Nano Toxicology Interlaboratory Experiment

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Introduction

Experiment tested whether agreement among laboratories performing biological assay measurements can be achieved. Previous published results in the field of nano toxicology were contradictory.

Uncertainty analysis was *top down*, not bottom up as in the GUM.

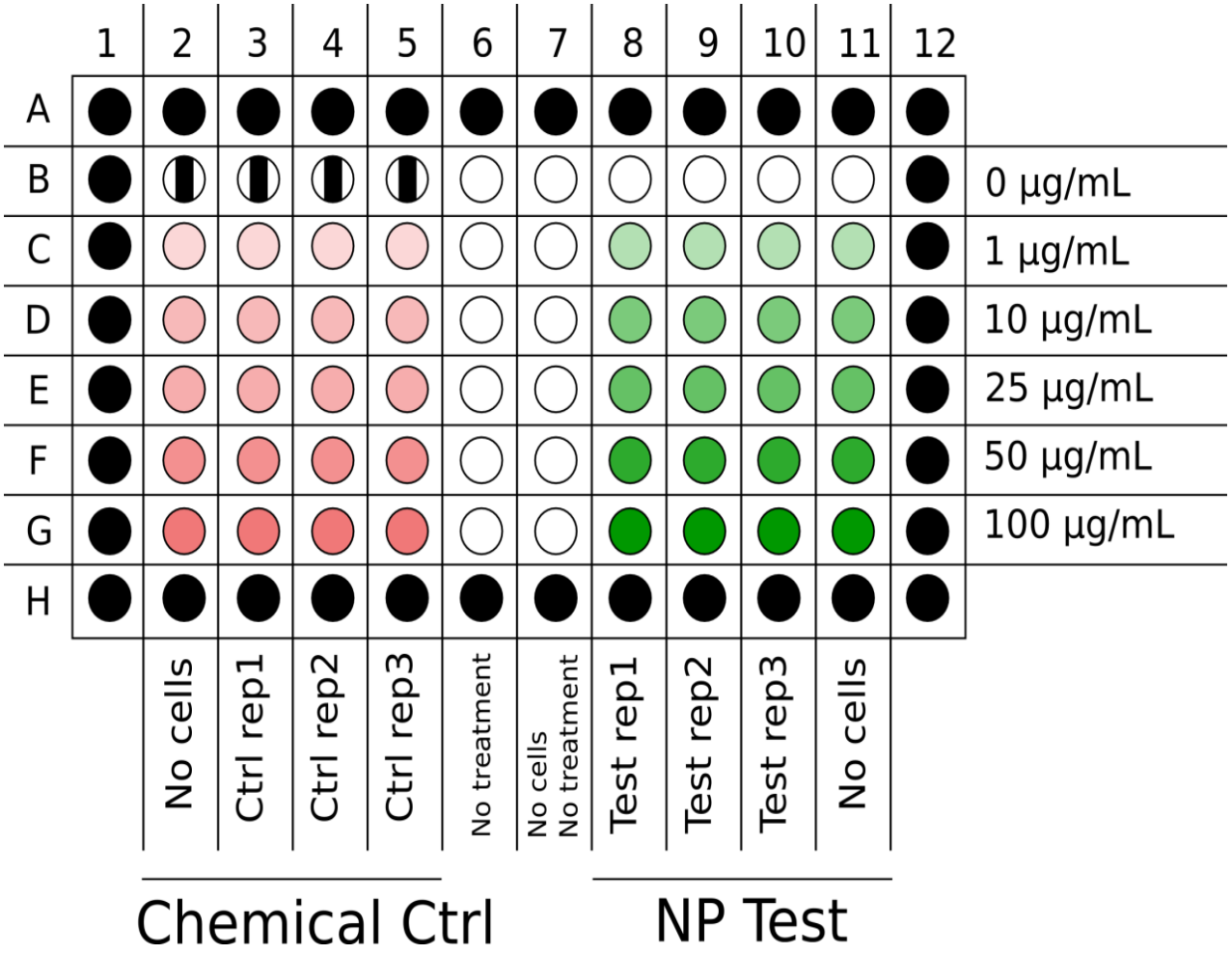
Experimental design and simple *data analysis* were critical for monitoring and assessing system performance.

Quantification of uncertainty was done using a *Bayesian hierarchical non-linear model* fitted using Markov Chain Monte Carlo methods.

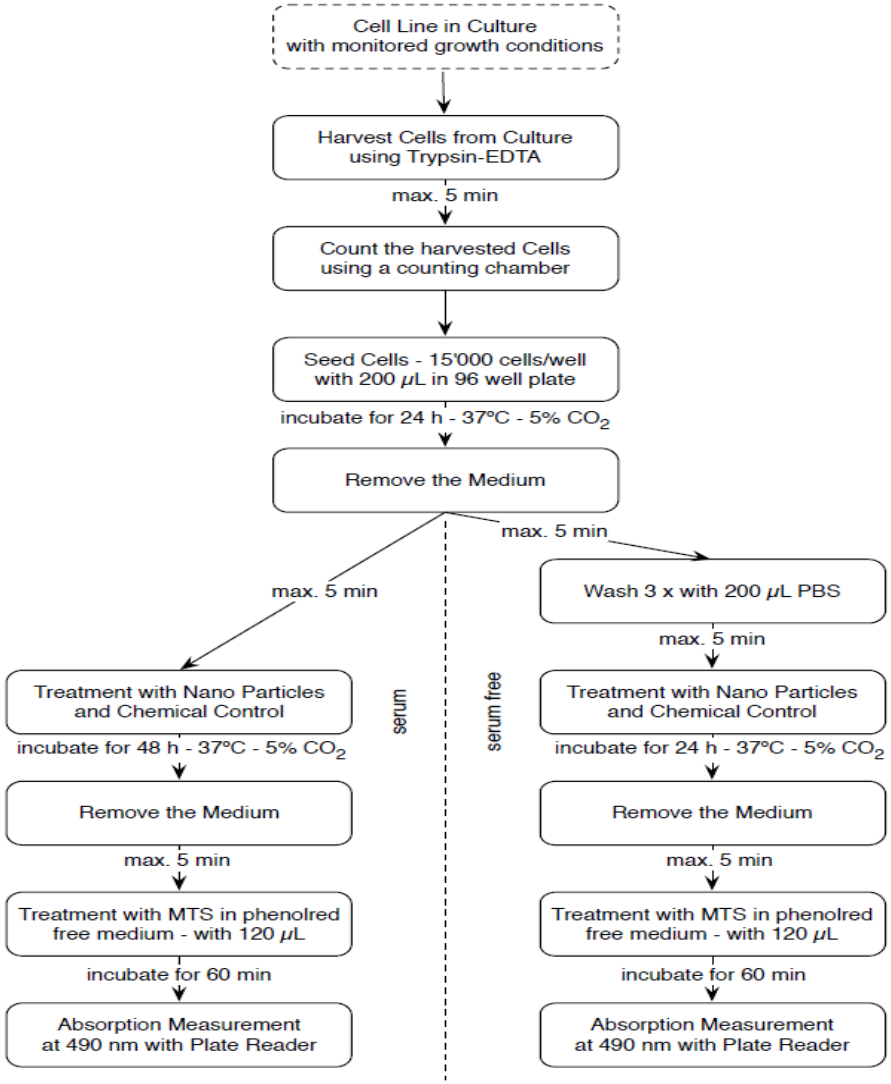
Details of the Experiment

- Cells treated with several different concentrations of nanoparticles or chemical control
- MTS cell viability assay
- Signal related to the number of metabolically active cells
- Measurement: absorbance at 490 nm
- Specifically designed plate
- Multiple cell lines
- Multiple laboratories

Plate Design



Process Flowchart for each laboratory Nano Particles



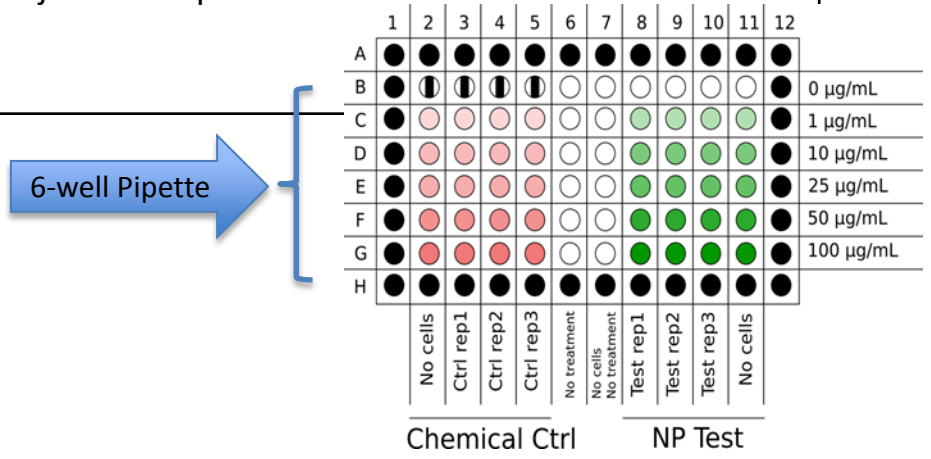
Sources of Variability

- Pipetting: seed density and chemical dosing
- Instrument performance
- Material handling

- Cell type
- Culture conditions
- Laboratory

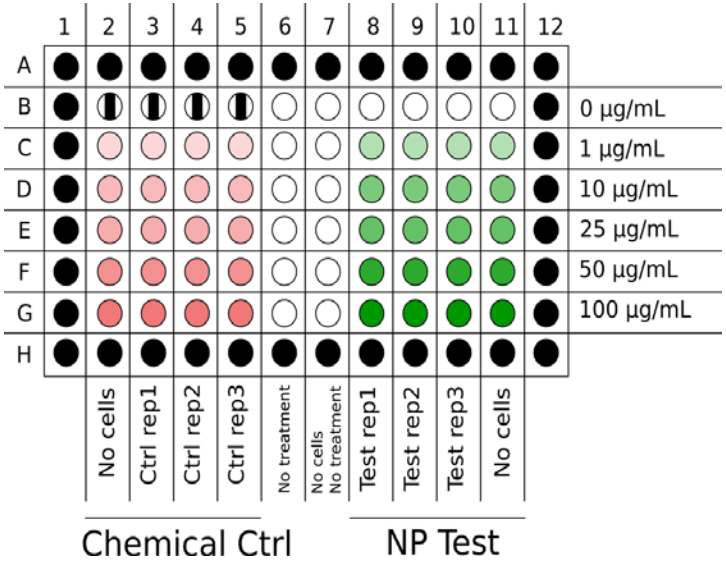
Plate Design: Assessment and control of Variability

	Brief Control Description
Column 2	Background correction for the chemical dosing.
Columns 3,4,5	Triplicate reference chemical control.
Column 6	**Within** multichannel pipetting variance. Non-treated cells seeded with a single ejection step.
Column 7	No cells but MTS reagent (last step of assay procedure).
Column 11	Background correction for the ENM dosing.
Black wells	Contain medium from the time of cell seeding on. Prevents edge effects that might occur during longer incubation times (i.e. evaporation).
Row B column 3,4,5 and 8,9,10	**Between** multichannel pipetting variance. Solvent treated cells seeded in different ejection steps.



Comparison of control measurements (no cells) two labs

	Lab A Mean (std) over all plates	Lab B Mean (std) over all plates
Column 2 (background CC)	0.056 (0.0007) OD	0.057 (0.004) OD
Column 7	0.057 (0.0009) OD	0.058 (0.003) OD
Column 11 (background NP)	0.20 (0.04) OD	0.23 (0.04) OD
Black cells	0.056 (0.0005) OD	0.058 (0.004) OD



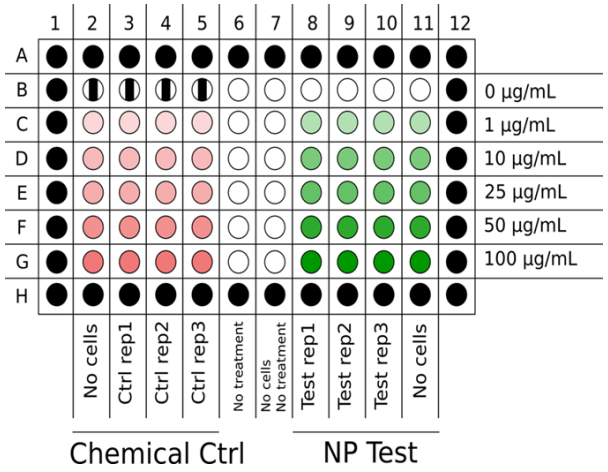
- Both labs appear to have good control of pipette volume on average
- Lab B is more variable

Column 6 - cells but no treatment (maximum absorbance)

Plate	Lab A Mean (std) OD	Lab B Mean (std) OD
1	2.29 (0.11)	2.10 (0.1)
2	2.24 (0.11)	2.43 (0.1)
3	2.18 (0.05)	2.47 (0.13)
4	2.23 (0.10)	2.82 (0.12)
5	2.19 (0.04)	2.80 (0.45)
6	2.17 (0.03)	

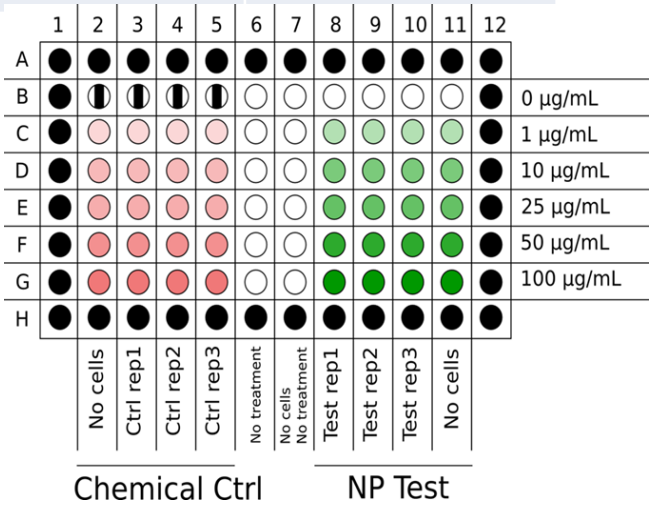
Within pipette variability reflected in std.
Plate to plate variability reflected in differences between means of plates.

- Suggests initial cell density (i.e. cell counting) is in less control in lab B
- May need to be more specific in the protocol instructions



Row B - cells but no treatment (maximum absorbance)

Plate	Lab A Mean (std) OD Col 3,4,5	Lab A Mean (std) OD Col 8,9,10	Lab B Mean (std) OD Col 3,4,5	Lab B Mean (std) OD Col 8,9,10
1	2.30 (0.08)	2.43(0.05)	2.15 (0.14)	2.48(0.05)
2	2.27 (0.01)	2.26(0.06)	2.54 (0.25)	2.45(0.15)
3	2.26 (0.05)	2.21(0.004)	2.47 (0.13)	2.36(0.12)
4	2.54 (0.05)	2.22(0.02)	2.77 (0.12)	2.98(0.10)
5	2.22 (0.04)	2.17(0.03)	2.72 (0.13)	2.78(0.12)
6	2.18 (0.01)	2.26(0.02)		



Between pipette variability reflected in std. **Plate to plate** variability reflected in differences between plate means.

Adjustments to measurements for dose response estimation

- Each plate has 3 sets of dose response measurements for CC (cols 3, 4, 5)

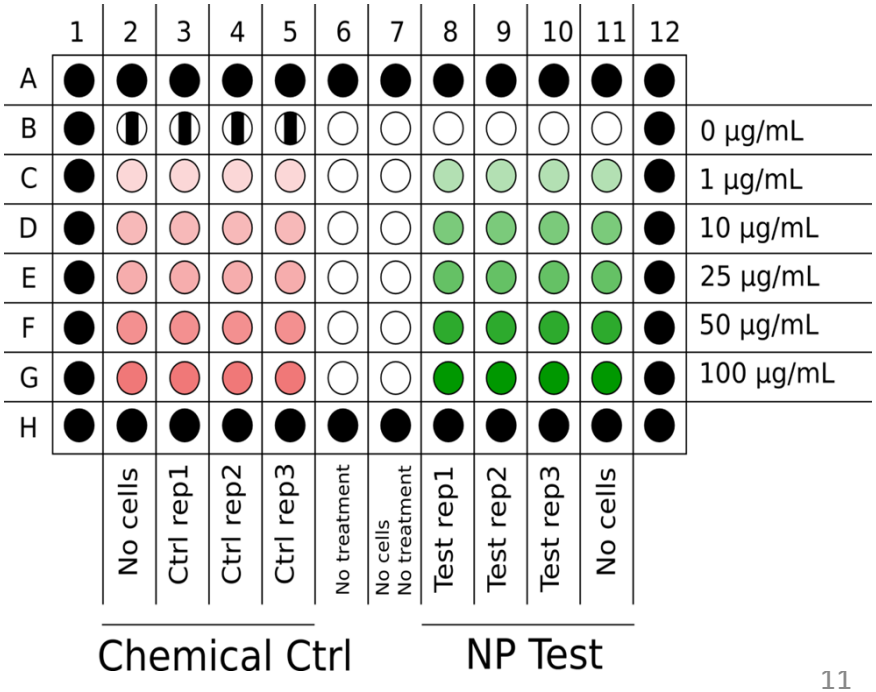
subtract background in col 2 and divide by (maximum absorbance in col 6-black cell absorbance)

- Each plate has 3 sets of dose response measurements for NP (cols 8, 9, 10)

subtract background in col 11 and divide by (maximum absorbance in col 6-black cell absorbance)

Dividing by row entries of column 6 minimizes the effect of **within** pipette variability (confounded with effect of dose), better for Lab A.

Alternate approach: divide by entry in row B minimizes effect of **between** pipette variability. This approach could be better for Lab B.

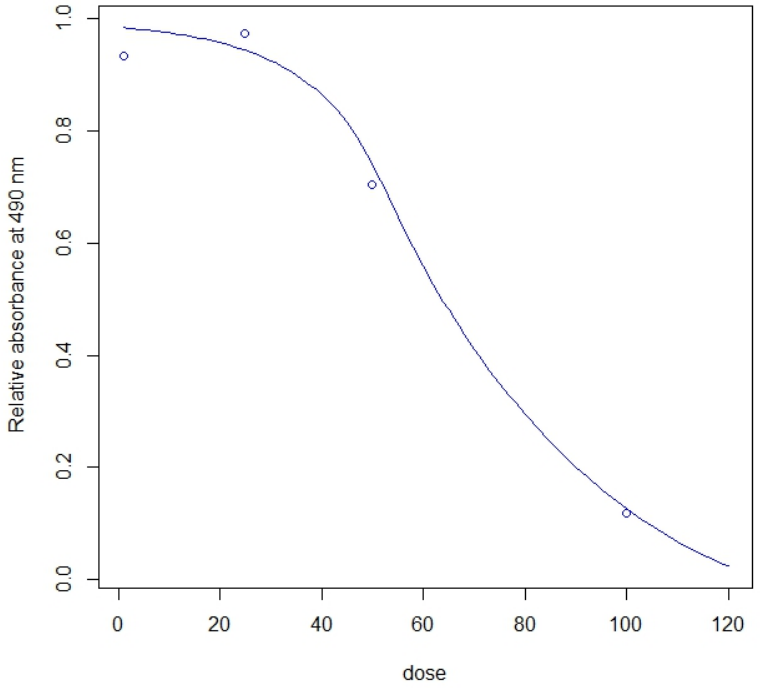


Analysis of Dose Response (statistical model)

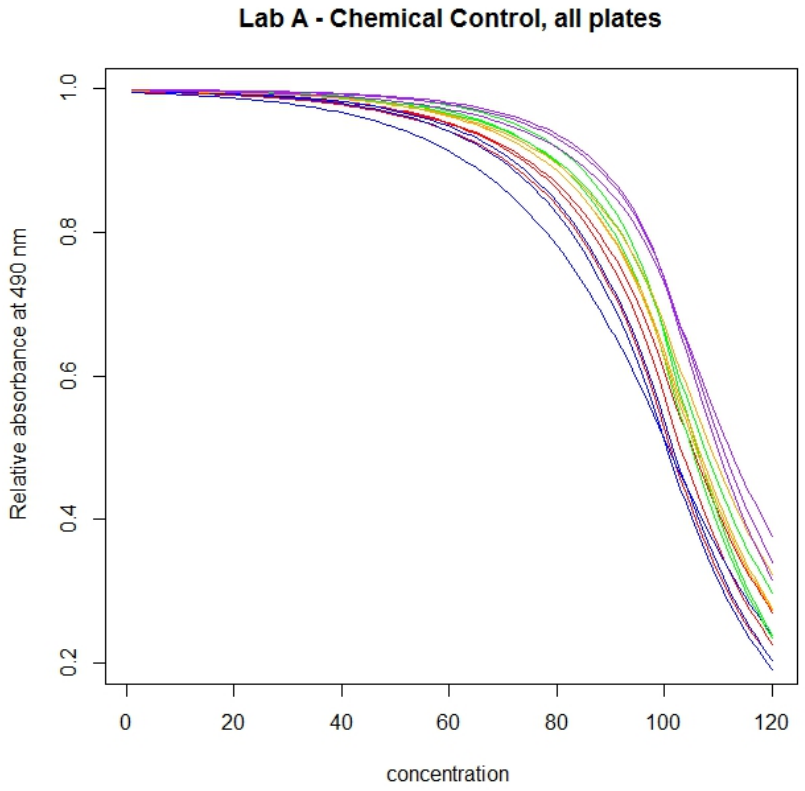
$$E(1-r_i) = \frac{\gamma}{1+e^{\frac{(\alpha - x_i)}{\beta}}}$$

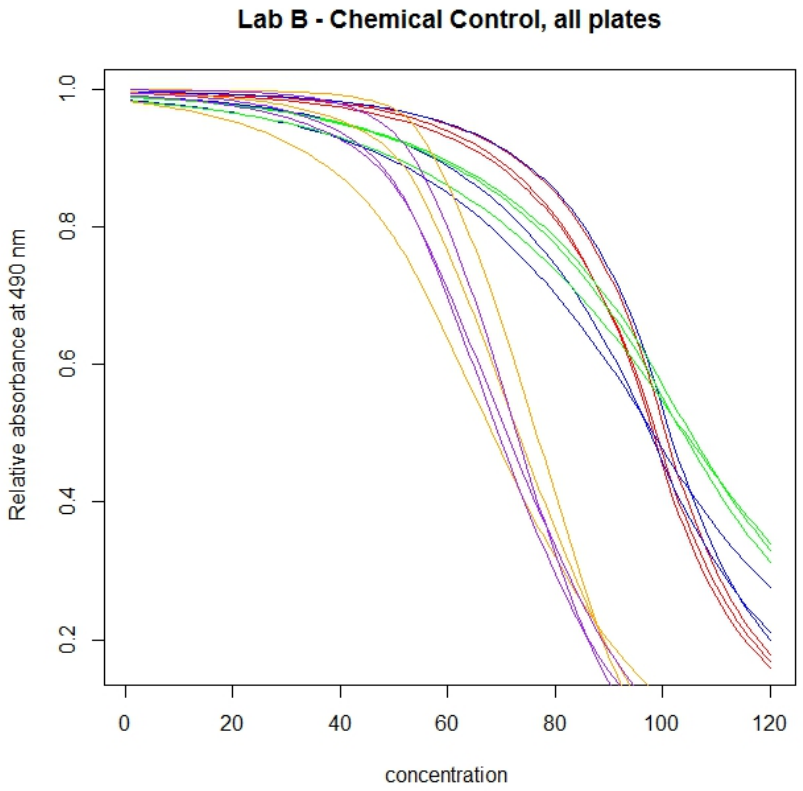
r_i is the normalized response,
x_i is the dose

Fitted using MCMC



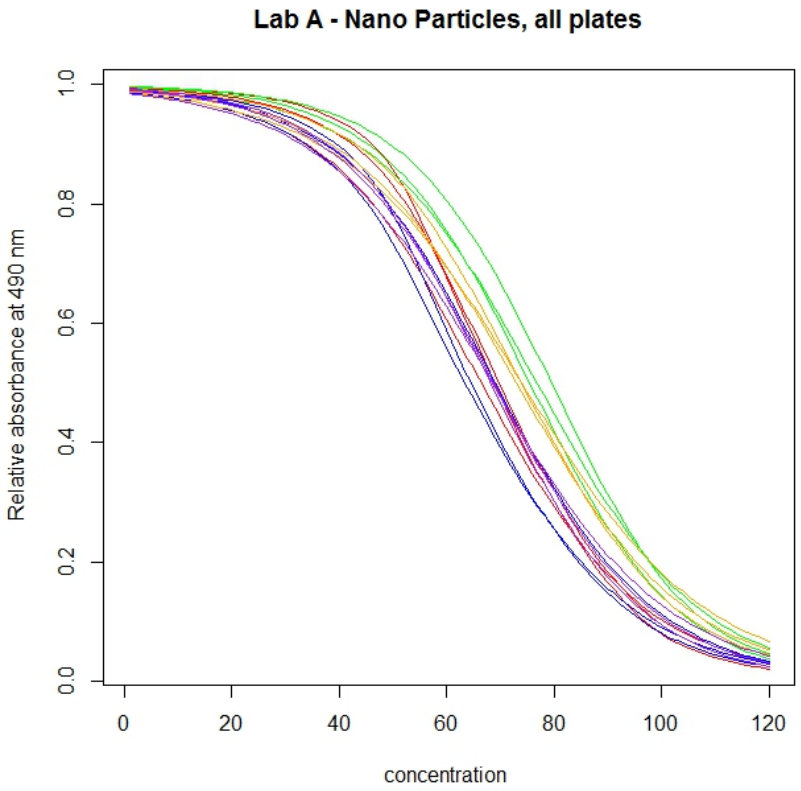
Chemical Control Comparison
Multiple plates, 3 curves per plate

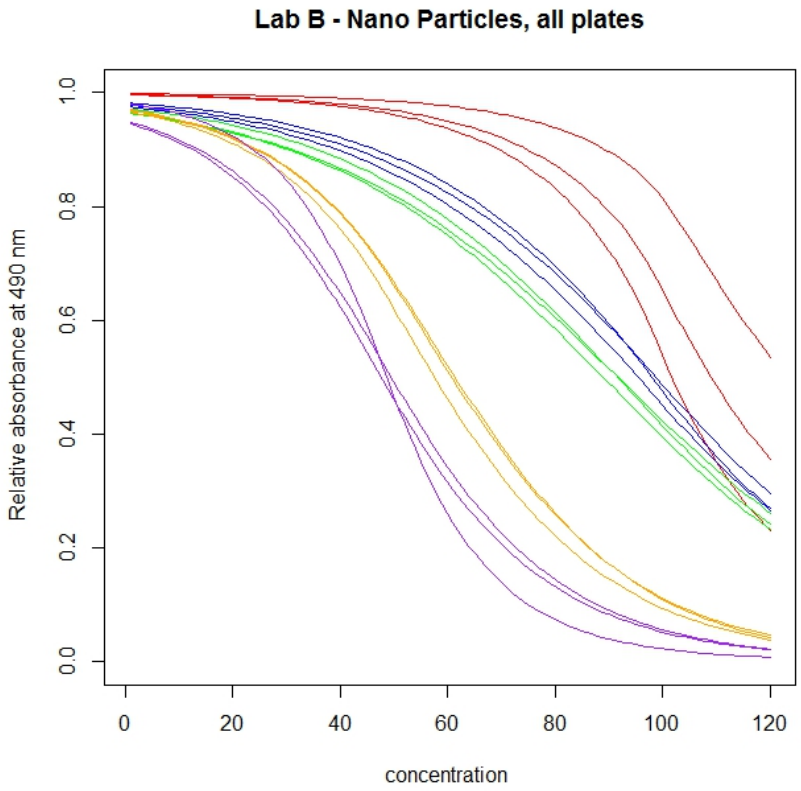




Replicate curves on each plate are similar –variability due to **between** pipette seeding and chemical dosing can be captured by combining the 3 replicates

Nano particles Comparison
Multiple plates, 3 curves per plate



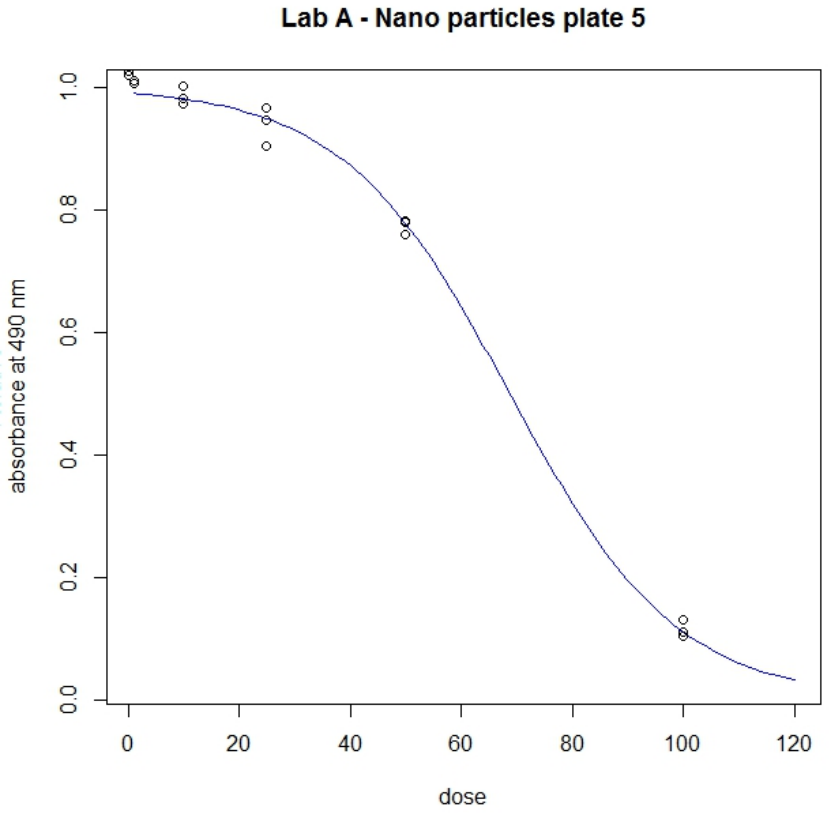


Replicate curves on each plate are similar –variability due to **between** pipette seeding and chemical dosing can be captured by combining the 3 replicates

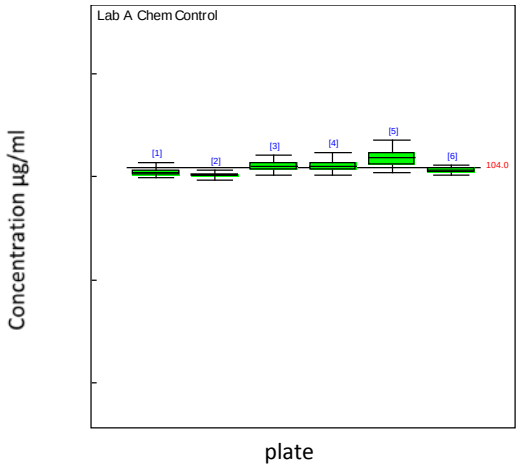
Combine reps on each plate (col 3, 4, 5 or 8, 9, 10)

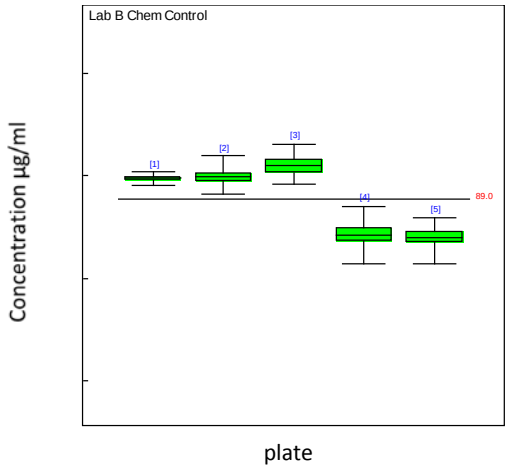
Model for each plate:

$$E\left(1-r_{ij}\right)=\frac{\gamma}{1+e^{\frac{(\alpha-x_i)}{\beta}}},\quad j=1,2,3$$



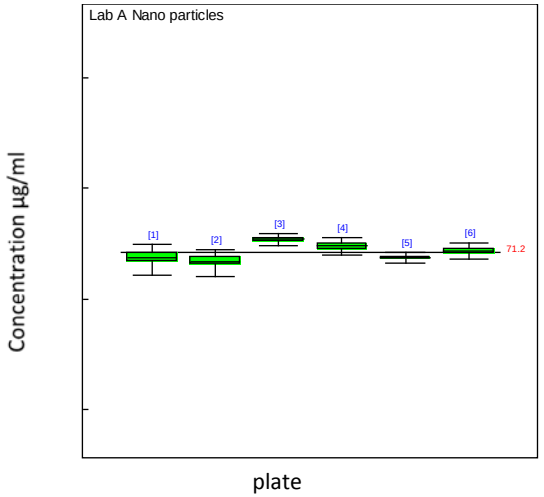
EC_{50} control and nanoparticles per plate





Row B

Plate	Lab A Mean (std) Col 3,4,5	Lab B Mean(std) Col 3,4,5
1	2.30 (0.08)	2.15 (0.14)
2	2.27 (0.01)	2.54 (0.25)
3	2.26 (0.05)	2.47 (0.13)
4	2.54 (0.05)	2.77 (0.12)
5	2.22 (0.04)	2.72 (0.13)
6	2.18 (0.01)	



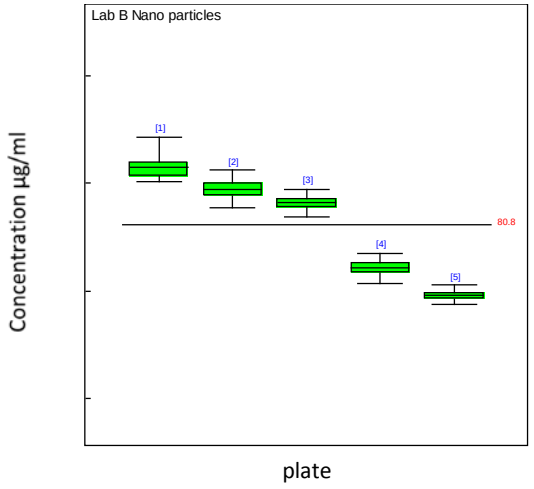
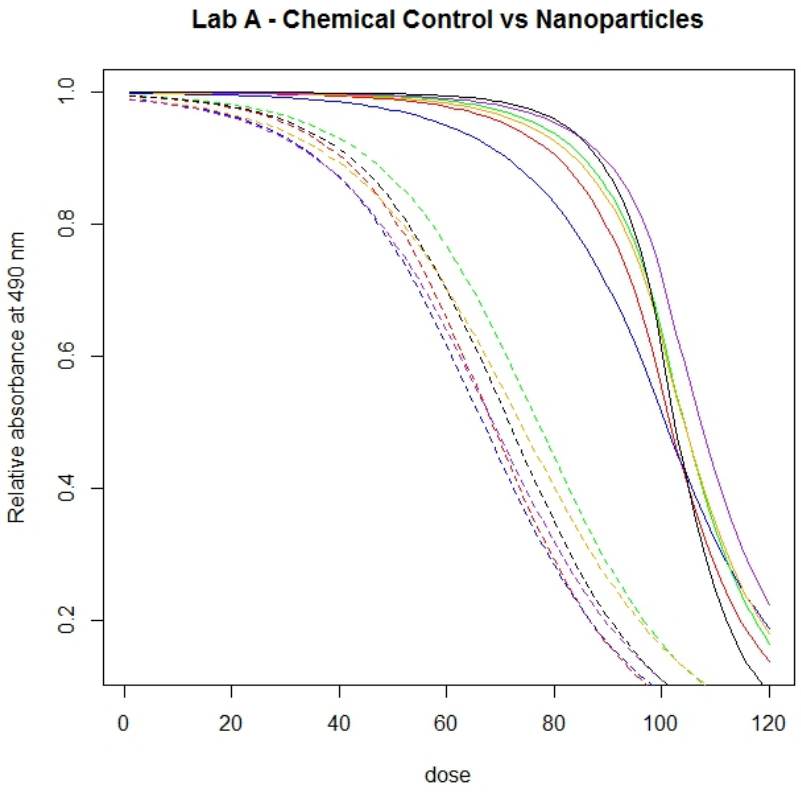
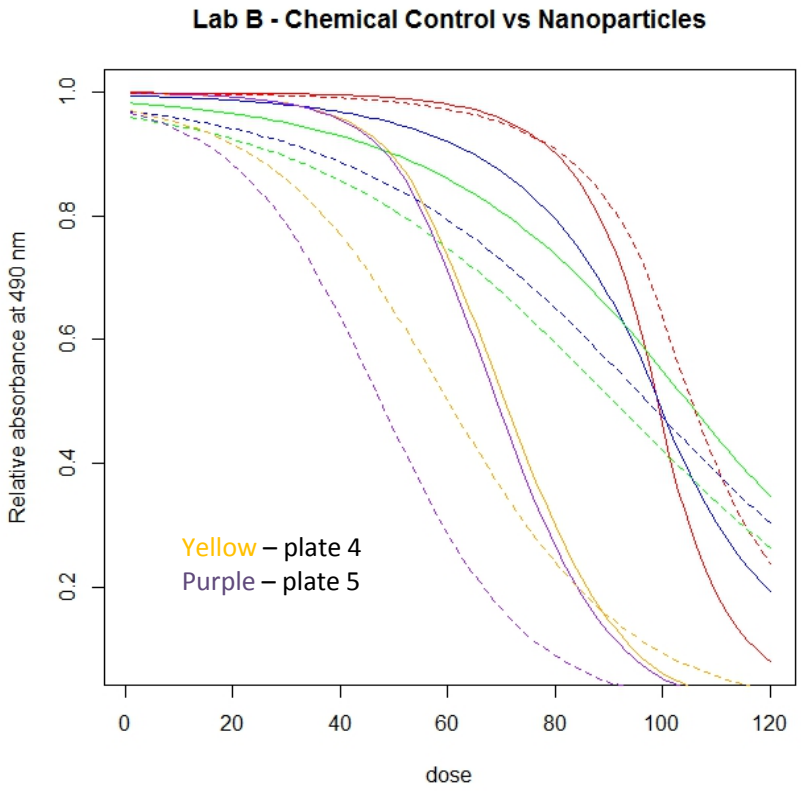


Plate	Lab A Mean (std) Col 8,9,10	Lab B Mean(std) Col 8,9,10
1	2.43(0.05)	2.48(0.05)
2	2.26(0.06)	2.45(0.15)
3	2.21(0.004)	2.36(0.12)
4	2.22(0.02)	2.98(0.10)
5	2.17(0.03)	2.78(0.12)
6	2.26(0.02)	

Uncertainty from **between** pipette effects and other unknown factors

Chemical Control vs. Nano Particles
Replicate Plate curves





Lot more variability among plates for Lab B - 2 separate sets?
Also, relationship between CC and NP curves according to plate for Lab B.

Model for Consensus curve

Lab A – all plates behave well but there is some plate to plate variability which can be captured using a hierarchical model:

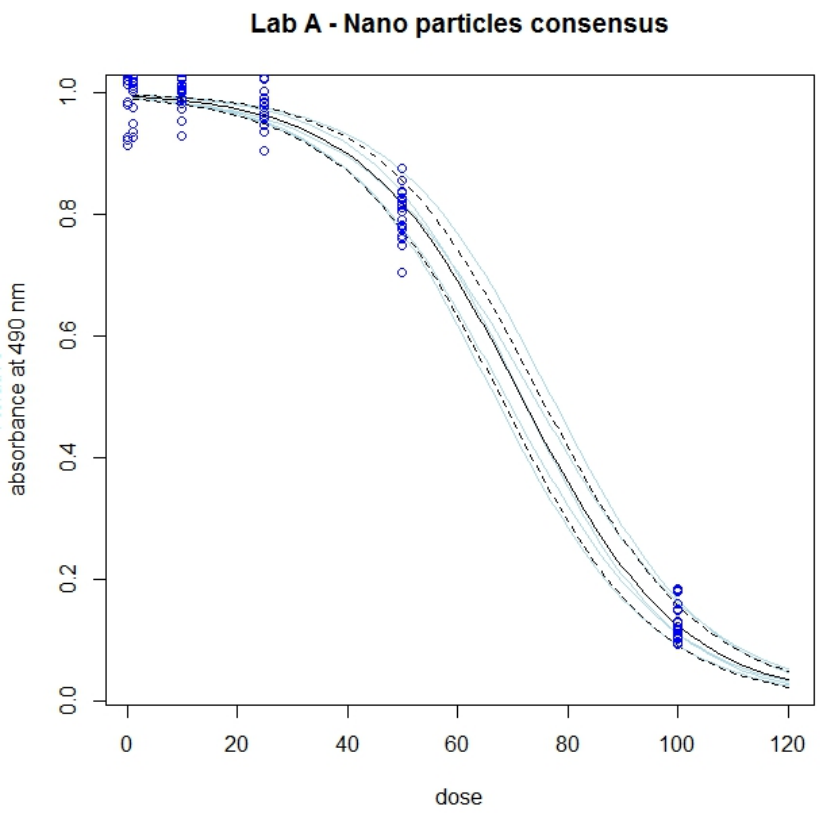
$$E\left(1-r_{ij}\right)=\frac{\gamma_i}{\left(1+e^{\left\{{(\alpha_i-x_j)}/{\beta_i}\right\}}\right)} \text{ for } i=1,\cdots,5 \text{ and } j=1,\cdots 6$$

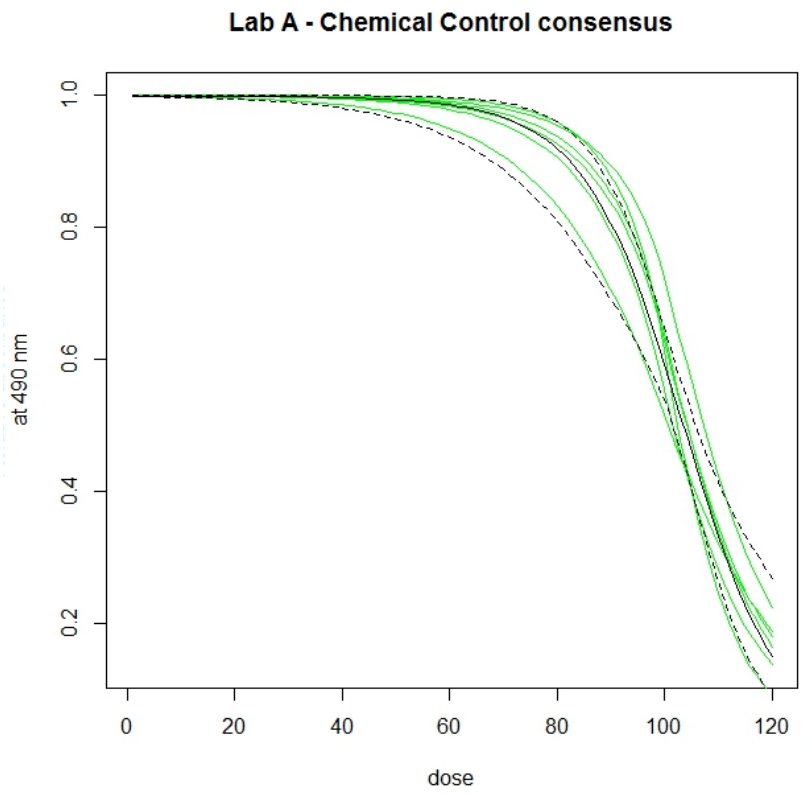
$$\alpha_i \sim N(\alpha,\sigma_\alpha),\ \log(\beta_i)\sim N(\beta,\sigma_\beta),\ \log(\gamma_i)\sim N(\gamma,\sigma_\gamma)$$

Consensus:

$$E\left(1-r_i\right)=\frac{e^{\gamma}}{1+e^{\frac{(\alpha-x_i)}{e^{\beta}}}}$$

Consensus curves with 95% HPD, estimation via MCMC





Uncertainty from **between** pipette effects plus “plate” effects

Lab B

- Has two subsets of similar plates, not clear how or if these should be combined to form a consensus.

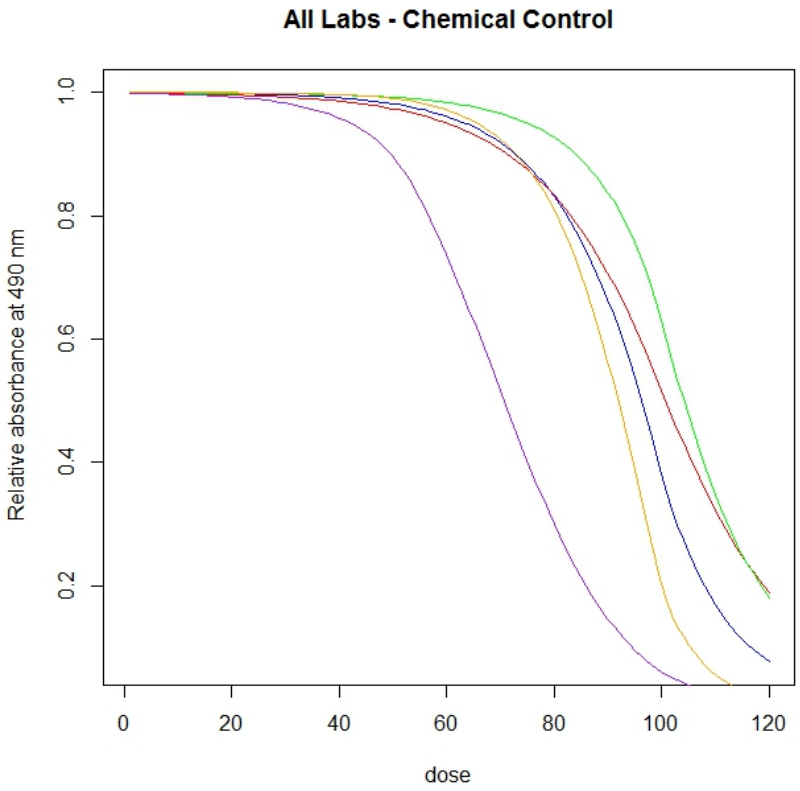
- The set that has similar values of the control variables to Lab A has curves that are less similar than the other set.

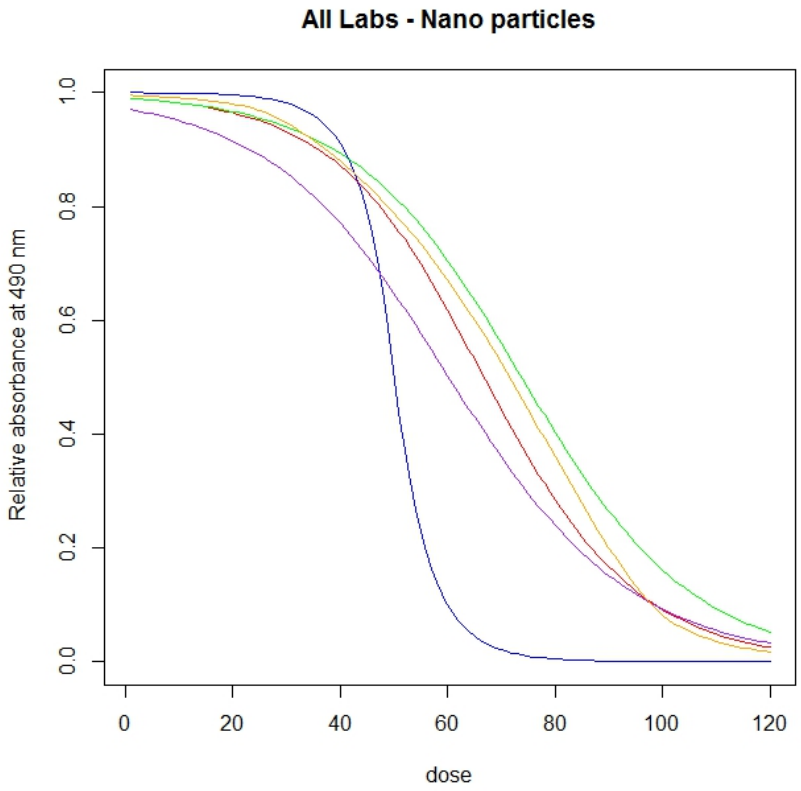
Interlaboratory study

- 5 laboratories
- 2 cell lines
- 2 types of cell growing conditions

- Separate analyses for cell lines and growing conditions
- Good overall agreement among labs with some outliers.

Lab Consensus curves
single cell line, single growing condition





Conclusions

- It is possible to achieve agreement among laboratories making biological assay measurements.
- Careful monitoring of the protocol is necessary.
- Top-down Uncertainty analysis using MCMC is viable.

Potential Experimental Design improvements:

- More plates
- Concentrations close to anticipated EC_{50}
- Control treatment with “known” EC_{50} value

Potential Uncertainty analysis improvements:

- Bottom-up uncertainty evaluation for individual absorbance measurements combined with the top-down approach