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

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Technical Framework Manuscript

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

**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

Technical Framework For High Quality NAMs

Based on biological relevance (e.g., AOP), capacity to fulfill a testing need, capacity to have quality sufficiently improved

Scientifically relevant NAMs

Conceptual evaluation of sources of technical variability in the assay: 1) flow charts; 2) cause & effect (C&E) analysis; 3) assay design; 4) check sheets

Within laboratory evaluation of assay performance: 1) control charting; 2) evaluate the applicability domain; 3) robustness testing; 4) scatterplots; 5) histograms

Statistical data analysis

Determination of method transferability (if needed)

High quality, robust method

Robustness testing can evaluate each of the branches.

Example: flow chart

1. Add acetonitrile to solvent system and negative control wells

2. Add solvent system (50 % Phosphate buffer: 50 % acetonitrile) to wells

3. Add positive chemical control or test chemicals to relevant wells

4. Add the probe molecule (NBT or PDA) to relevant wells, and cover plate with plate seal

5. Place the plate in the plate reader, and take kinetic measurements for 50 min.

*Control measurements should cover each step in the flow chart*

**Example: plate design**

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- SS - Blank (Solvent System)
- NC - Negative Control
- PC - Positive Control (serial dilution)
- 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*

Example: control charting
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

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

http://dx.doi.org/10.14573/altex.1906252
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](https://doi.org/10.3390/toxics10050257); figure from [https://doi.org/10.14573/altex.2205081](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
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.

\[
T = \sqrt{\frac{(\bar{NC} - \bar{S}) - (\bar{TC} - \bar{TCB})}{\frac{(sd_{NC}^2)}{n_{NC}} + \frac{(sd_{S}^2)}{n_{S}} + \frac{(sd_{TC}^2)}{n_{TC}} + \frac{(sd_{TCB}^2)}{n_{TCB}}}}}
\]

NC – Negative Control
S – NC/PC Blank
TC – Test Compound
TCB – Test Compound Blank
sd – standard deviation
n – number of replicates
Effect (or in our case Depletion)
Cumulative Uncertainty
Statistical approaches: call line based on t-value

The call line ($t_{\text{critical}}$ value for $\alpha=0.005$) 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