

Technical Framework for Enabling High-Quality Measurements in New Approach Methodologies (NAMs)

Elijah Petersen

National Institute of Standards and Technology (NIST)

Technical Framework Manuscript

*ALTEX, accepted manuscript
published July 15, 2022
doi:10.14573/altex.2205081*



Technical Framework for Enabling High-Quality Measurements in New Approach Methodologies (NAMs)

Elijah J. Petersen,¹ John T. Elliott,¹ John Gordon,² Nicole C. Kleinstreuer,³ Emily Reinke,⁴ Matthias Roesslein⁵ and Blaza Toman¹

¹National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA; ²US Consumer Product Safety Commission, Rockville, MD, USA; ³National Institute of Environmental Health Sciences, National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, Research Triangle Park, NC, USA; ⁴U.S. Army Public Health Center, Aberdeen Proving Ground, MD, USA; ⁵Empa, Swiss Federal Laboratories for Material Testing and Research, Particles-Biology Interactions Laboratory, St. Gallen, Switzerland

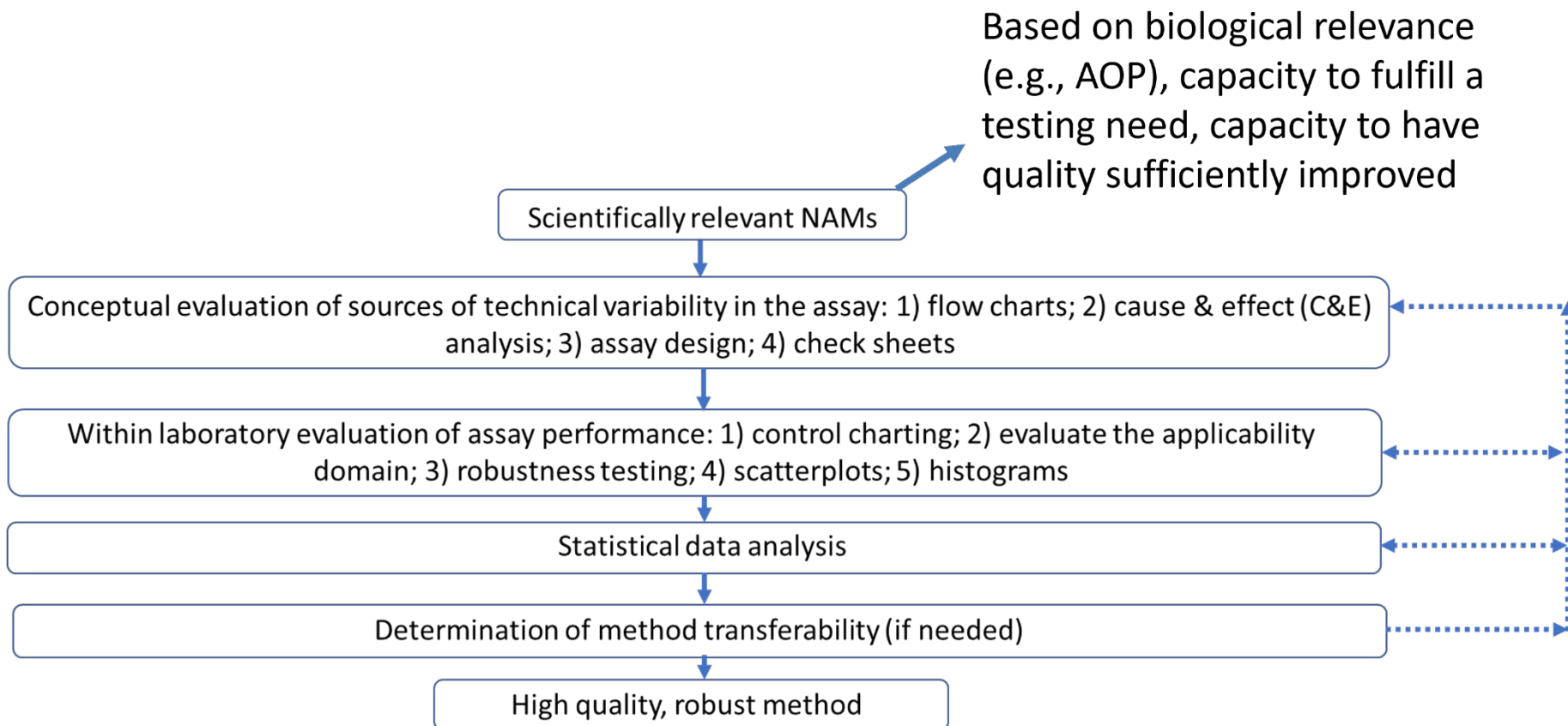
Technical Framework for High-Quality NAMs

Collaborative project with CPSC, NICEATM, DOD, EMPA, NIST

- To yield reproducible NAM results across time and among laboratories, the framework includes a series of inter-related steps that describe
 - How to apply basic quality tools (cause-and-effect analysis, flow charts, control charts, etc) to improve confidence in NAMs
 - Approaches for adding statistical confidence to decisions based on NAM results
 - There may be tradeoffs though with more controls potentially leading to higher costs

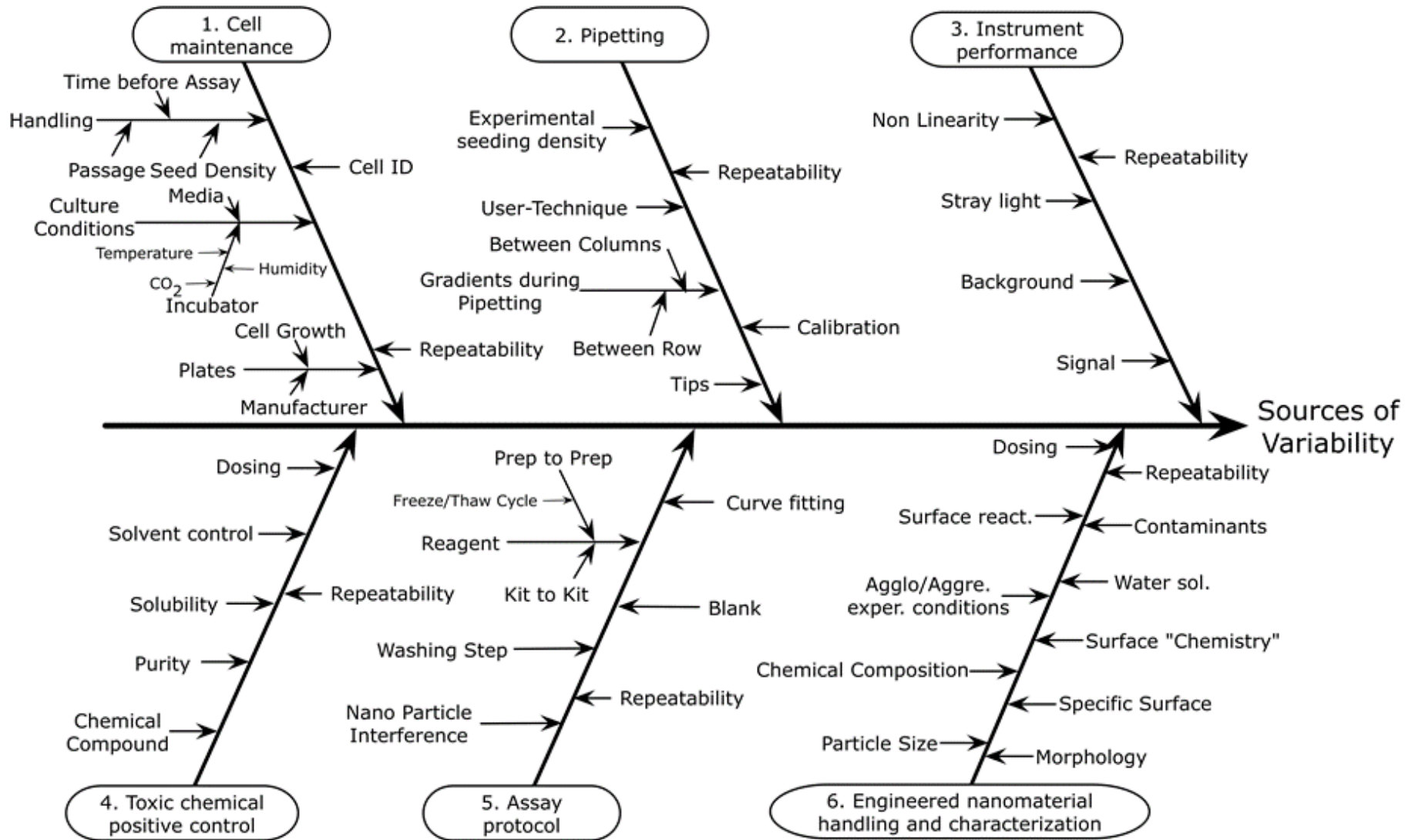
Petersen, E. J., Elliott, J. T., Gordon, J., Kleinstreuer, N., Reinke, E, Roesslein, M., Toman, B. 2023, Altex, 40 (1), 174-186. <https://doi.org/10.14573/altex.2205081>

Technical Framework for High-Quality NAMs (continued)



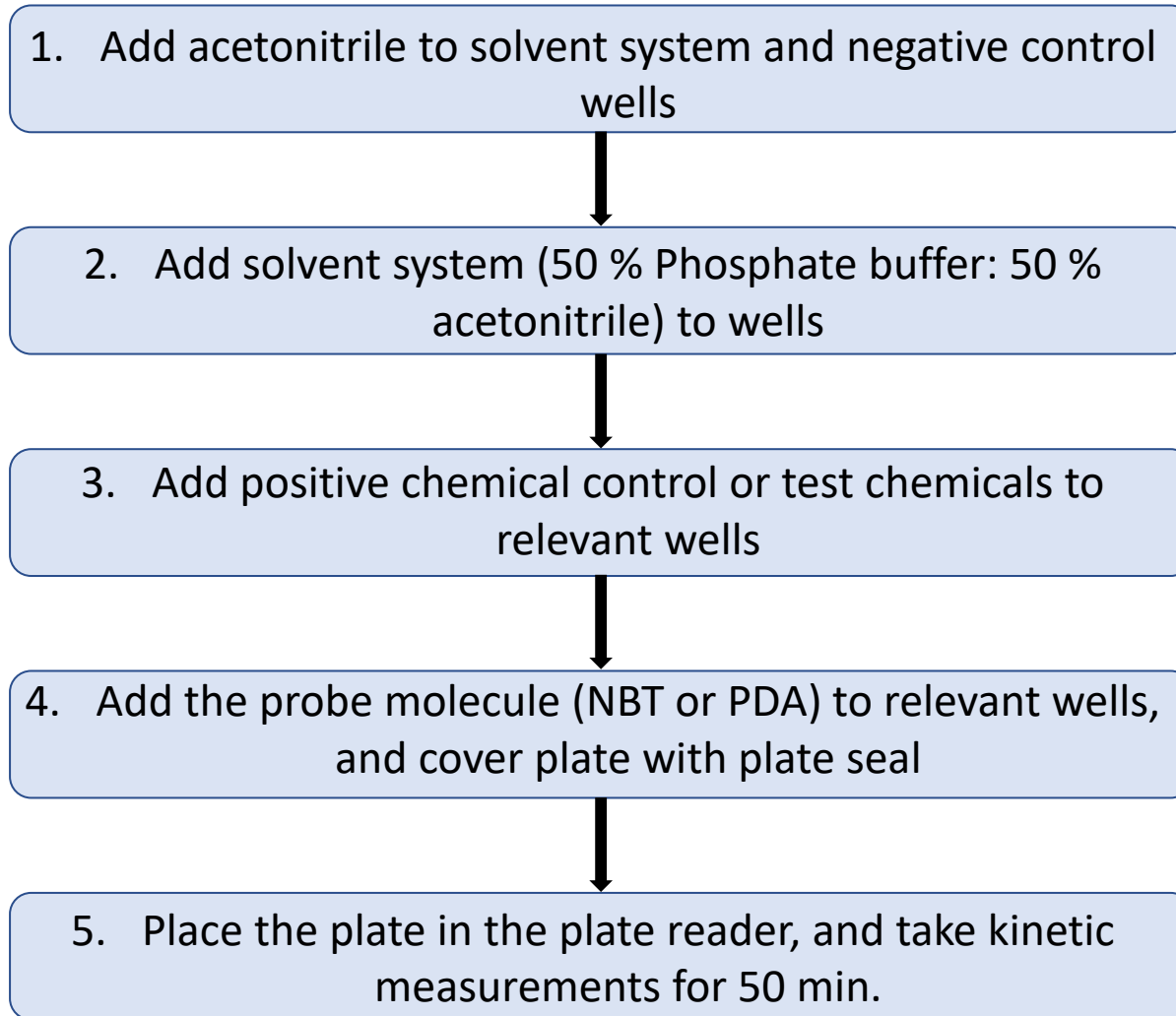
Petersen, E. J., Elliott, J. T., Gordon, J., Kleinstreuer, N., Reinke, E, Roesslein, M., Toman, B. 2023, Altex, 40 (1), 174-186. <https://doi.org/10.14573/altex.2205081>

Example: cause-and-effect analysis



Robustness testing can evaluate each of the branches

Example: flow chart



Control measurements should cover each step in the flow chart

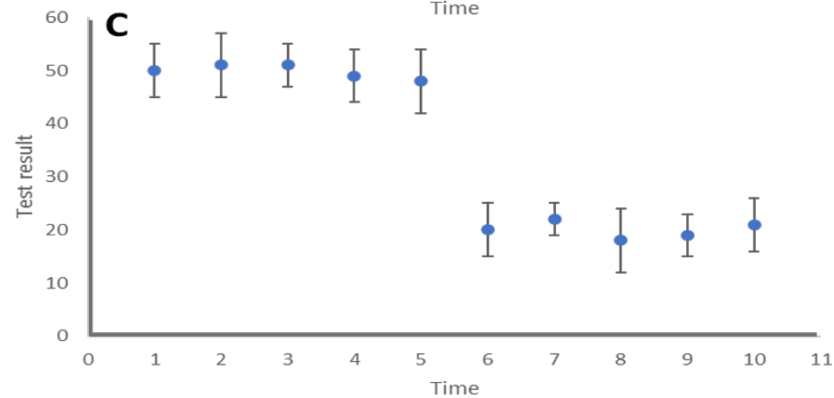
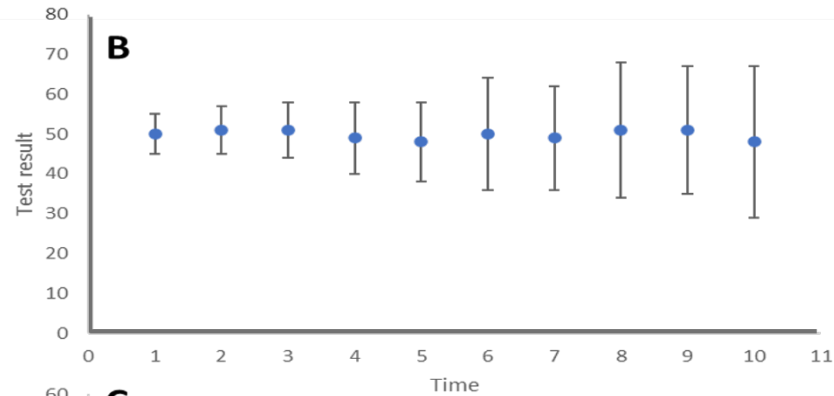
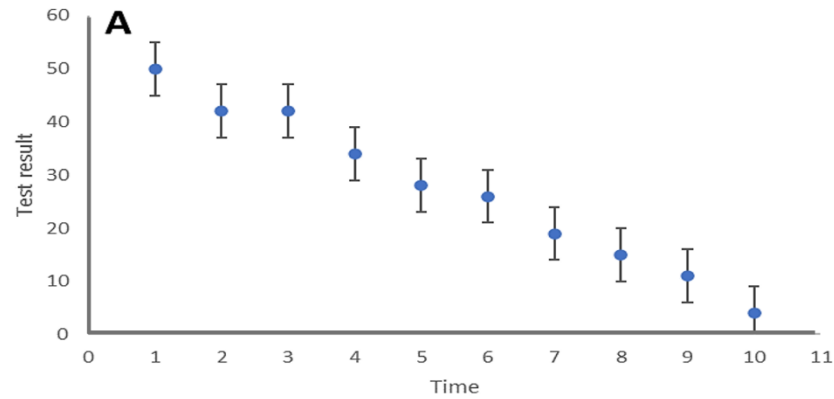
Example: plate design

	1	2	3	4	5	6	7	8	9	10	11	12
A	SS	NC	NC	NC	NC	NC	NC	NC	NC	●	●	●
B	SS	NC	PC	PC	PC	TC	TC	TC	TC			
C	SS	NC	PC	PC	PC	TC	TC	TC	TC			
D	SS	NC	PC	PC	PC	TC	TC	TC	TC			
E	SS	NC	PC	PC	PC	TC	TC	TC	TC			
F	SS	NC	PC	PC	PC	TC	TC	TC	TC			
G	SS	NC	PC	PC	PC	TC	TC	TC	TC			
H	SS	NC	PC	PC	PC	TC	TC	TC	TC			

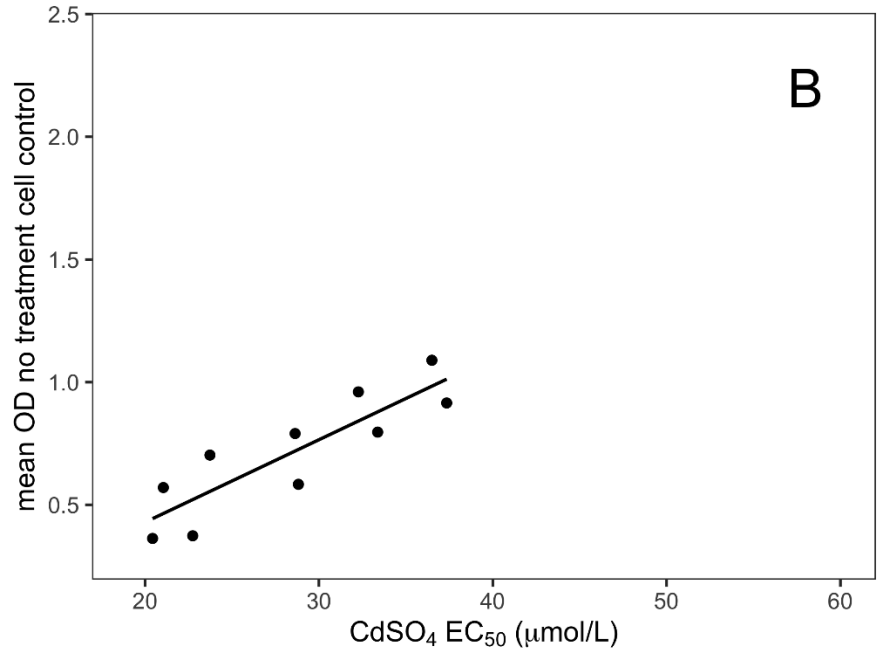
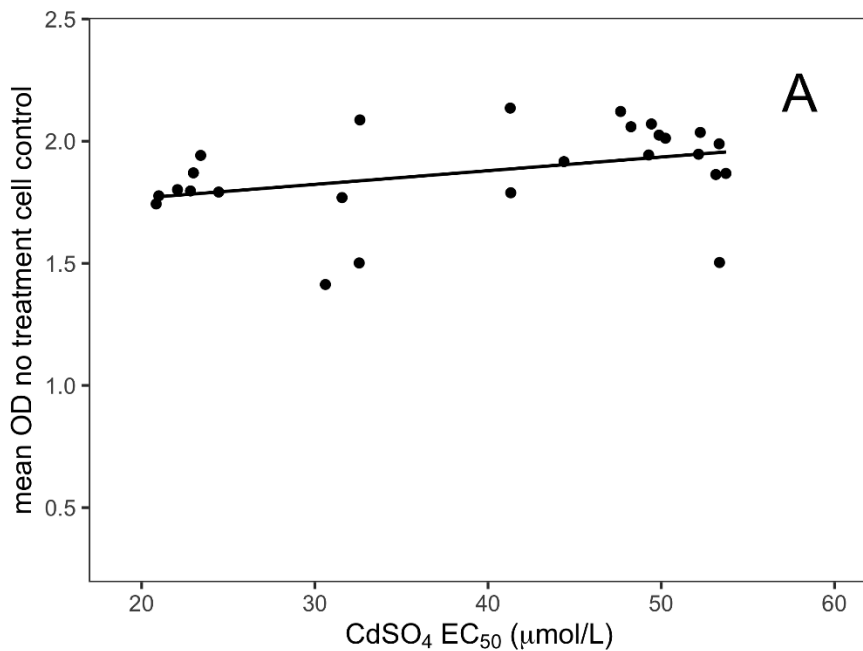
- SS - Blank (Solvent System)
- NC - Negative Control
- PC - Positive Control (serial dilution)
- TC TC TC TC TC TC TC - Test chemicals
- | - Test chemical interference wells
- - Wells without added reagents

Control measurements evaluate key sources of variability each time the assay is performed

Example: control charting



Example: scatter plot

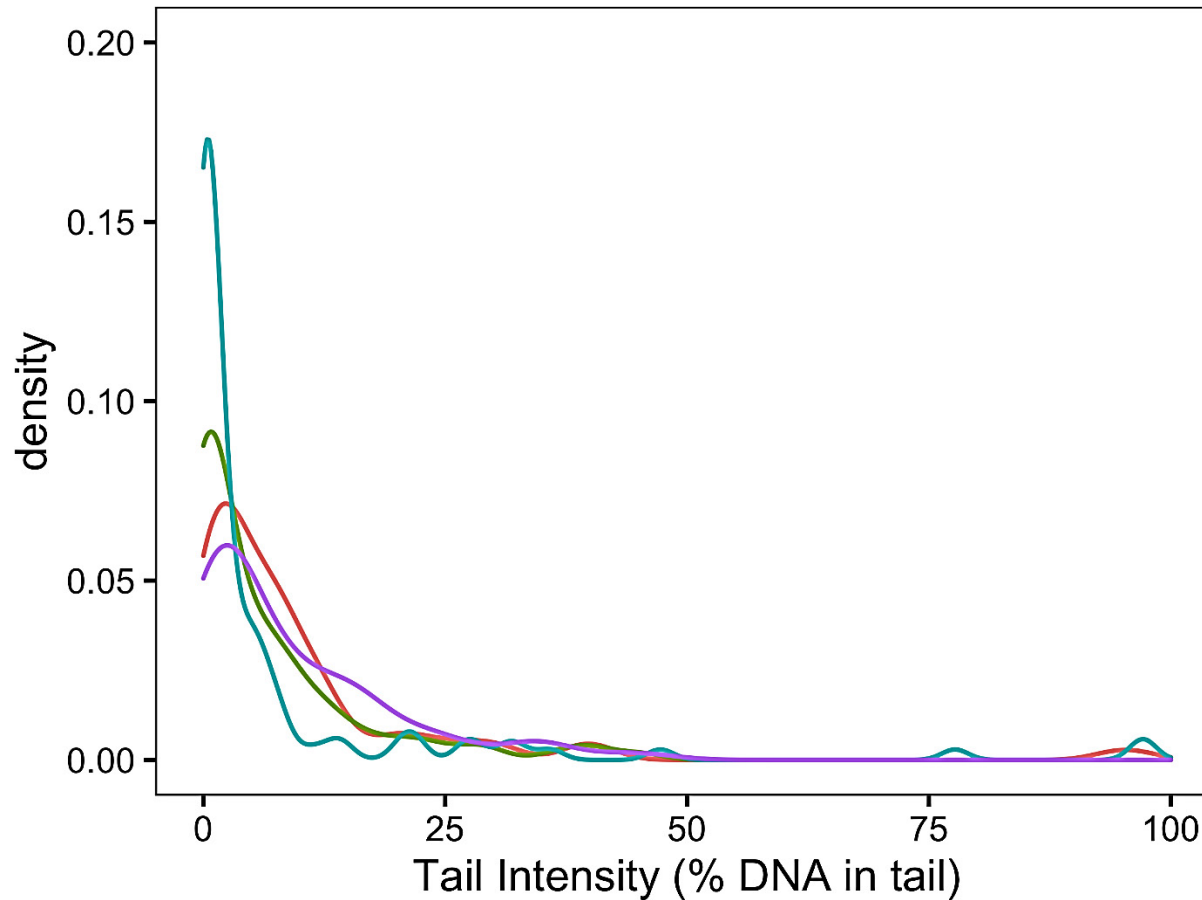


There is either a lack of an interaction between the EC₅₀ values (part A) or an interaction (part B) depending upon the range of mean OD values which reflect the number of cells.

Ranges in specifications can be set to avoid interactions among variables

Elliott, J. T., Rosslein, M., Song, N. W., Toman, B., Kinsner-Ovaskainen, A., Maniratanachote, R., Salit, M. L., Petersen, E. J., Sequeira, F., Lee, J., Kim, S. J., Rossi, F., Hirsch, C., Krug, H. F., Suchaoin, W., Wick, P. Toward achieving harmonization in a nano-cytotoxicity assay ρ measurement through an interlaboratory comparison study, **2017**, *Altex*, 34(2), 201-218.

Example: histogram



If the data do not have a Gaussian distribution, different statistical approaches may be needed

Cassano, J. C., Roesslein, M., Kaufmann, R. et al. (2020). A novel approach to increase robustness, precision and high-throughput capacity of single cell gel electrophoresis. *ALTEX - Alternatives to Animal Experimentation*, 3, 95-109.

<http://dx.doi.org/10.14573/altex.1906252>

Summary

- Quality tools enable more confidence in measurement systems
- Technical framework focused on quality in NAMs
- Plate design allows direct encoding of control measurements for each test sample
- Statistical evaluation can yield a call with the likelihood of false positive/false negative decisions
- Possibly facilitates standardization and adoption of test methods