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

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Technical Framework Manuscript

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Bench Marks!

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

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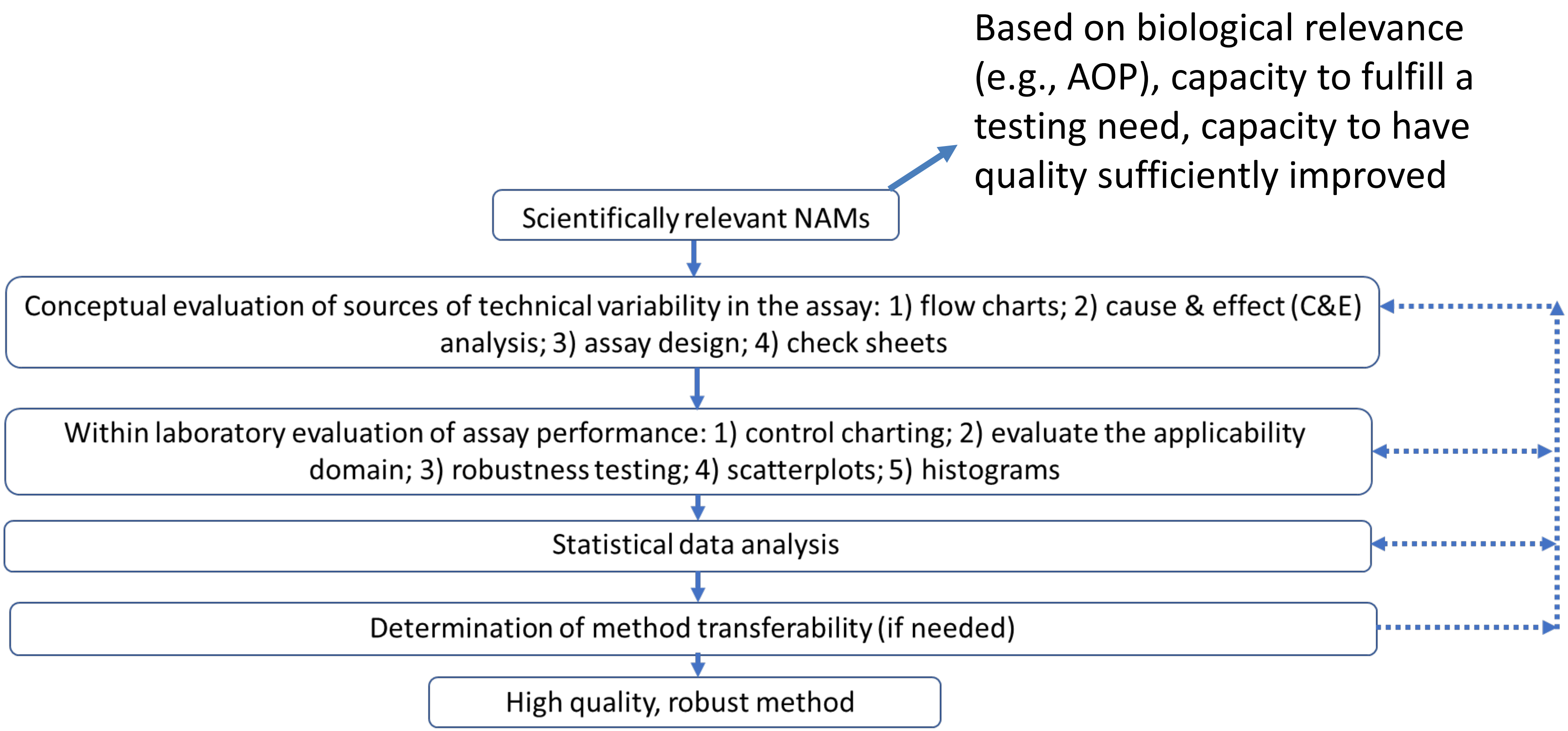
Technical Framework for High Quality NAMs

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

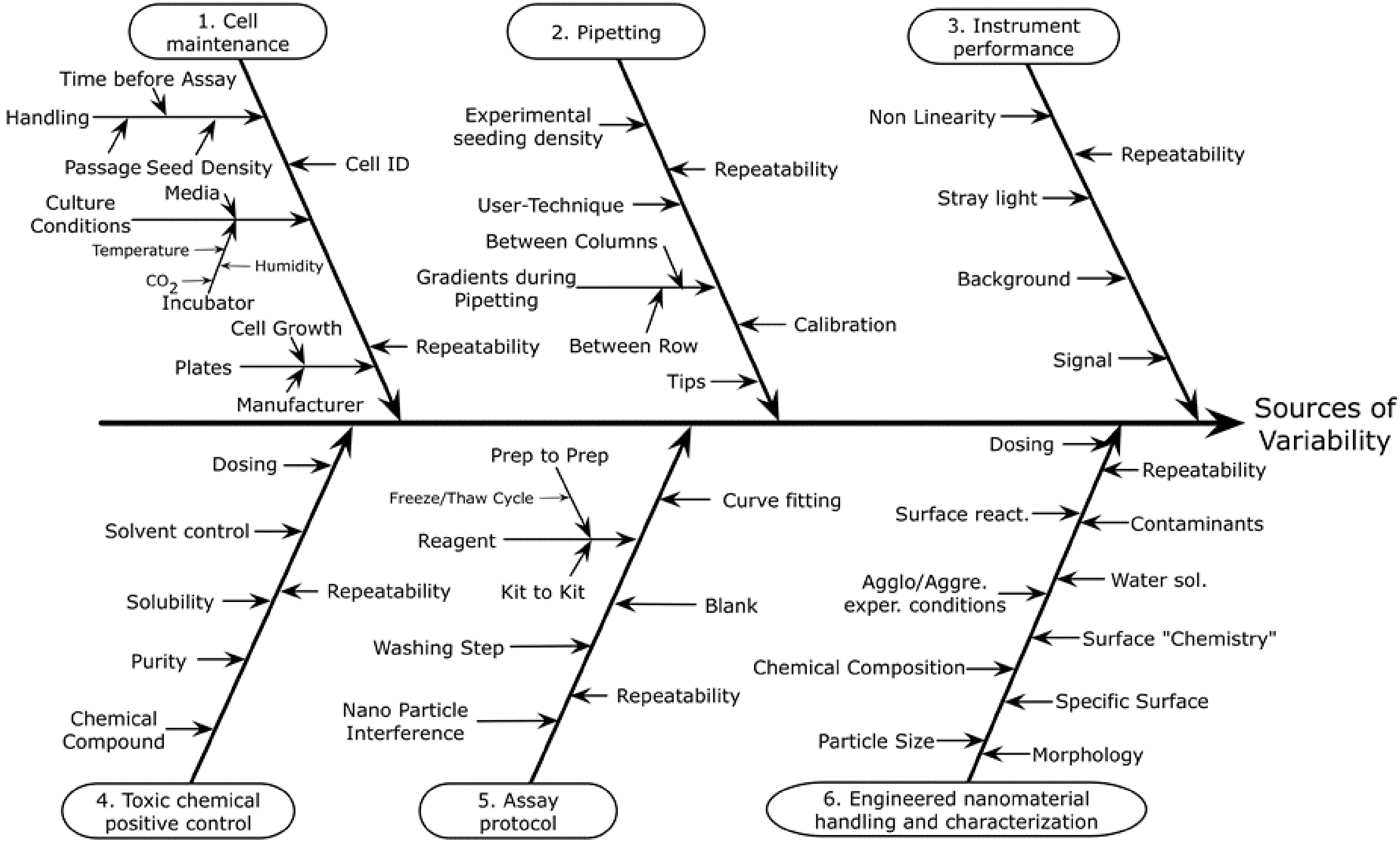
Petersen, E. J., Elliott, J. T., Gordon, J., Kleinstreuer, N., Reinke, E, Roesslein, M., Toman, B. 2022, Altex, in press. https://doi.org/10.14573/altex.2205081

Technical Framework For High Quality NAMs



Petersen, E. J., Elliott, J. T., Gordon, J., Kleinstreuer, N., Reinke, E, Roesslein, M., Toman, B. 2022, Altex, in press. https://doi.org/10.14573/altex.2205081

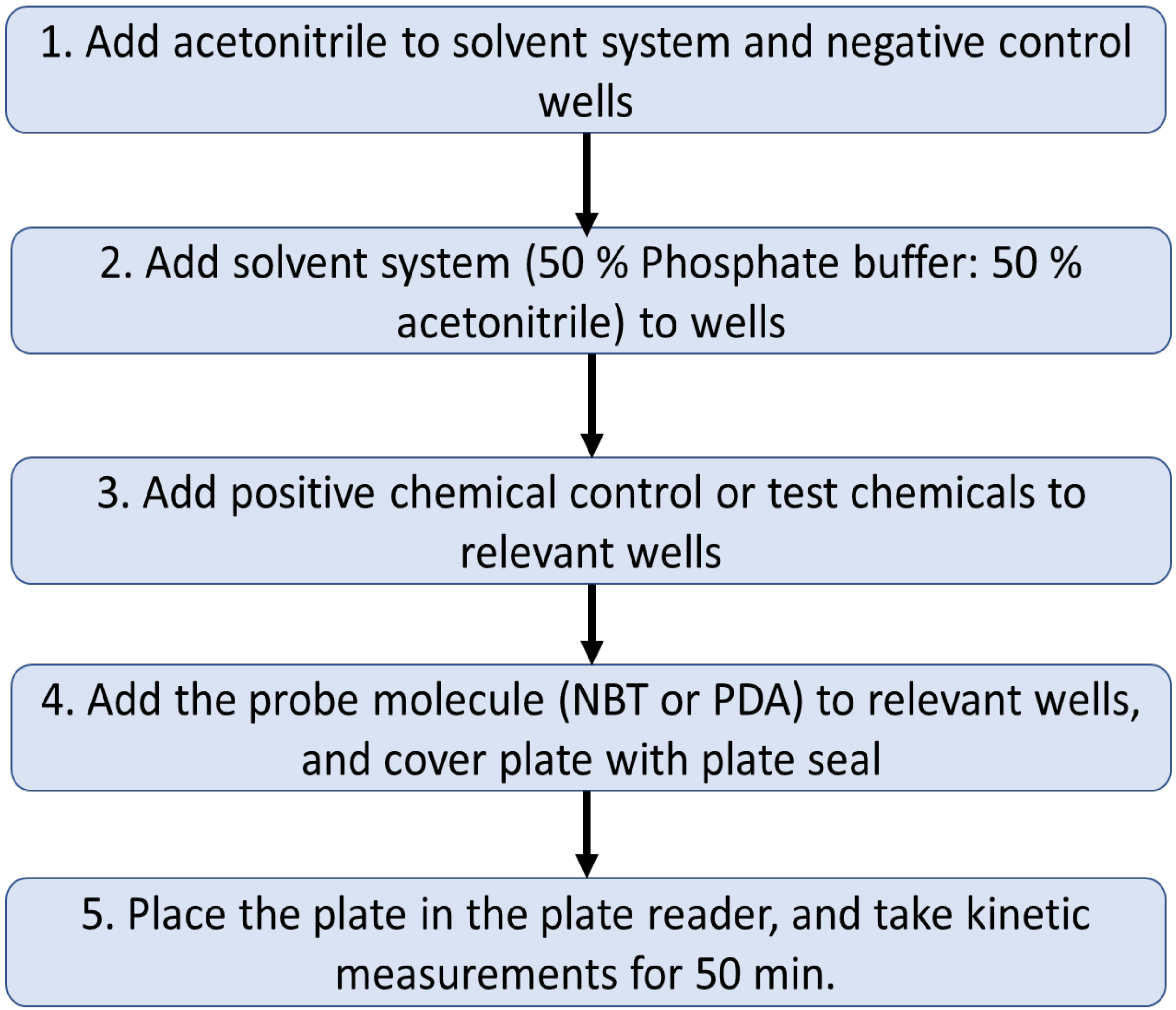
Example: cause-and-effect analysis



Robustness testing can evaluate each of the branches

Rosslein, M., Elliott, J. T., Salit, M., Petersen, E. J., Hirsch, C., Krug, H. F., Wick, P. The use of cause-and-effect analysis to design a high-quality nano-cytotoxicity assay, Chemical Research in Toxicology, **2015,** 28(1), 21-30.

Example: flow chart



Control measurements should cover each step in the flow chart

Petersen, E.J., Uhl, R., Toman, B., Elliott, J.T., Strickand, J., Truax, J., Gordon J. Development of a 96-Well Electrophilic Allergen Screening Assay for Skin Sensitization Using a Measurement Science Approach. Toxics, **2022**, 10(5), article number 257.

Example: plate design

SS - Blank (Solvent System)

NC - Negative Control

PC - Positive Control (serial dilution)

TC TC TC TC TC TC TC - Test chemicals

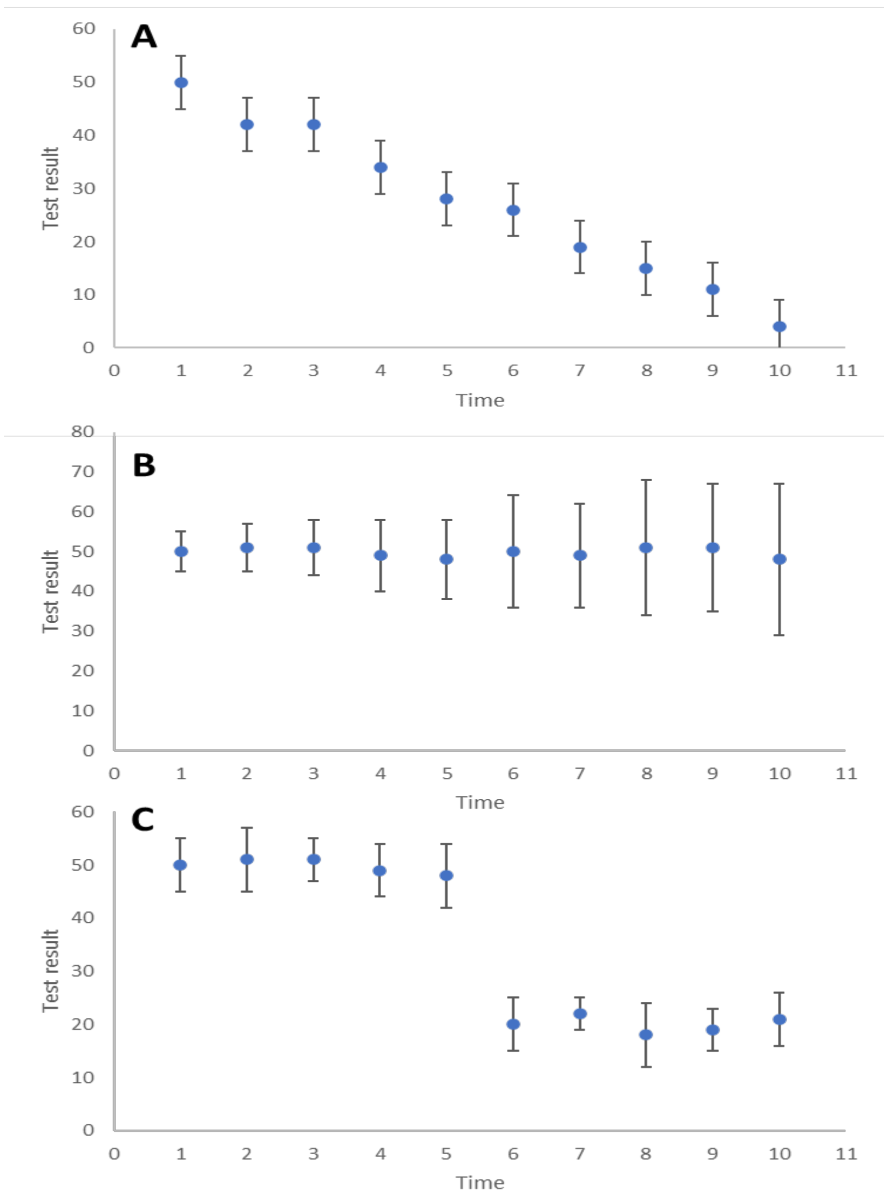
I - Test chemical interference wells

● - Wells without added reagents

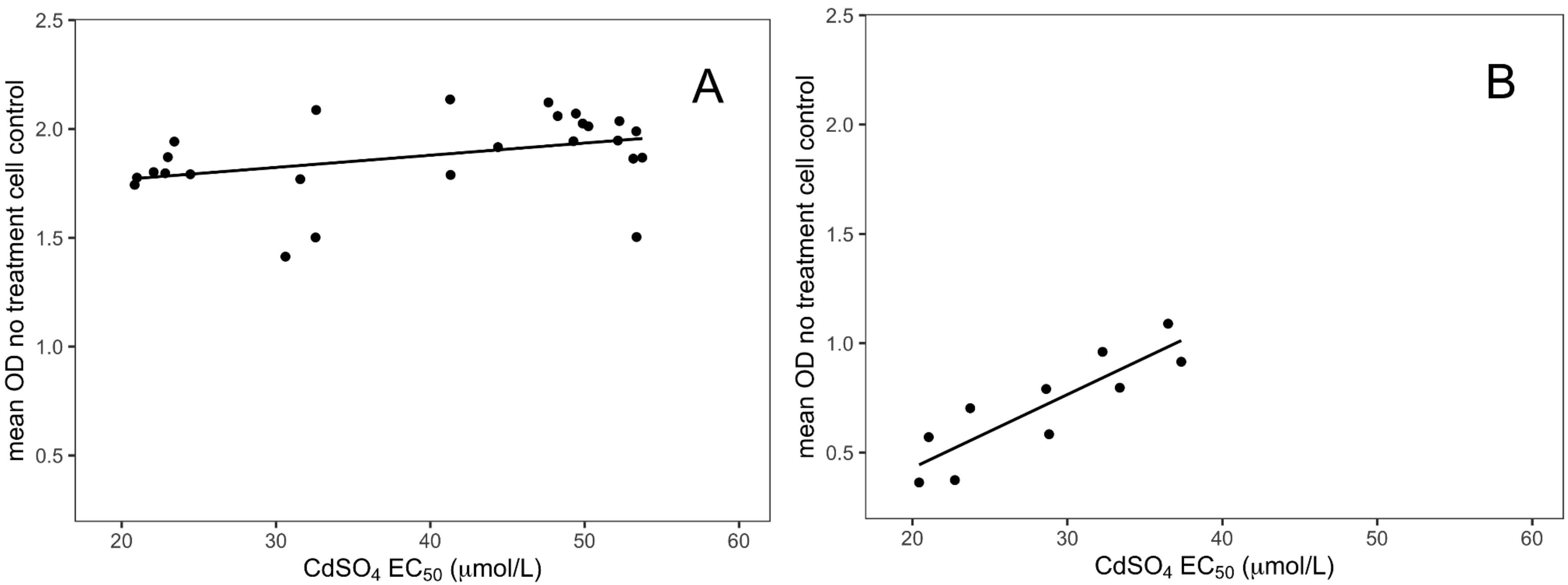
Control measurements evaluate key sources of variability each time the assay is performed

Petersen, E.J., Uhl, R., Toman, B., Elliott, J.T., Strickand, J., Truax, J., Gordon J. Development of a 96-Well Electrophilic Allergen Screening Assay for Skin Sensitization Using a Measurement Science Approach. Toxics, **2022**, 10(5), article number 257.

Example: control charting



Example: scatter plot

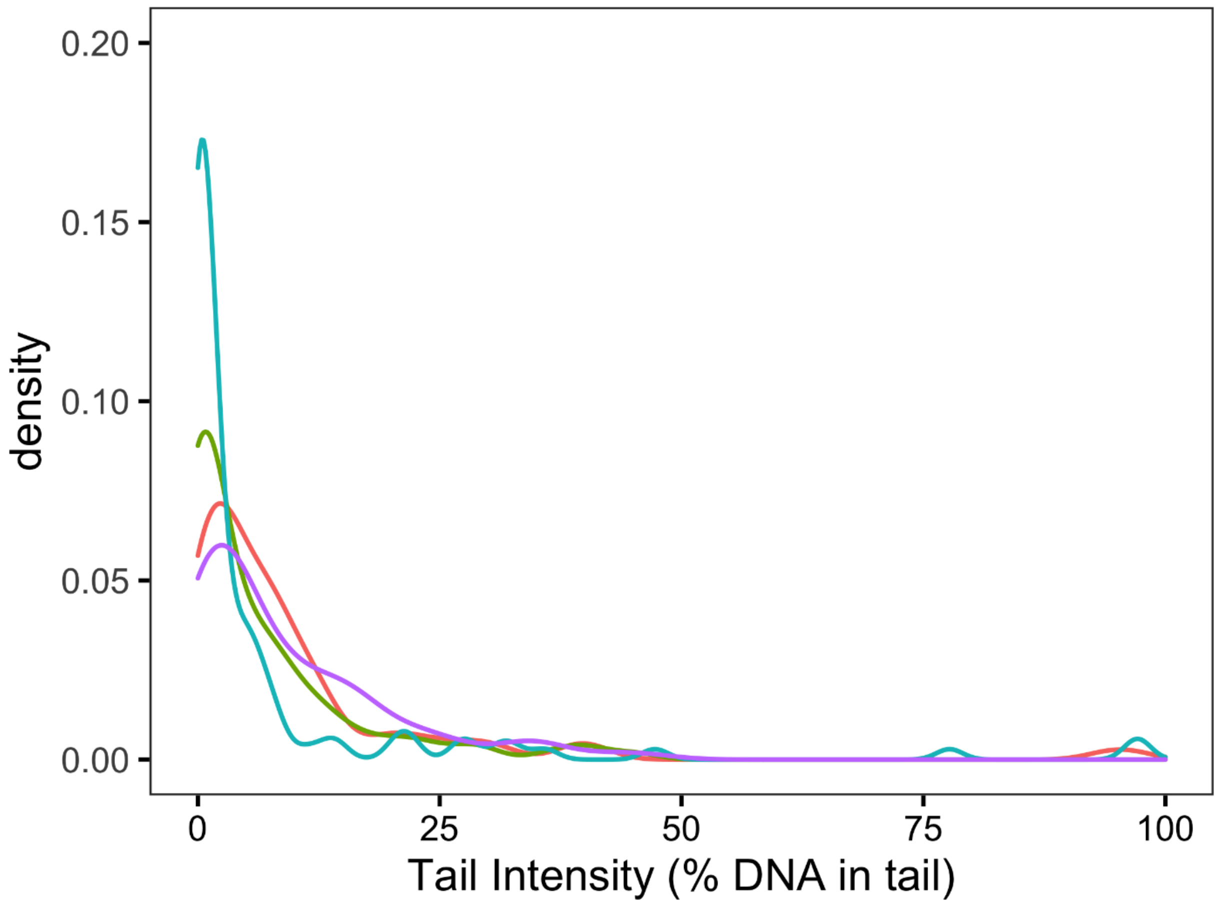


There is either a lack of an interaction between the EC_{50} values (part A) or an interaction (part B) depending upon the range of mean OD values which reflect the number of cells.

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 measurement through an interlaboratory comparison study, **2017**, Altex, 34(2), 201-218.

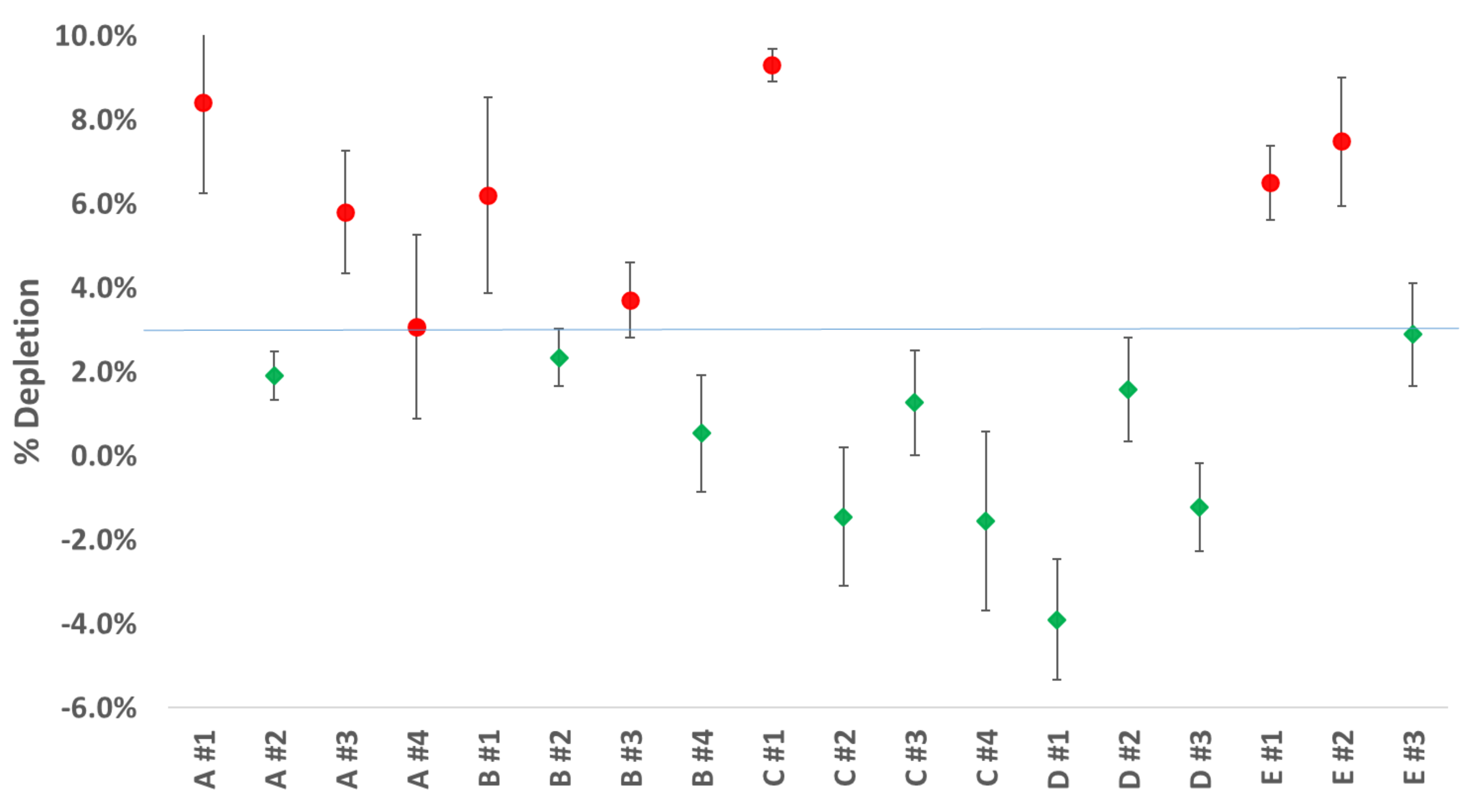
Example: histogram



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

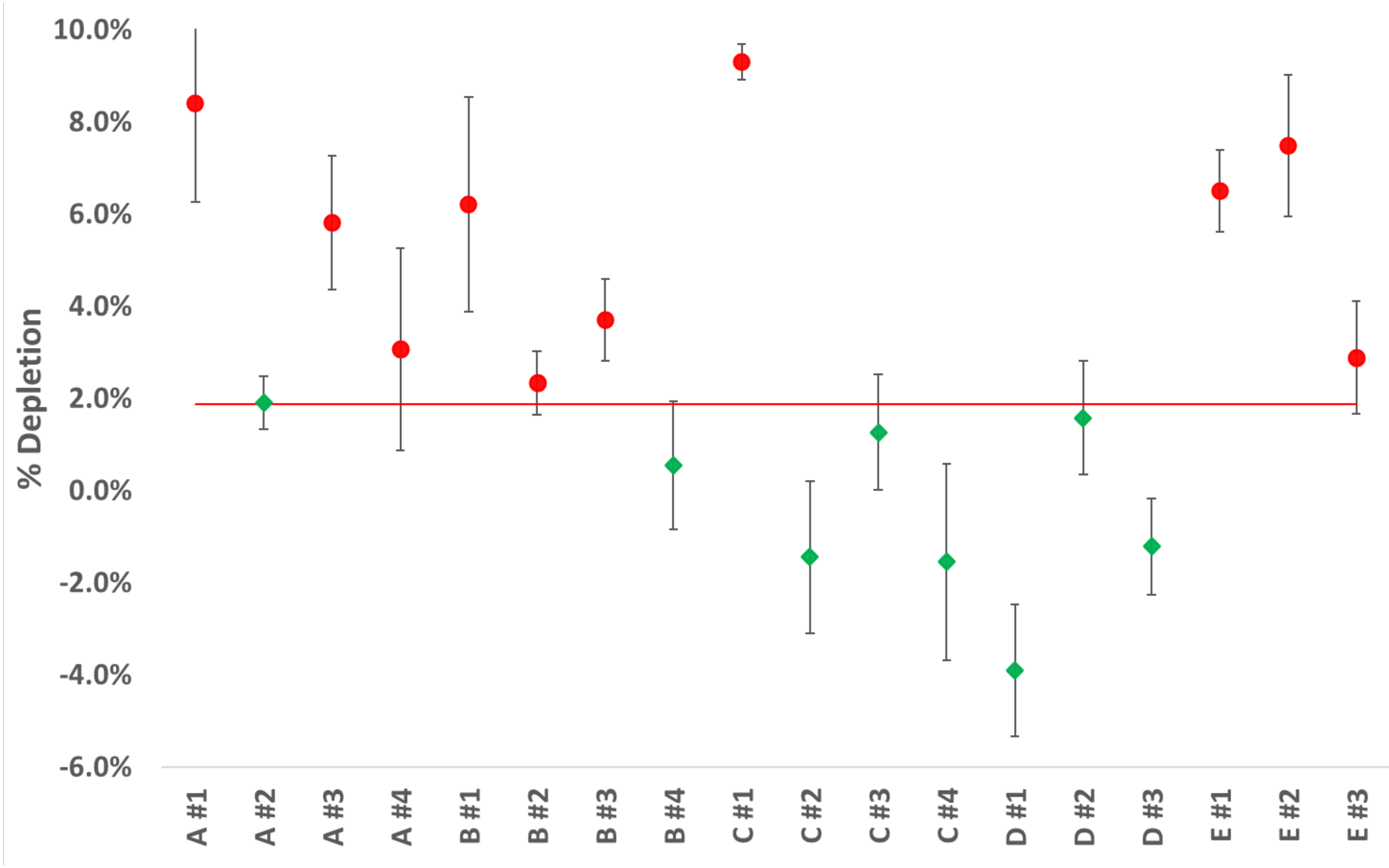
Statistical approaches: static call line



The call line is based on a set amount, in this case 3 %, regardless of the experimental uncertainty.

Data from https://doi.org/10.3390/toxics10050257; figure from https://doi.org/10.14573/altex.2205081

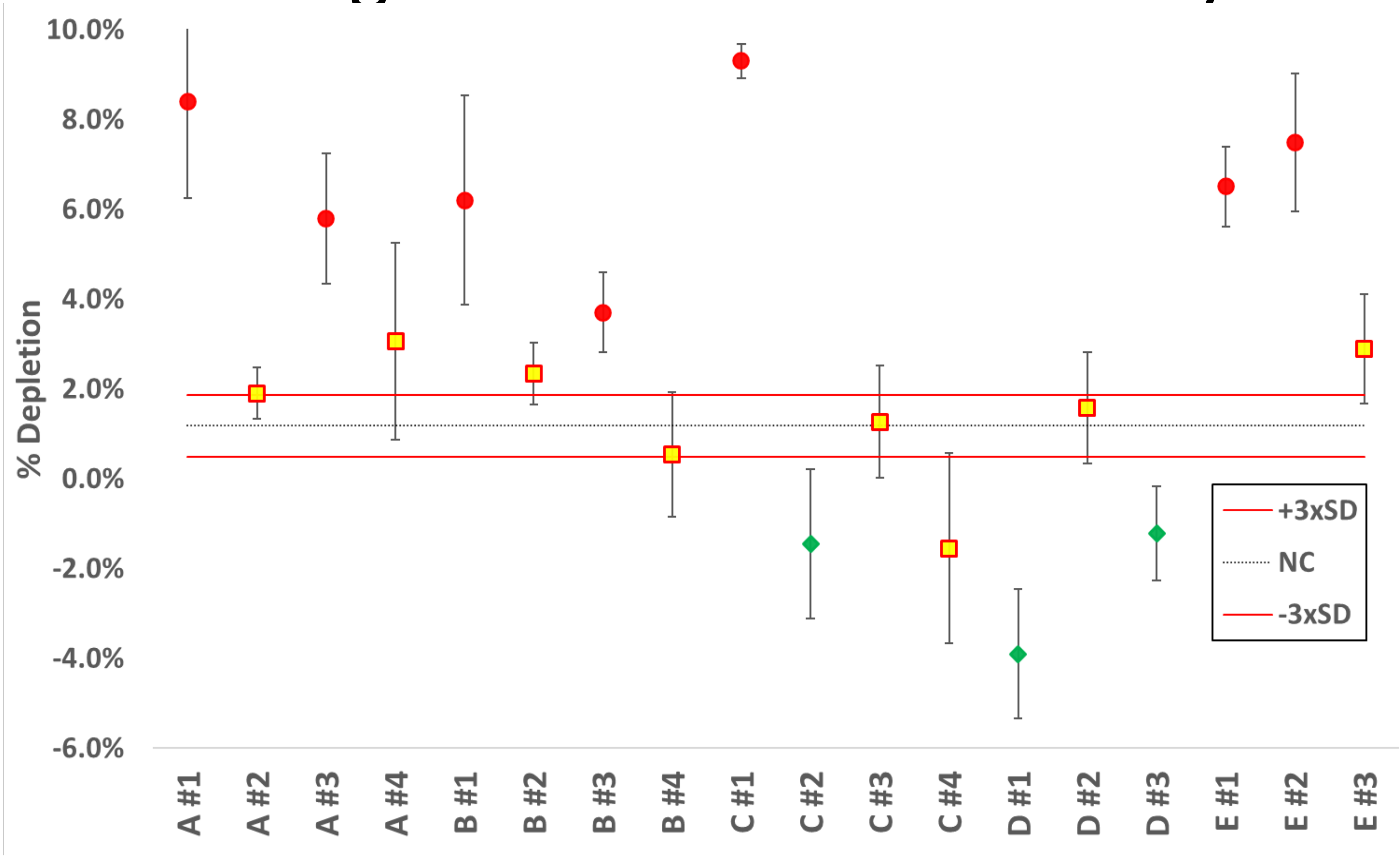
Statistical approaches: call line based on negative control uncertainty



The call line is based on the mean + 3 times the standard deviation of the negative control.

Data from https://doi.org/10.3390/toxics10050257; figure from https://doi.org/10.14573/altex.2205081

Statistical approaches: call line based on negative control uncertainty



The call line is based on mean ± 3 times the standard deviation of the negative control. If the 95 % confidence interval of the chemical in a run overlaps with the uncertainty band for the negative control, the data is called “borderline.”

Data from https://doi.org/10.3390/toxics10050257; figure from https://doi.org/10.14573/altex.2205081

Statistical evaluation

A T-score is calculated by taking the “Effect” and dividing by the standard error. In order to take all uncertainty into account, all sources of variability must be included in the calculation. In this case, we took into account the variability of the Negative Control, the NC/PC Blank, the test compound and the test compound Blank.

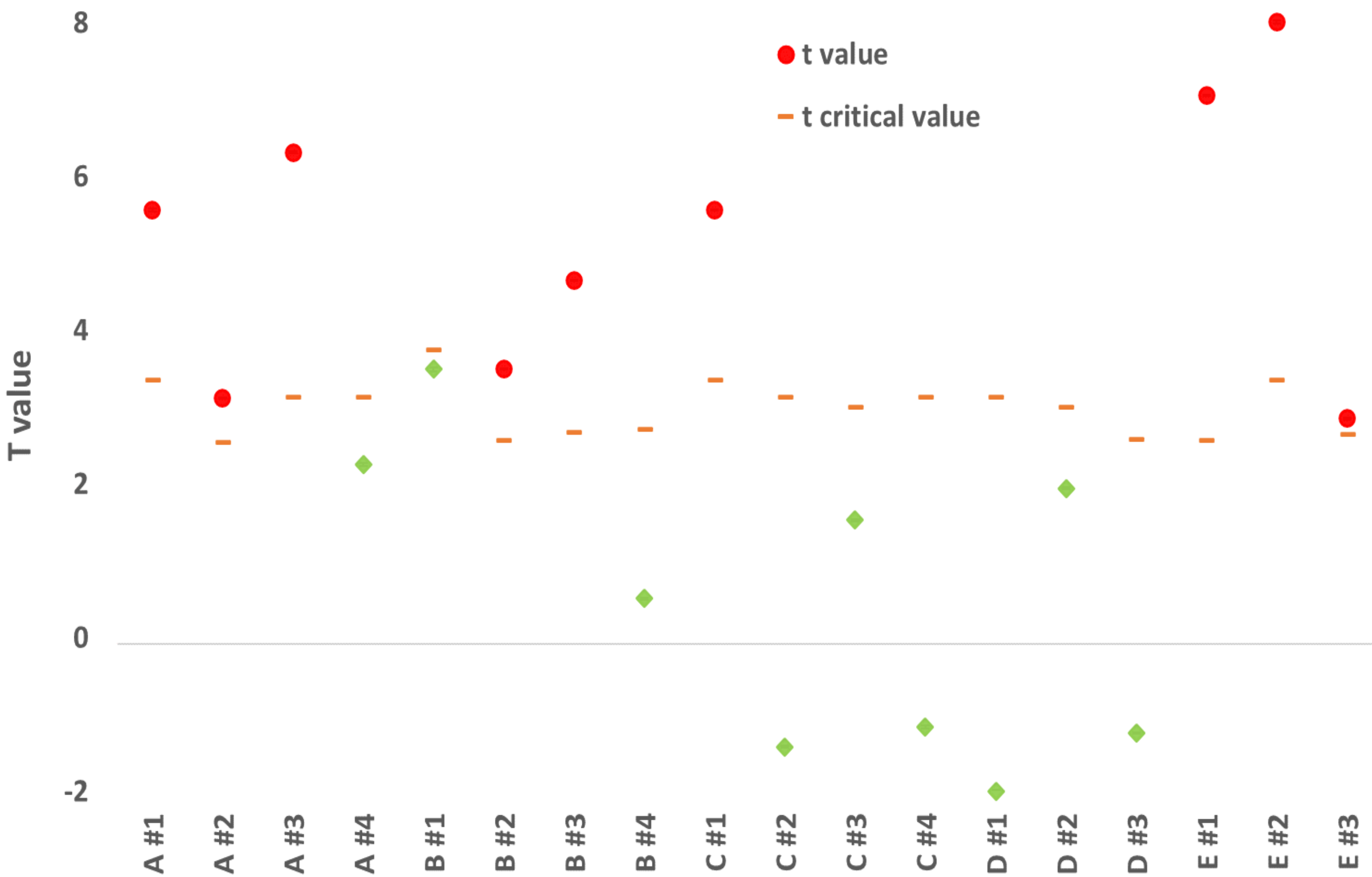
NC – Negative Control
S – NC/PC Blank
TC – Test Compound
TC_B – Test Compound Blank
sd – standard deviation
n – number of replicates

$$T = \frac{(\overline{NC} - \overline{S}) - (\overline{TC} - \overline{TC_B})}{\sqrt{\frac{sd_{NC}^2}{n_{NC}} + \frac{sd_S^2}{n_S} + \frac{sd_{TC}^2}{n_{TC}} + \frac{sd_{TC_B}^2}{n_{TC_B}}}}$$

Effect (or in our case Depletion)

Cumulative Uncertainty

Statistical approaches: call line based on t- value



The call line ($t_{critical}$ value for α=0.005) changes for every run based on propagated uncertainty in that run.

Data from https://doi.org/10.3390/toxics10050257; figure from https://doi.org/10.14573/altex.2205081

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