Measurement assurance tools and their application to alternative test methods

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Redesigning electrophilic allergen screening assay to increase confidence in the measurement (in collaboration with CPSC)

- Change from single cuvette assay to 96-well plate
- Instrumentation has now been set up at NIST for the plate reader, fluorometer, and spectrophotometer
- New plate design to include multiple process control measurements
- Measure positive control dose-response curve to provide more detailed information about assay performance
- Unique considerations for plate reader measurements related to testing a 50% organic solvent/50% phosphate buffer solution
- Comprehensive evaluation of sources of uncertainty

Flow chart

1. Add solvent system (50 % Phosphate buffer: 50 % acetonitrile), positive chemical control and test chemicals to relevant wells

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

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

Cause and Effect Diagram

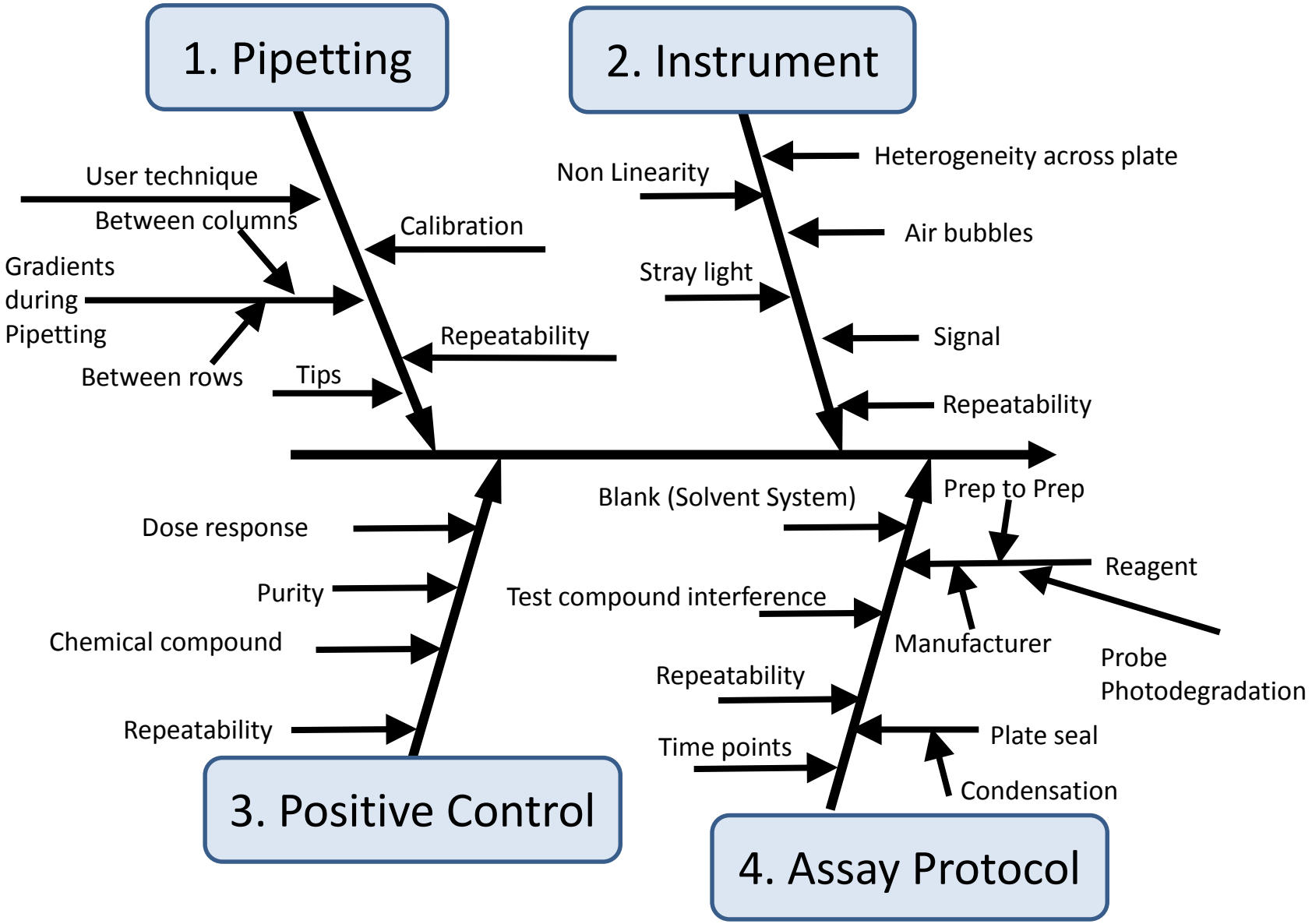
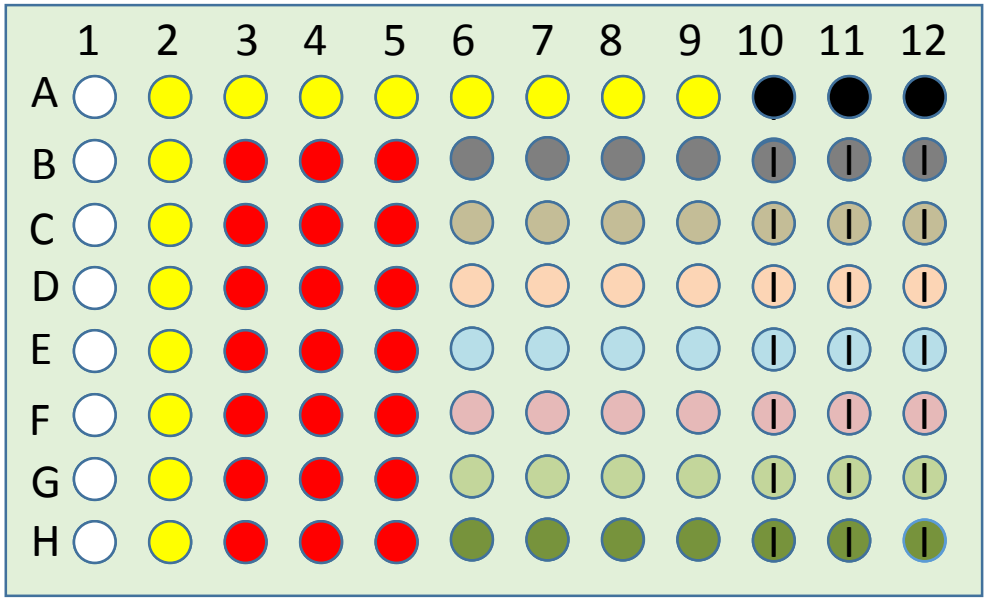


Plate Design for EASA assay



- Blank (Solvent System)
- Negative Control
- Positive Control (serial dilution)
- Test chemicals
- Test chemical interference wells

Process control measurements:

1. Within pipette step variability
2. Between pipette step variability
3. Solvent system (blanks)
4. Serial dilution of positive chemical control
5. Instrument performance/bubbles (680 nm)
6. Test chemical interference

- **Process control measurements encode quality onto the plate.**

Robustness testing

- Photodegradation of probe molecules
- Plate reader homogeneity and impact of pipetting direction
- Assay duration
- Potential for bias from bubbles in wells
- How to handle bias from test chemicals which absorb or fluoresce similarly to probe molecules
- Usage of polar and semipolar solvents
- Select positive controls based on ease of handling, low toxicity
- Initial test chemical concentration
- Performance of different types of plates and plate seals

- **A main goal was to select measurement parameters in the protocol that were scientifically defensible and based on data instead of expert judgement.**
- **Robustness testing and plate design revealed biases undetected during Phase 1 with cuvette assay**

Results

- Over 60 chemicals have been evaluated including 50 sent from NTP and 10 from Phase 1 with the cuvette assay
- Comparison to LLNA data yielded 75-80 % agreement similar to what was observed for published skin sensitization assays
- Bayesian statistical model is being developed based on all quantified sources of uncertainty
- Discussions ongoing about threshold for positive versus negative calls and uncertainty in making these decisions for each chemical tested

Future work

- Interlaboratory testing to evaluate transferability of assay
- Evaluate the applicability to nonpolar solvents which would enable the assay to be used for polar, semi-polar, and nonpolar solutions after extraction
- Evaluate the predictive capacity of the assay, or kinetic modifications of it, for assessing potency
- Evaluate the predictive capacity of the assay when used with other skin sensitization in vitro assays to predict LLNA results
- Evaluate use in 384 well plates for high throughput analysis

Updates on ASTM and ISO and other activities

- Development of a photocatalytic activity assay for use with nanomaterials (ISO TC 229, Vytas Reipa)
- Development of an in vitro phototoxicity assay for use with nanomaterials (under discussion in ISO TC 229, Vytas Reipa)
- Development of *in vitro* assays to support the development of nanotechnology-enabled medical products in collaboration with FDA (ASTM E56, Bryant Nelson)
- Involvement in National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) efforts to reduce animal testing during vaccine testing

Collaborators at NIST and CPSC for assay development and interlaboratory testing

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DOD
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NICEATM/ILS
Judy Strickland
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Meeting at NIST on March 8, 2019

NRC postdoc opportunity at NIST

Improving Measurement Assurance of *In Vitro* Toxicity Assays

Applications can be submitted on August 1 or February 1

2-year appointment

~ 72k stipend

Limited to US citizens

Contact Elijah Petersen (Elijah.Petersen@nist.gov) for more information