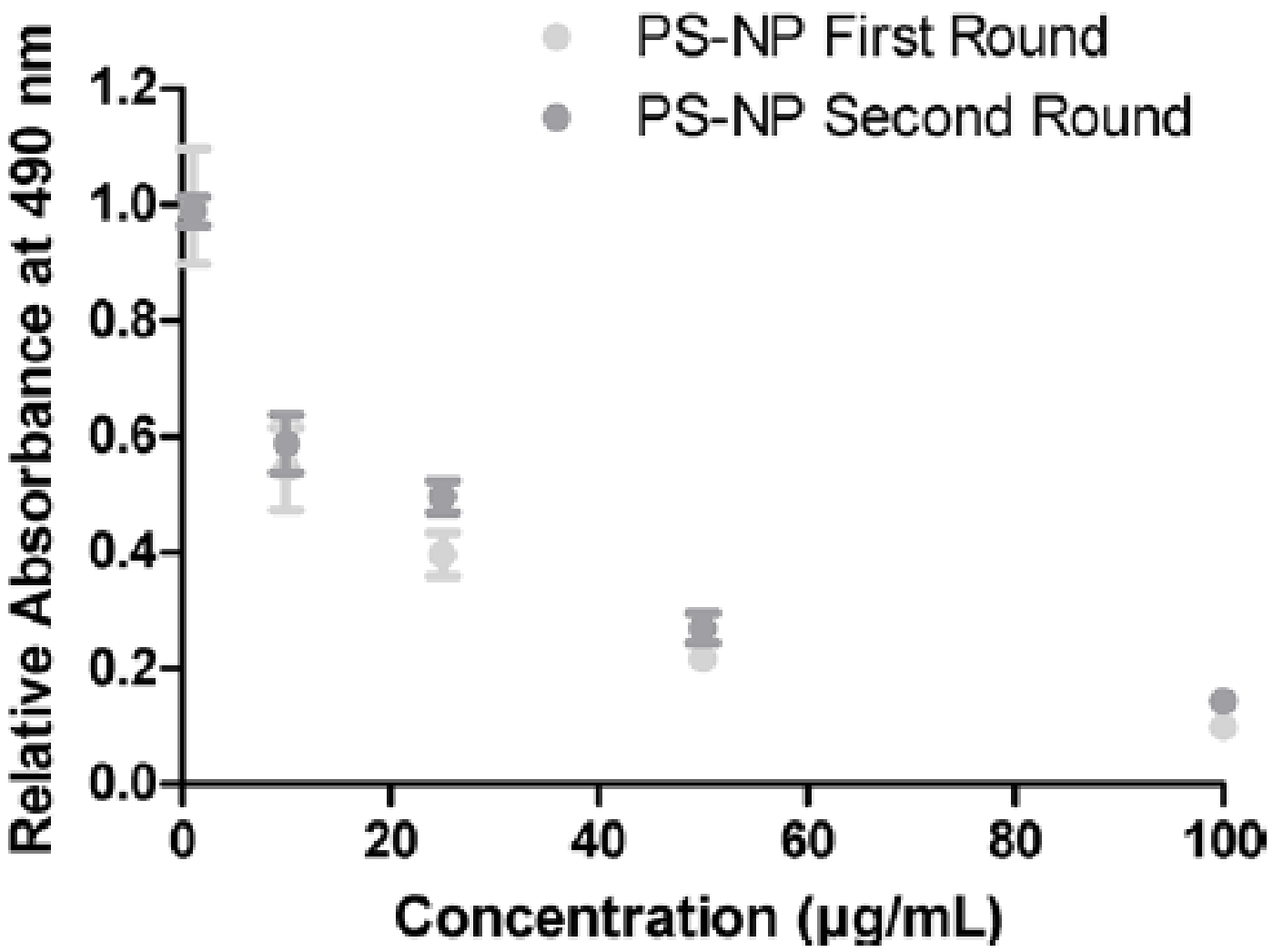


Cause and effect diagram for MTS assay

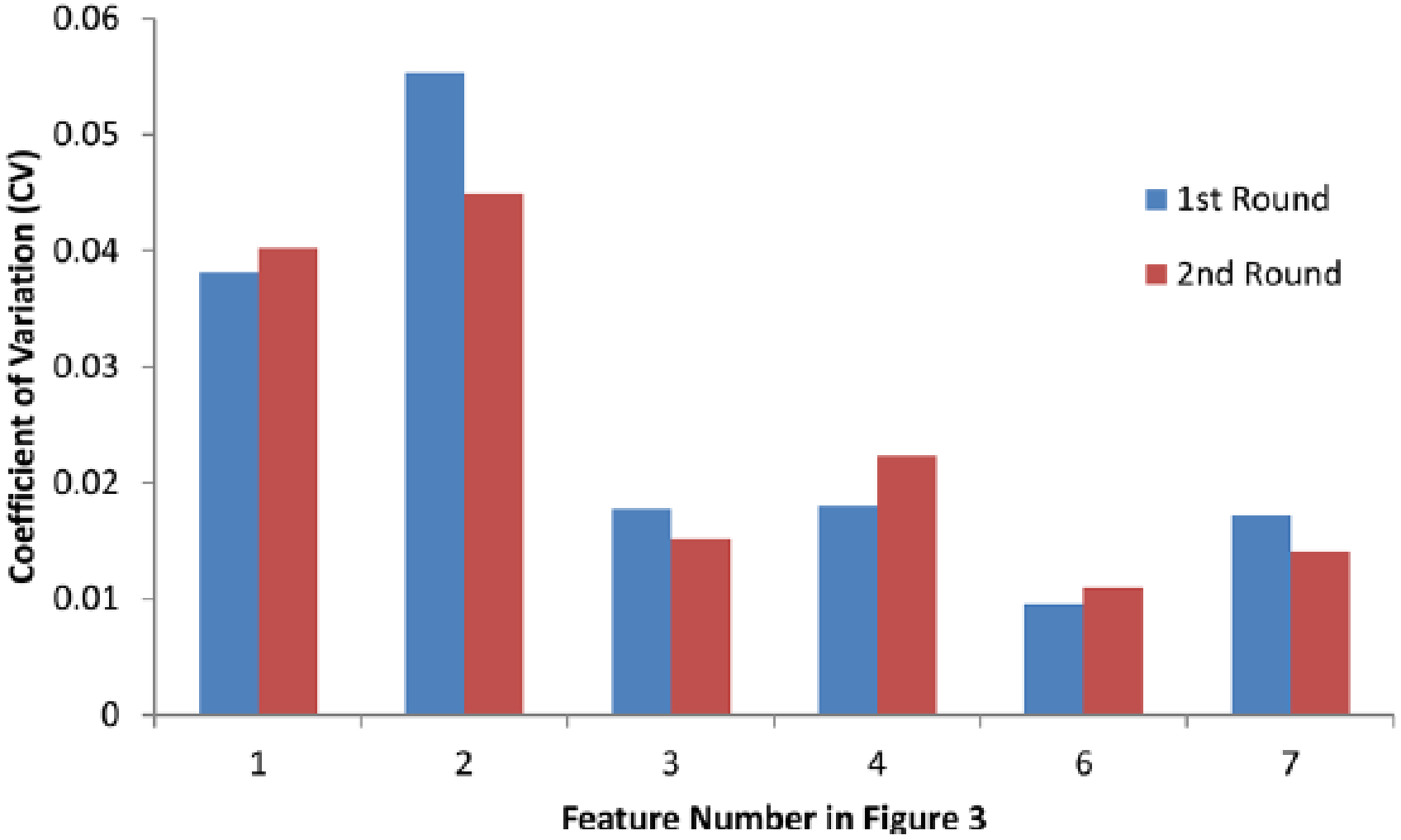
Design a new plate format with process control measurements



Results


PS-NP First Round
PS-NP Second Round
Relative Absorbance at 490 nm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
Concentration (µg/mL)

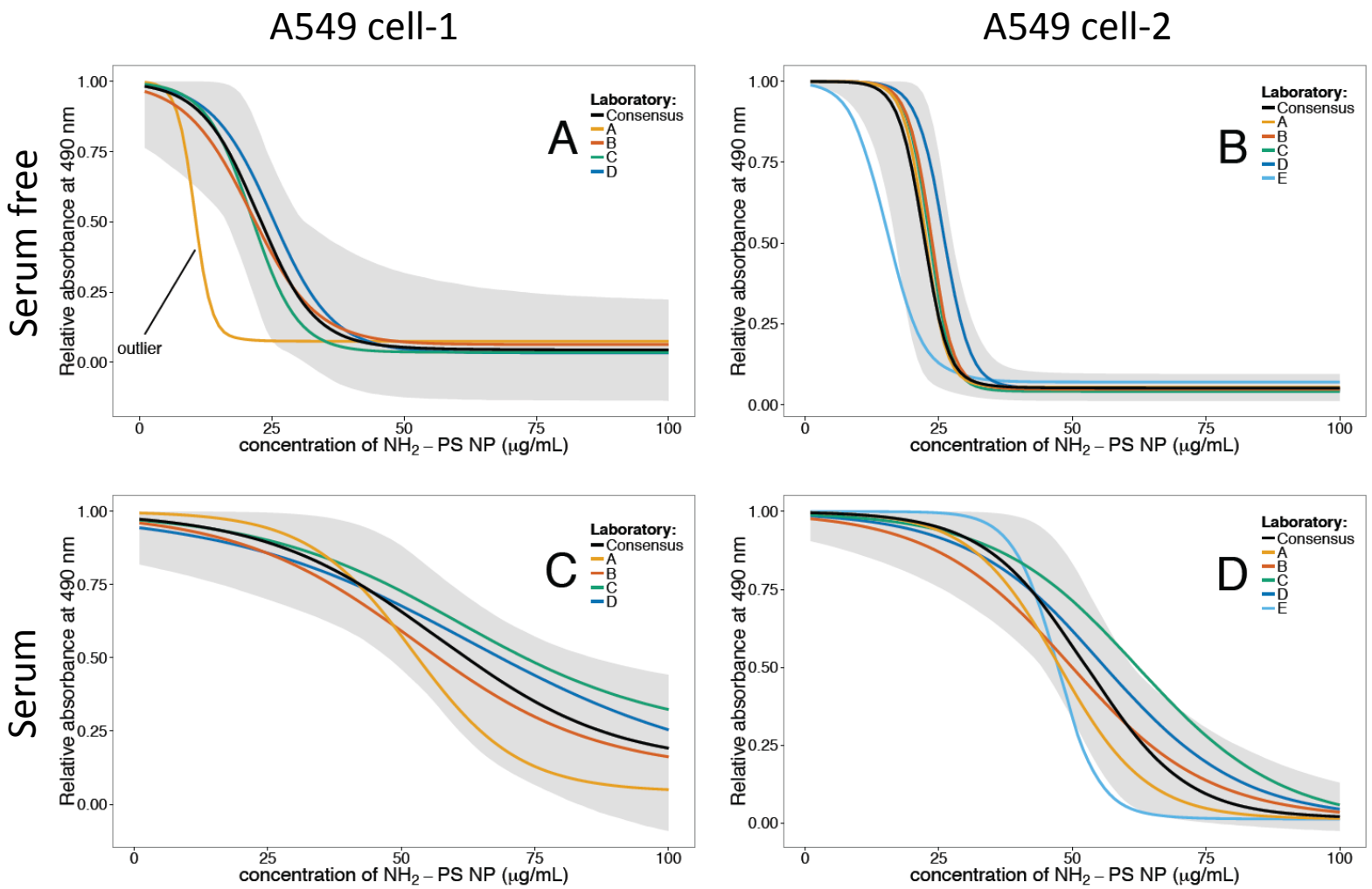
Results


0.06
0.05
0.04
0.03
0.02
0.01
0
Coefficient of Variation (CV)
1st Round
2nd Round
1
2
3
4
6
7
Feature Number in Figure 3

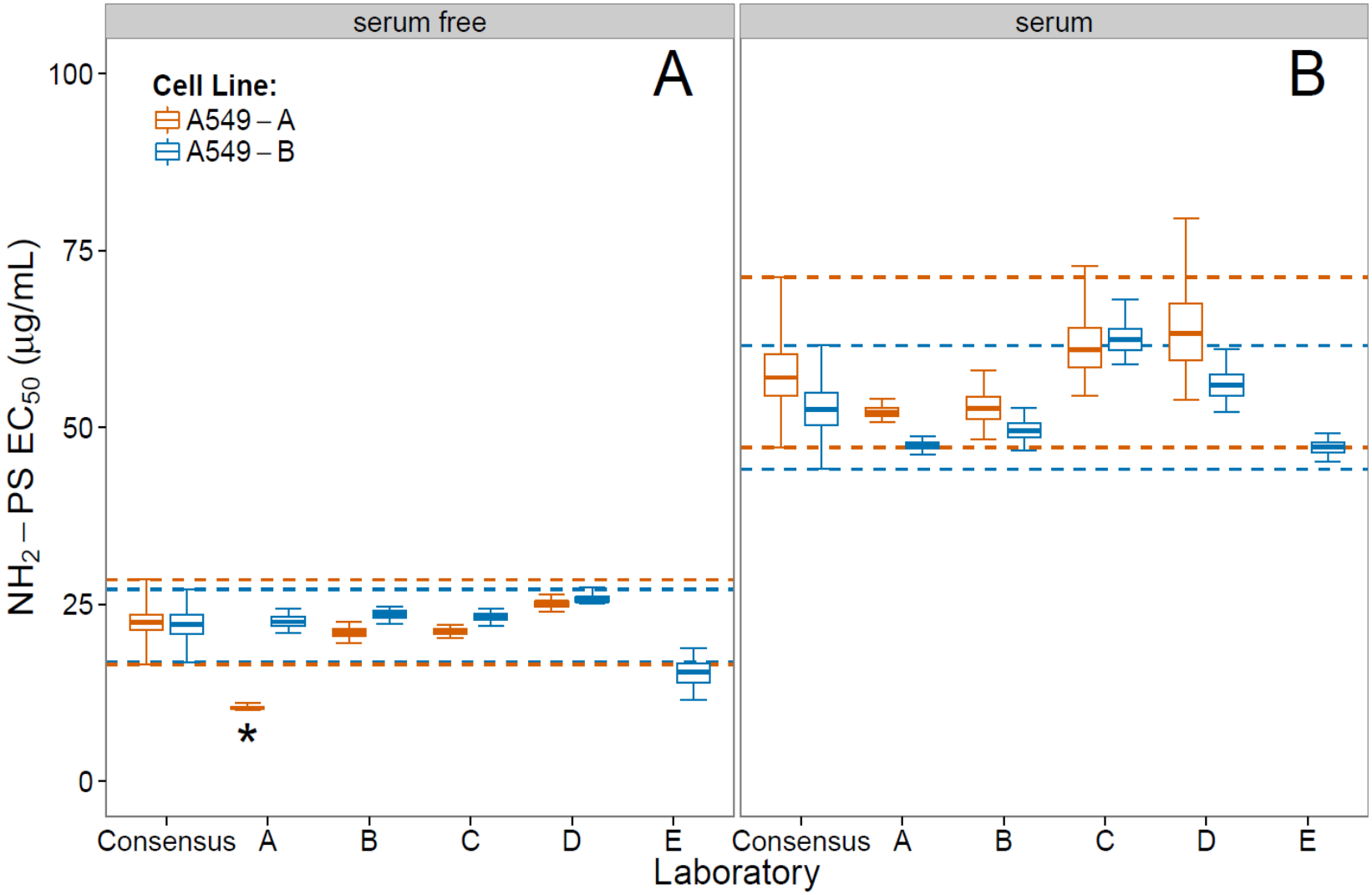
Interlaboratory comparison

- 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 ($CdCl_2$)

Dose Response Curves NP



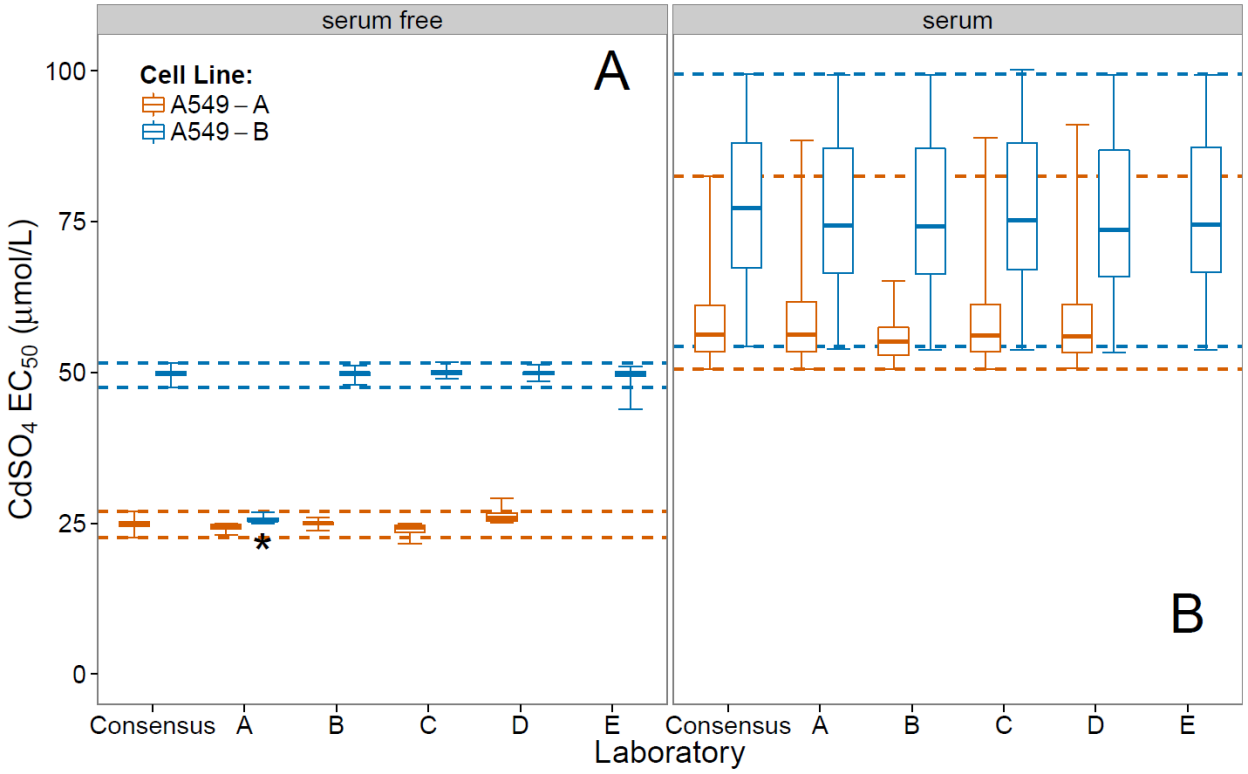
NP EC50 values



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

Lets look at the controls

- Chemical Process Control- tests overall measurement system



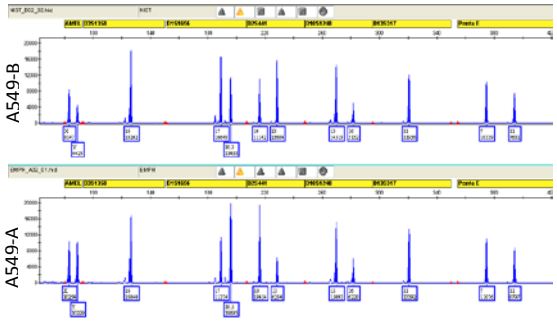
Serum free conditions, variability less than with NP

Differences between cell lines

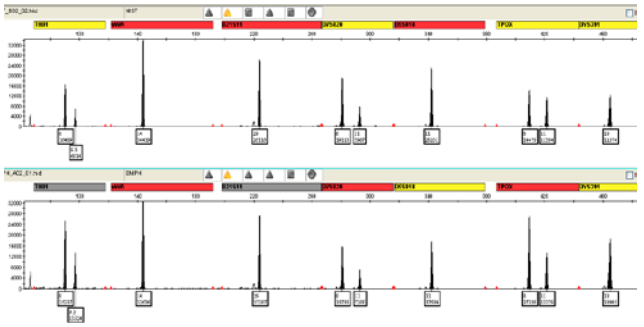
Cell line differences?

- Cell ID

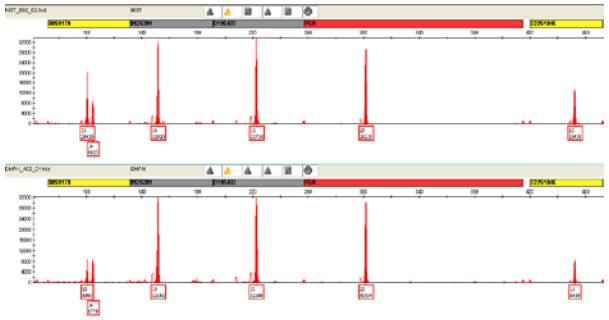
a. FAM dye



b. NED dye

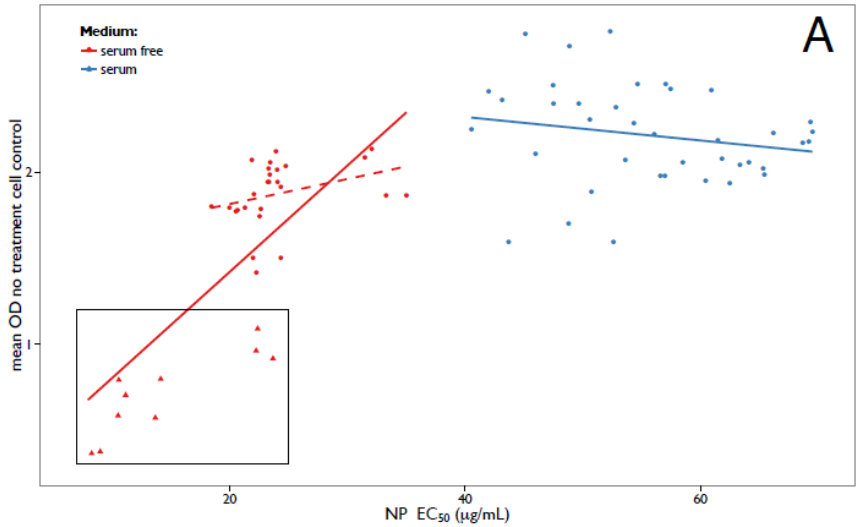


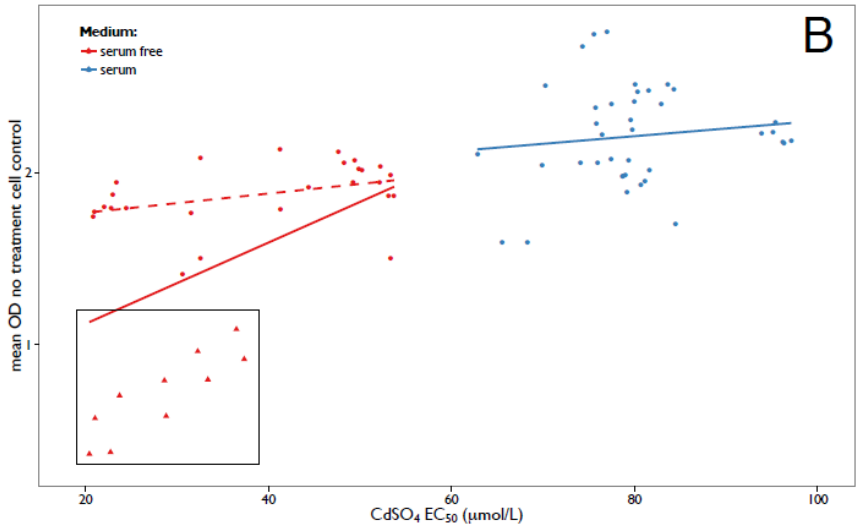
c. PET dye



d. VIC dye

How sensitive are we to cell seeding variability

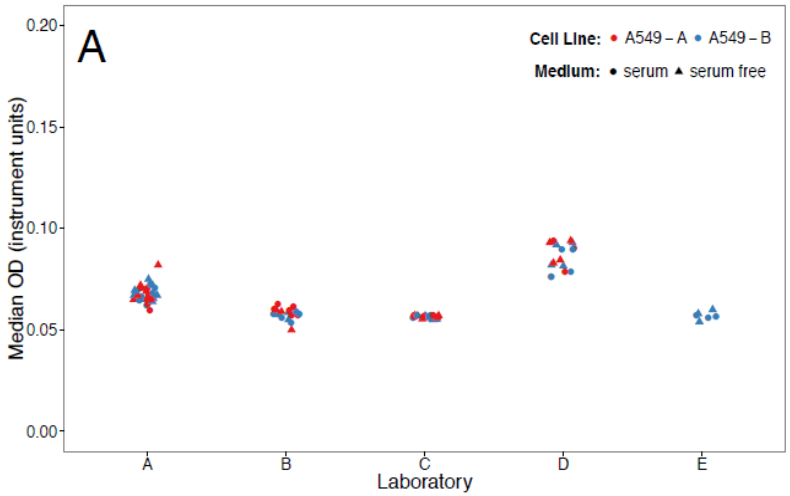


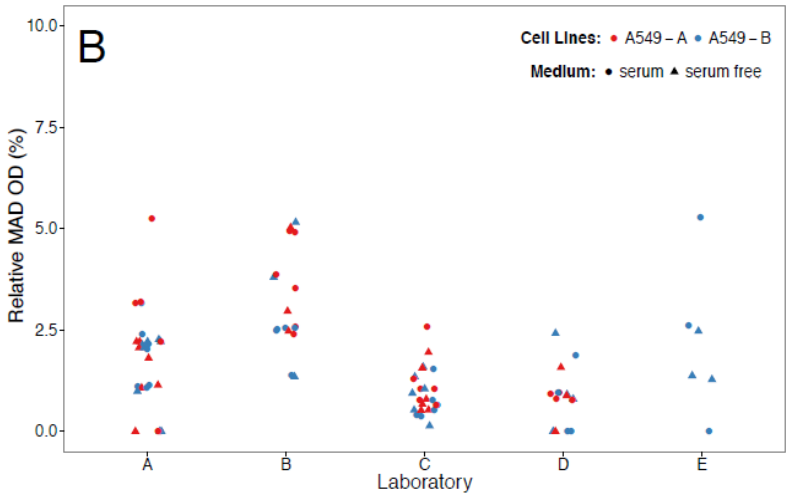


- Correlation between no-treatment cells and NP EC50
- If outliers are removed, no strong correlation
- Suggests that within this range of cell seeding variability (OD=1.5-2.5) no big effect on EC50

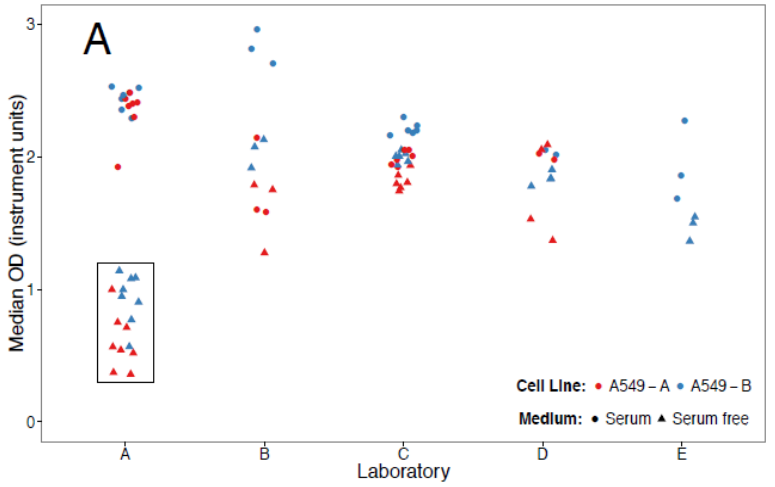
Pipetting volumes and cells

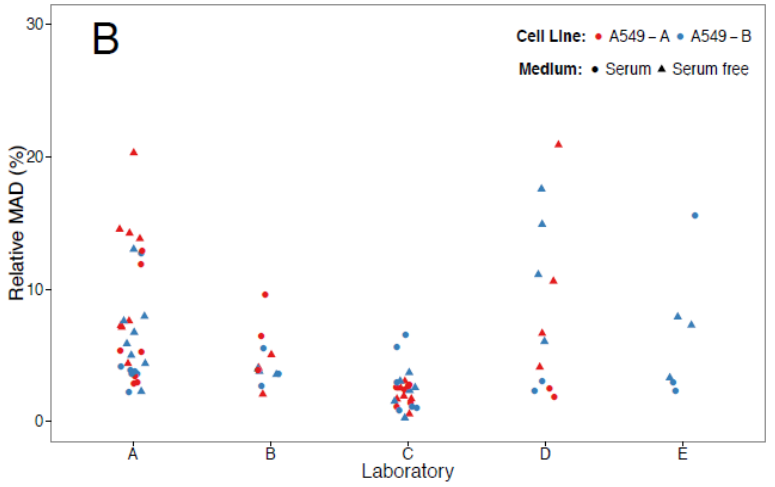
Within pipette volume control





Within pipette cell control





Variability in pipetting volumes<< variability in pipetting cells

Specification of process controls:

Control	Serum free: target value	Serum free: range	Serum free: variability	Serum: target value	Serum: range	Serum: 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%	0.06 OD	0.05-0.09 OD	< 6%
Control 3B [1)] Background Chemical Control B2-G2	0.06	0.05-0.09	<6%	0.06	0.05-0.09	<6%
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	

Conclusions:

- Interlab data with process controls presents a powerful view of a biological assay
- The findings regarding the sources of variability in this assay may be relevant for other cytotoxicity assays
- Check cell line ID. May affect controls and not test result
- The process used to quantify the sources of variability and generate test specifications can be used with other assays
- Meeting specifications provides evidence that the test procedure is as expected. “Accept test result”
- Adds Measurement Assurance to a Cell Assay

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