

# 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?

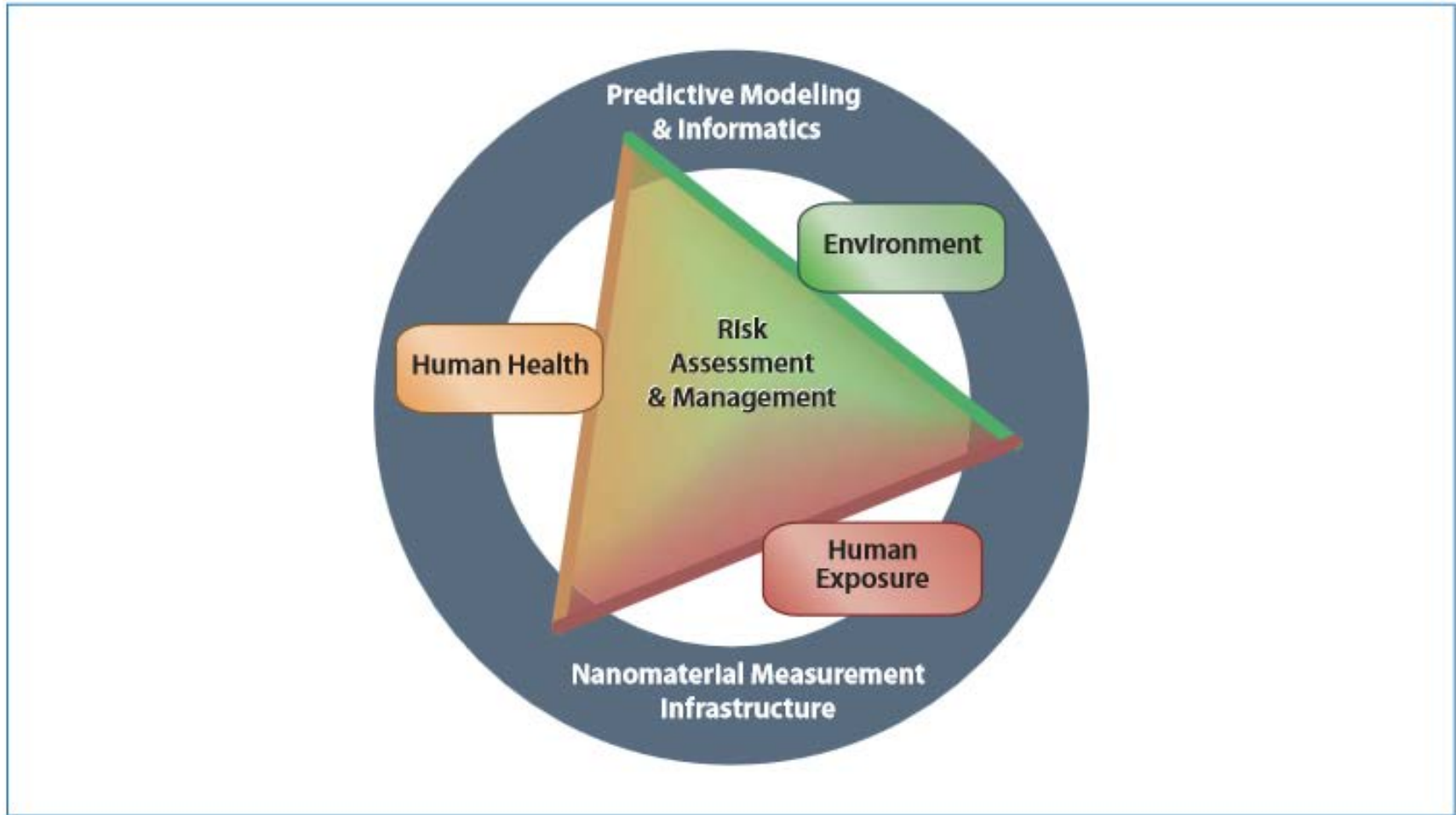
# What can we do to increase confidence in the measurement

- 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

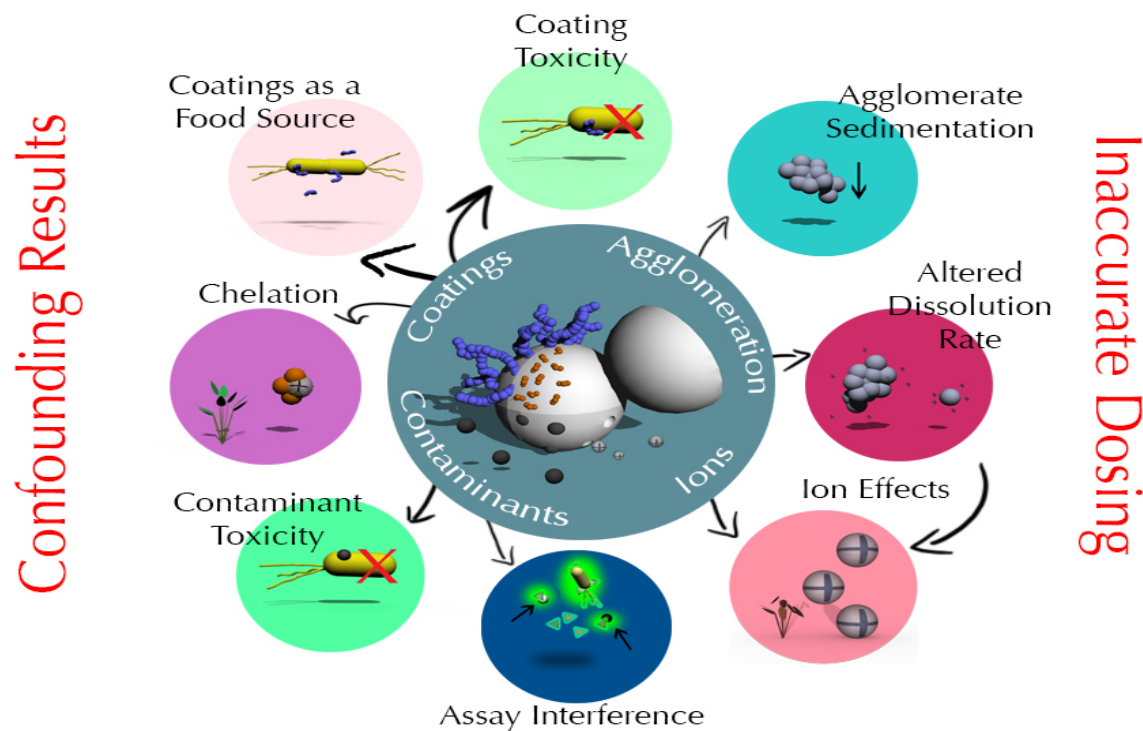
# NIST Role in Nano-Environmental Health & Safety



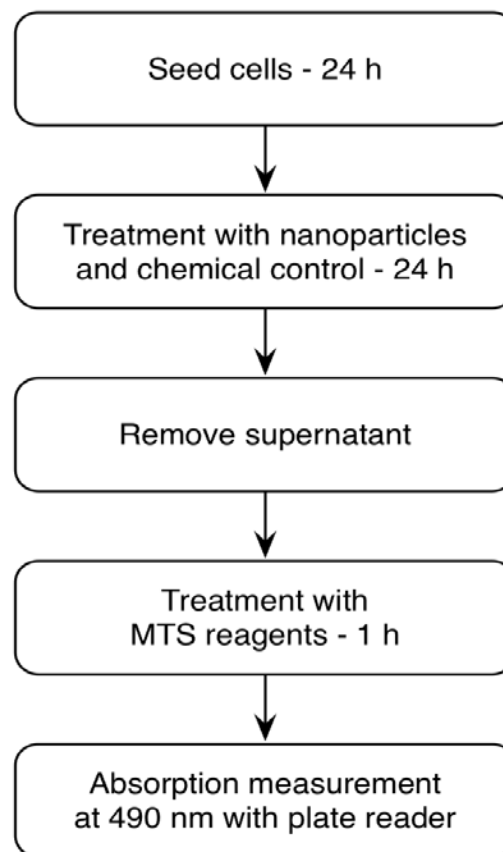
**National Nanotechnology Initiative 2011 Environmental Health and Safety Research Strategy**

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

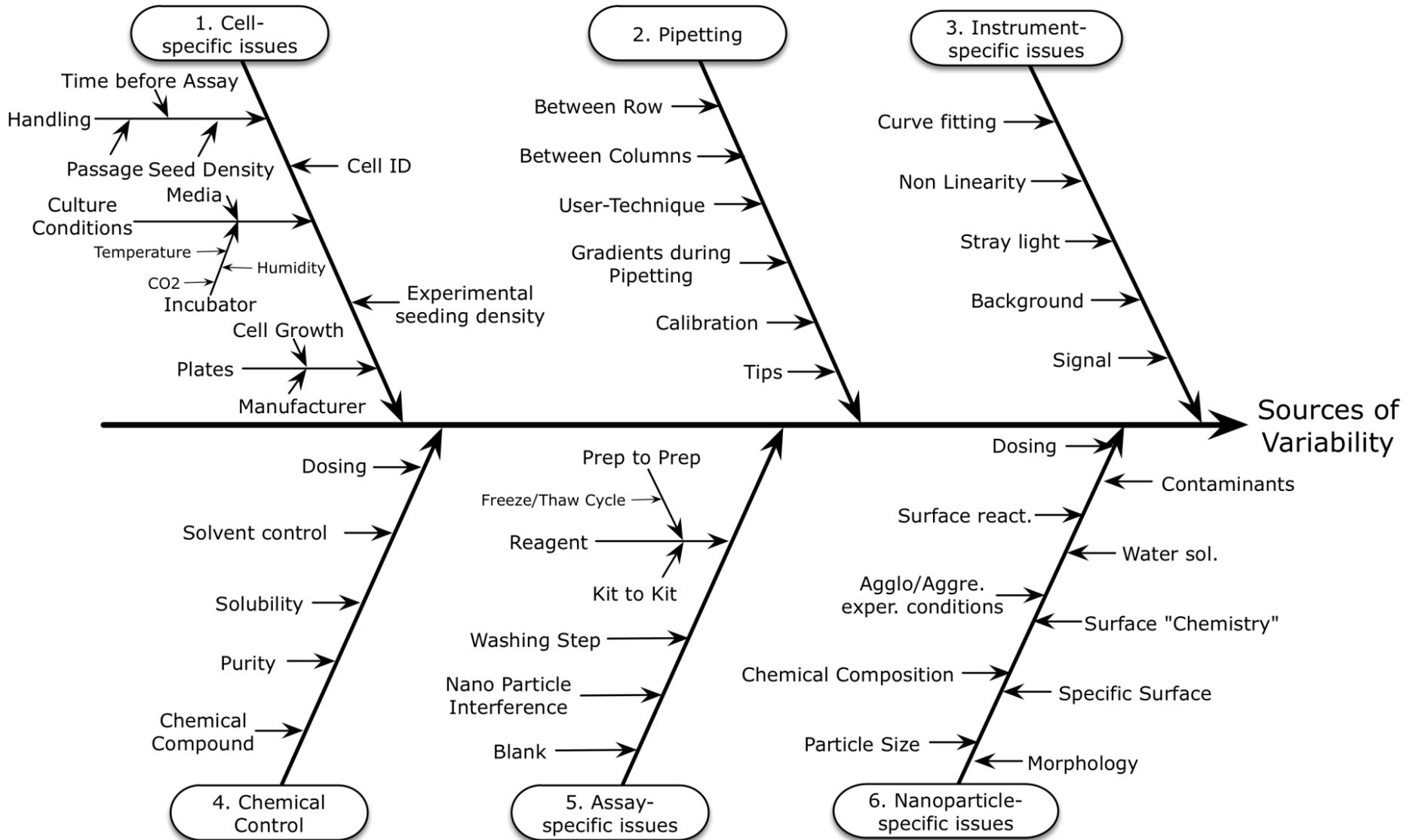
Elijah J. Petersen,<sup>†,\*</sup> Theodore B. Henry,<sup>‡,§,||</sup> Jian Zhao,<sup>⊥</sup> Robert I. MacCuspie,<sup>#,∇</sup> Teresa L. Kirschling,<sup>○</sup> Marina A. Dobrovolskaia,<sup>◆</sup> Vincent Hackley,<sup>#</sup> Baoshan Xing,<sup>⊥</sup> and Jason C. White<sup>¶</sup>



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



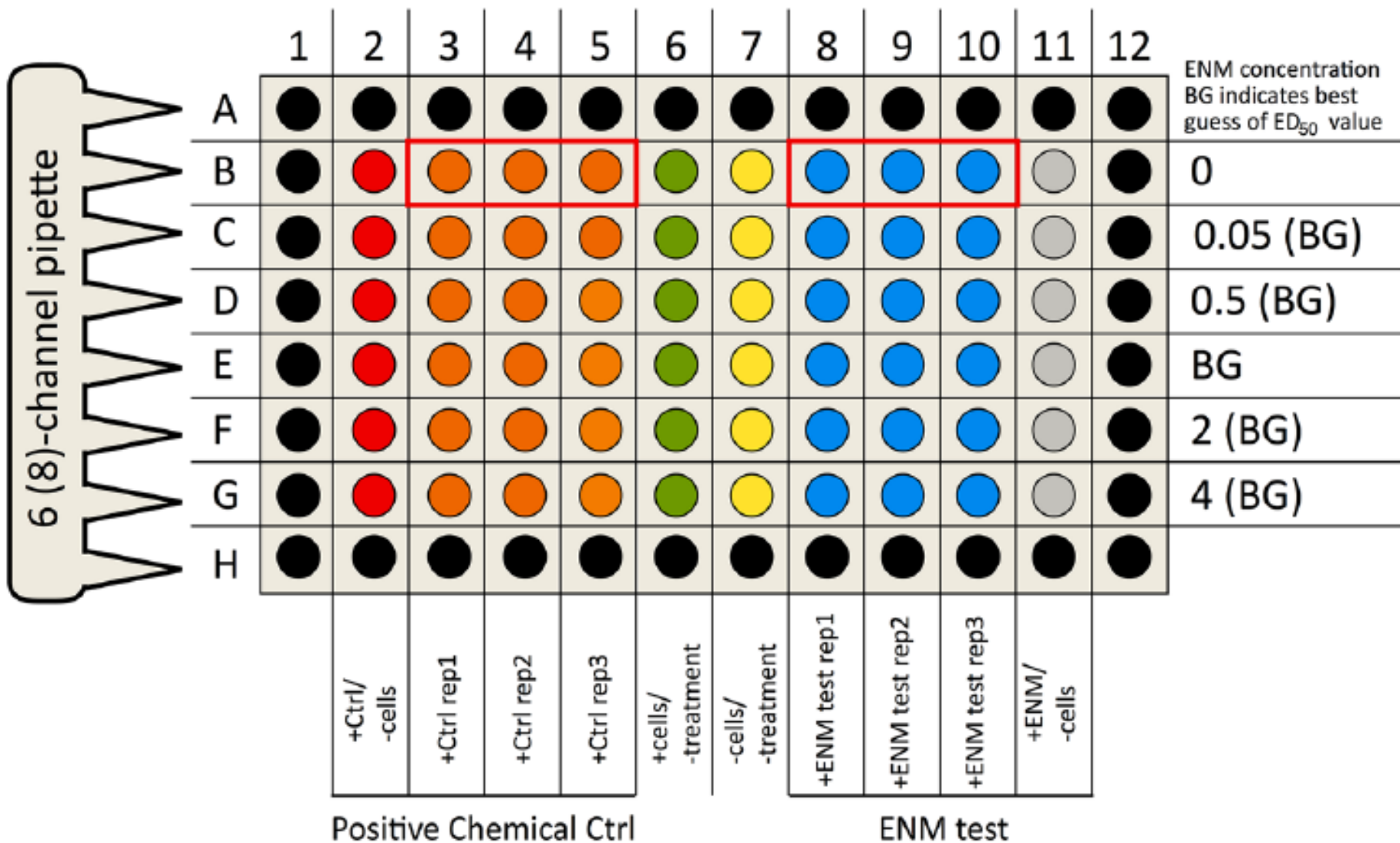
# Find sources of variability in assay



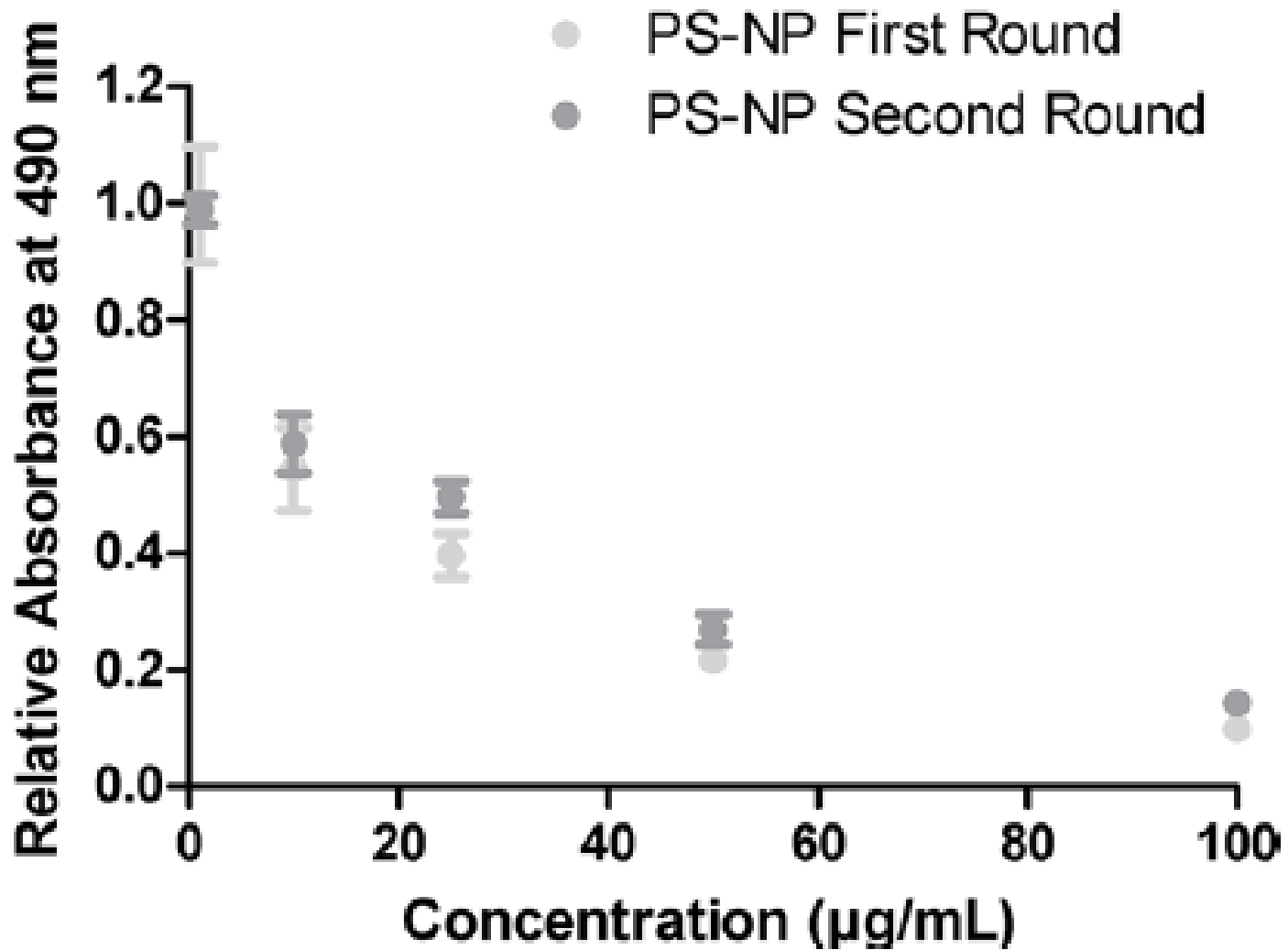
Cause and effect diagram for MTS assay



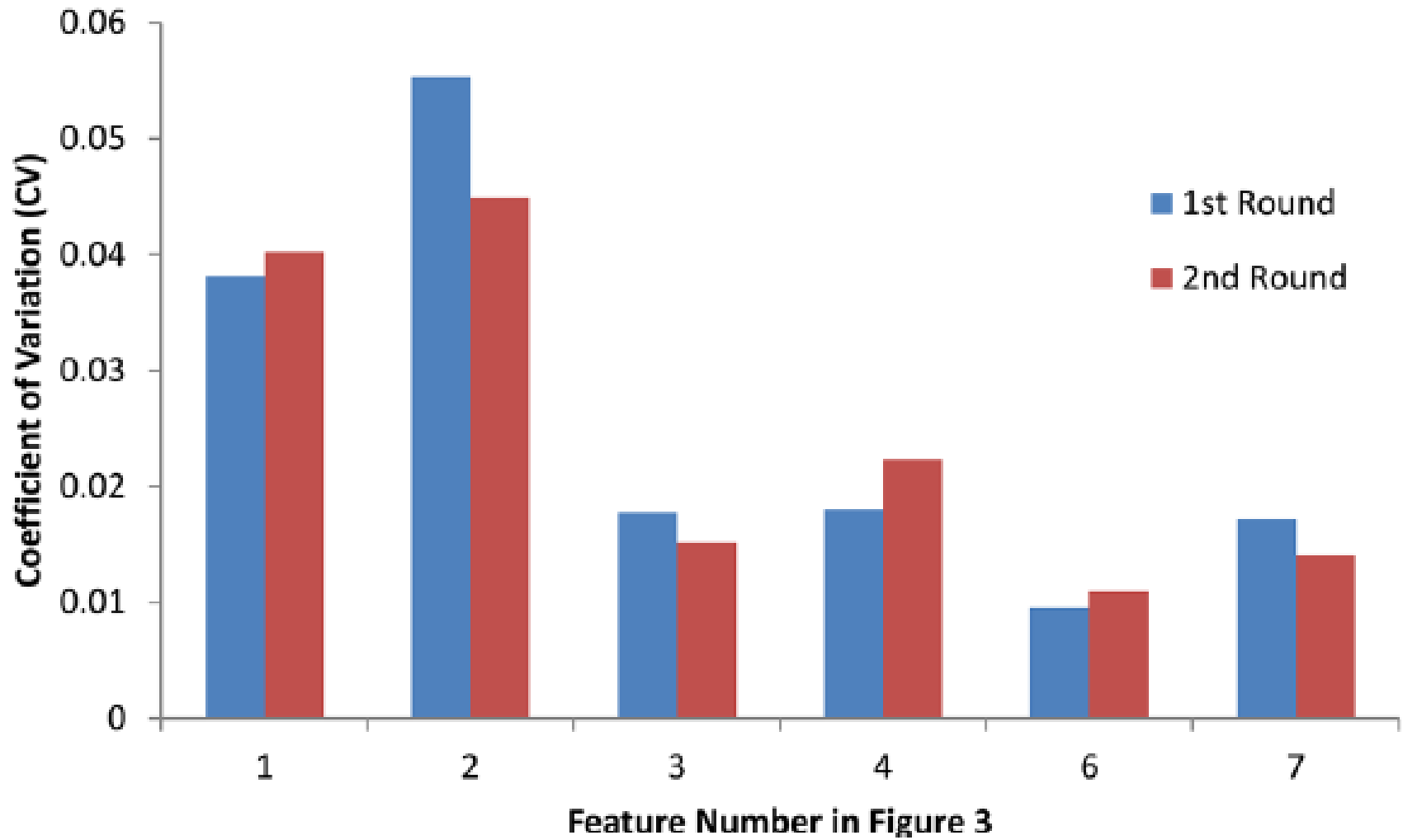
# Design a new plate format with process control measurements



# Results



# Results



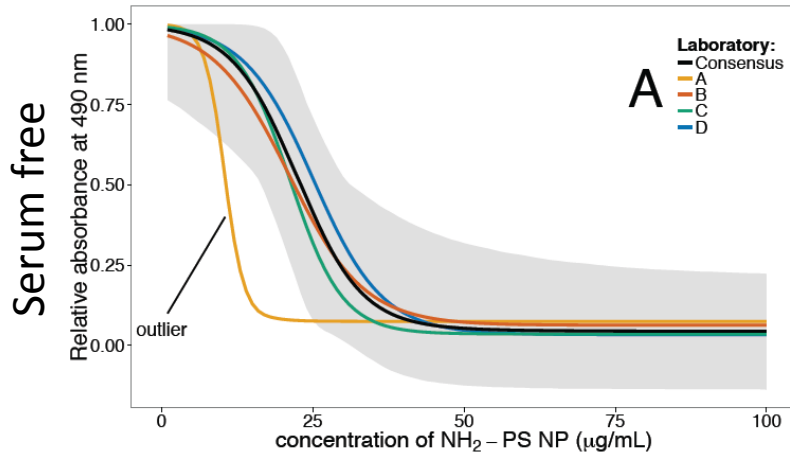
# Interlaboratory comparison

- 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 ( $\text{CdCl}_2$ )

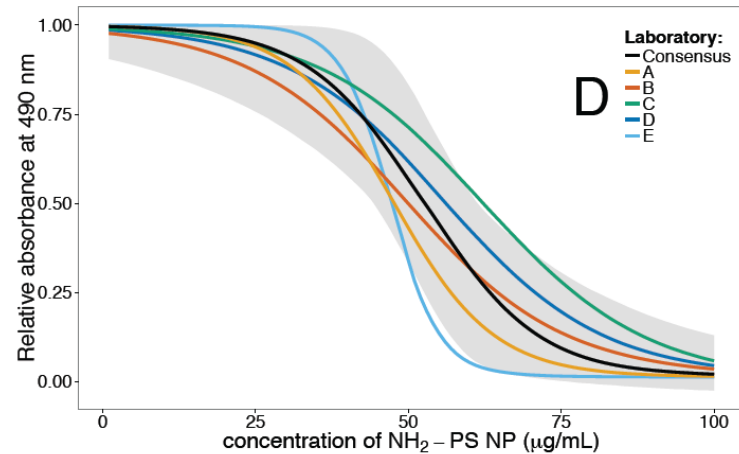
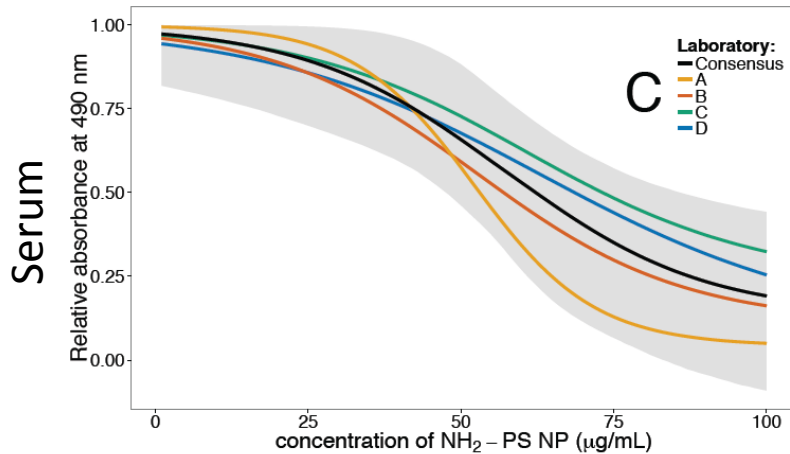
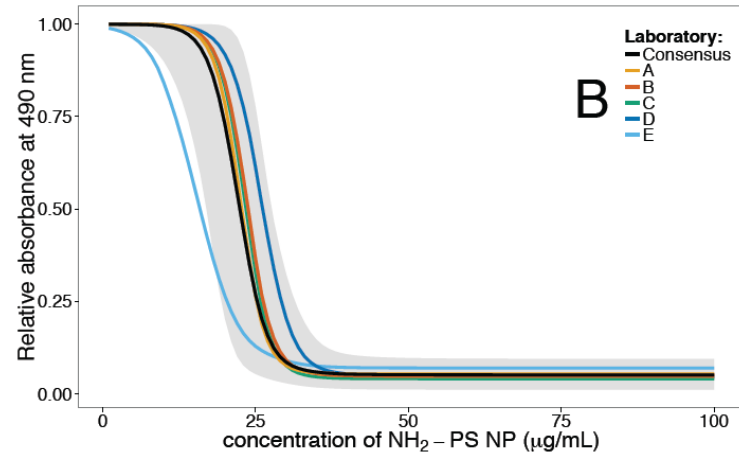


# Dose Response Curves NP

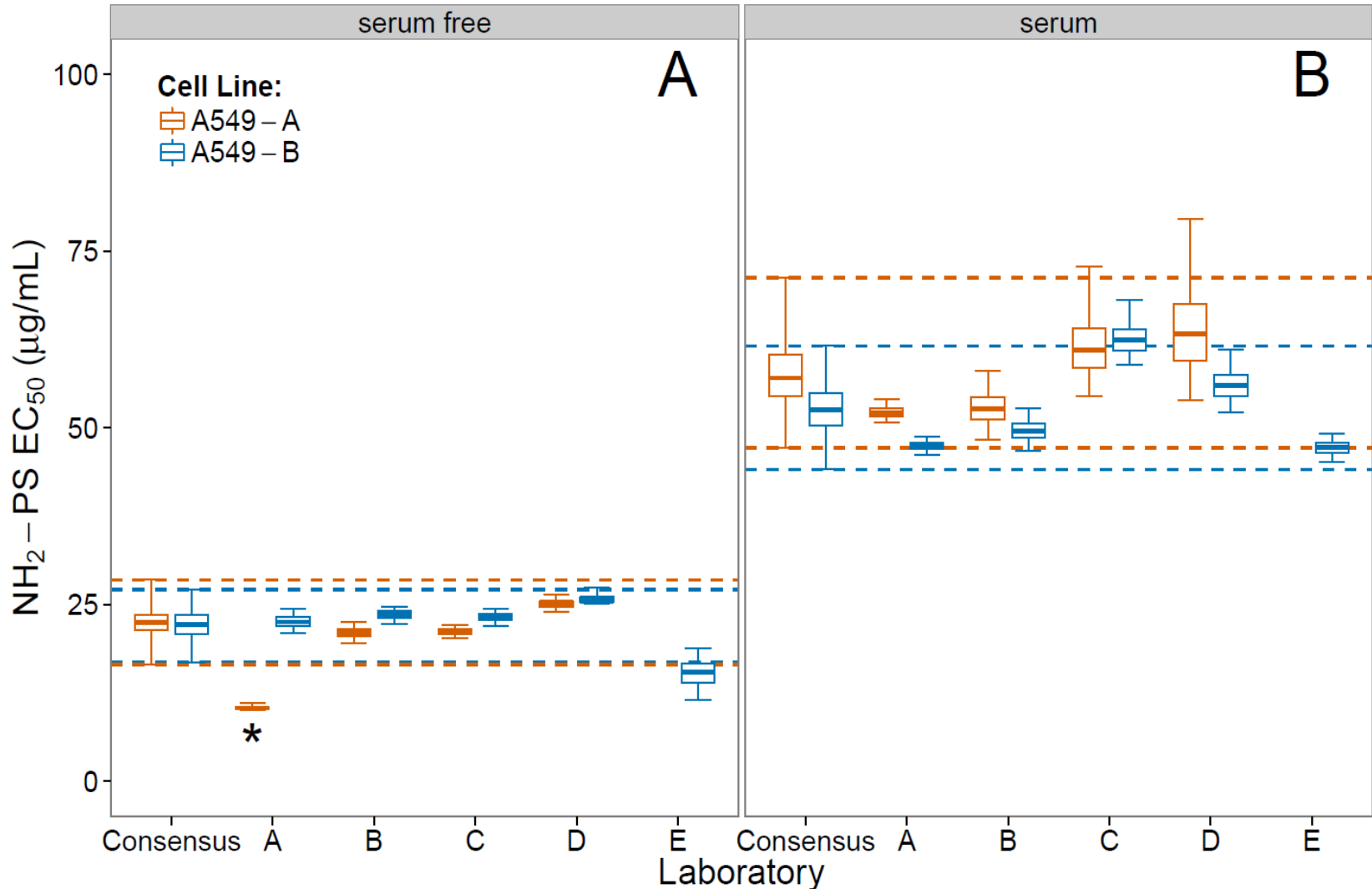
A549 cell-1



A549 cell-2



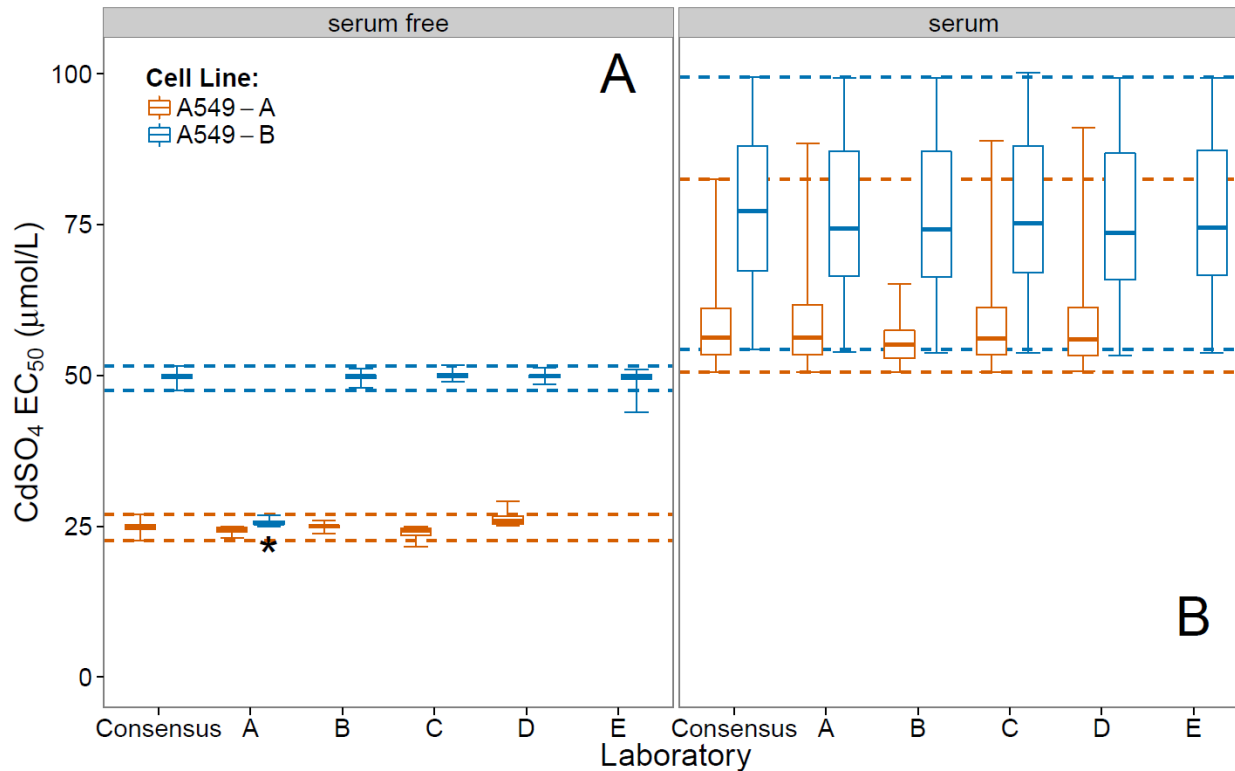
# NP EC50 values



- 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

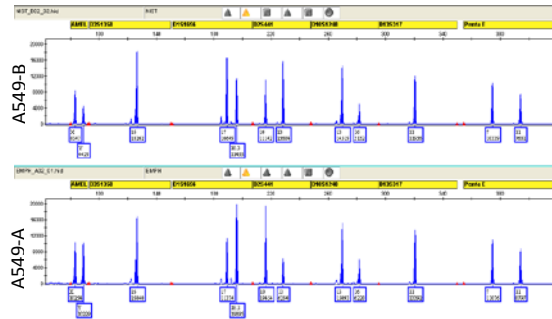


Serum free conditions, variability less than with NP  
Differences between cell lines

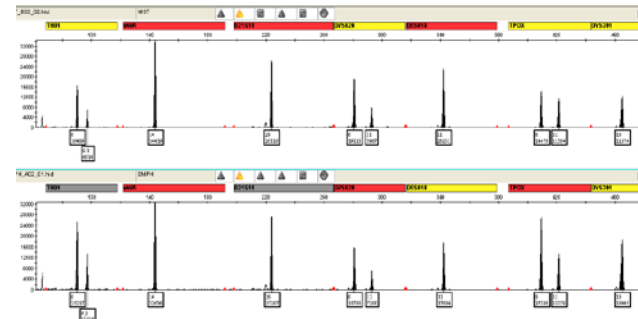
# Cell line differences?


- Cell ID

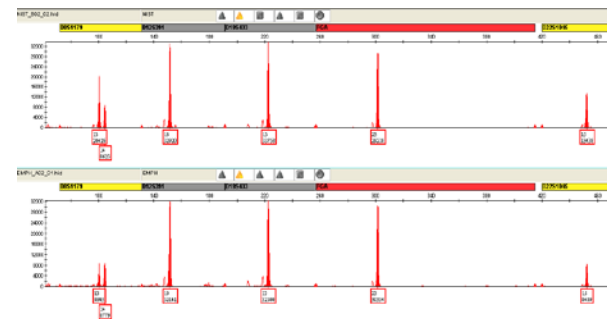
a. FAM dye



b. NED dye



c. PET dye

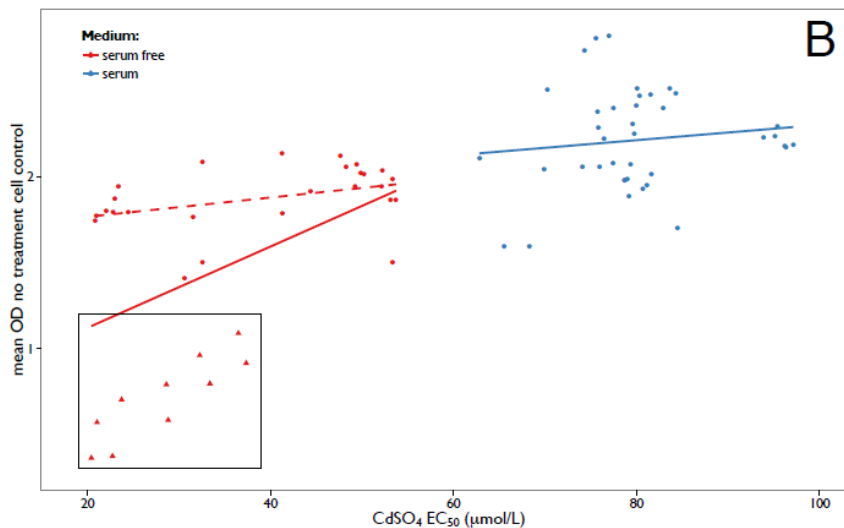
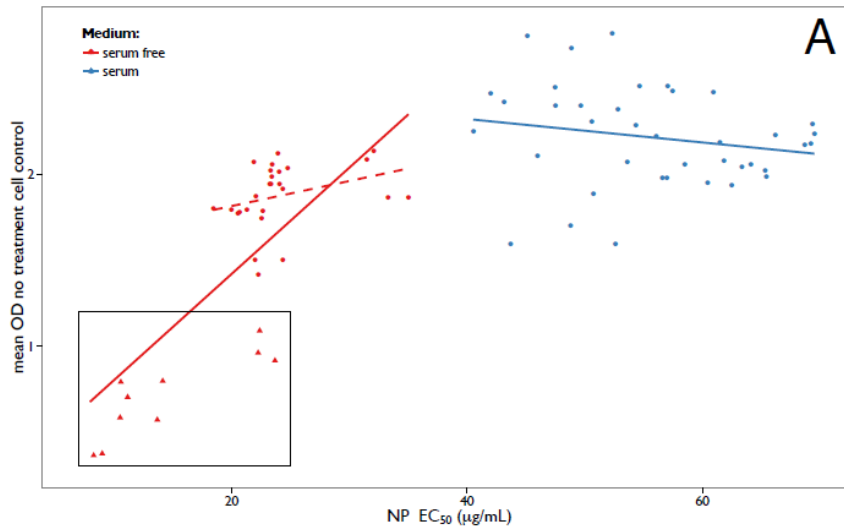


d. VIC dye





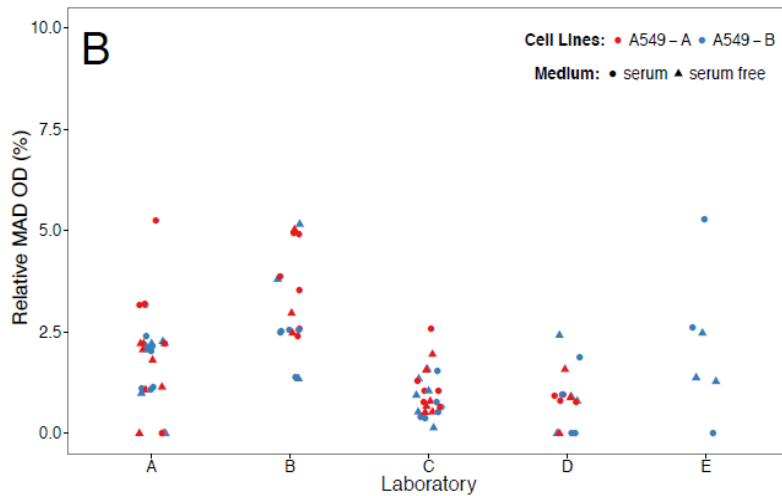
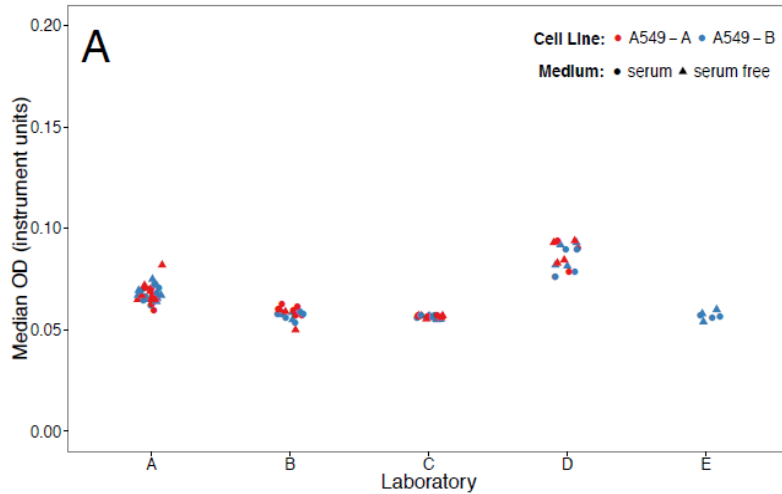
# How sensitive are we to cell seeding variability



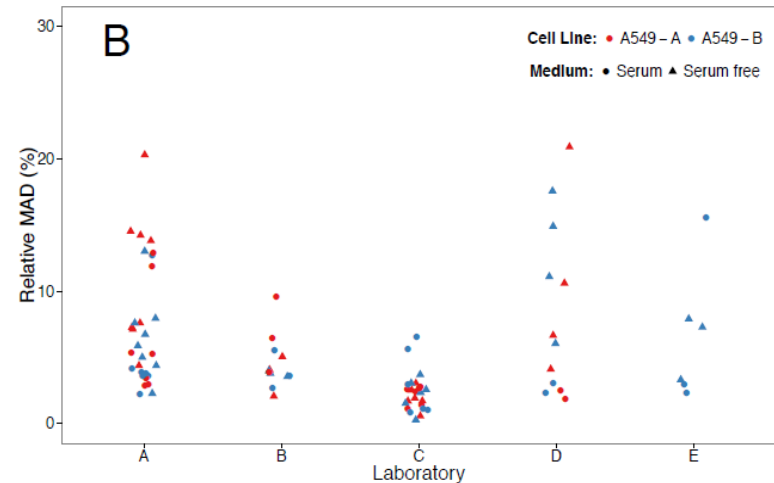
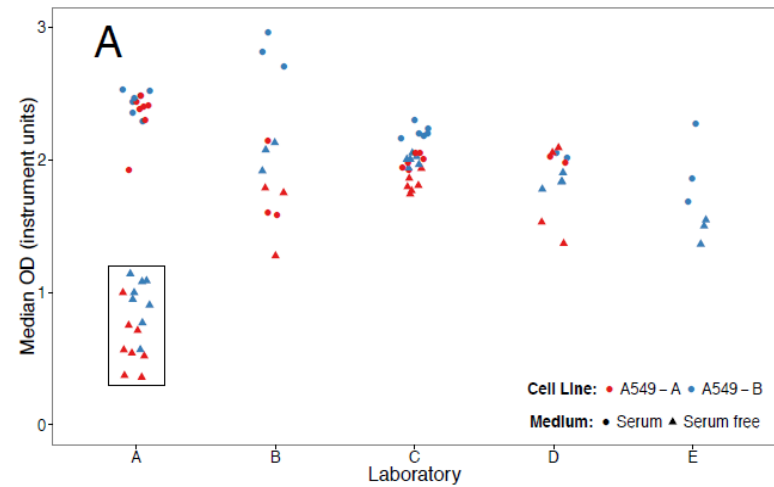
- Correlation between no-treatment cells and NP EC<sub>50</sub>
- If outliers are removed, no strong correlation
- Suggests that within this range of cell seeding variability (OD=1.5-2.5) no big effect on EC<sub>50</sub>

# Pipetting volumes and cells

Within pipette volume control



Within pipette cell control



Variability in pipetting volumes  $\ll$  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 <sup>1)</sup> Background Chemical Control B2-G2	0.06	0.05-0.09	<6%	0.06	0.05-0.09	<6%
Control 3C <sup>2)</sup> Background NP B11-G11						
Control 4 <sup>3)</sup> 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

# Collaborators

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