NIST at ICCVAM: Tools to improve confidence in alternative test methods

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Some key focuses at NIST

• **Measurements**
  • Develop new measurement methods
  • Improve accuracy/precision of measurements

• **Reference Materials**
  • Well-defined materials for use as a reference when making measurements
  • Enables inter-lab comparability
  • Physical artifacts for calibrating instruments

• **Standards**
  • Documentary standards, ASTM, ISO
  • Reference data (chemical spectra)
  • Technical Notes: “Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results” (GUM)

• **Biology/biotechnology**
  • Cell-related measurements and technology (~1990)
  • Cytotox measurements, organism measurements (~2005)
Measurement Assurance in Biological Assays

Cause and Effect Analysis: A new approach for developing robust nano-bio assays
Workshop hosted by EMPA (Switzerland) on June 18 & 19, 2015
16 participants in attendance from 3 countries

Evaluated five in vitro assays for use with nanoparticles:
- MTS assay (cell viability)
- DCF-DH assay (ROS generation)
- Flow cytometry assay (quantification of viable, necrotic, or apoptotic cells)
- Comet assay (genotoxicity)
- ELISA assay for IL-8 (inflammation response)

For each assay, we developed a flow chart, cause-and-effect analysis, and control experiments
MTS cell viability assay

Summary Instructions:

1. Receive NP, serum, cells, chemical control
2. Negative control- no treatment
3. Positive control- 100 uM CdCl2
4. Manufacturer’s protocol
5. Cell proliferation rate- 21h
6. Normalize treatment to no-treatment well
Identify sources of variability using cause & effect analysis

1. Cell-specific issues
   - Time before Assay
   - Handling
   - Passage Seed Density
   - Media
   - Culture Conditions
   - Incubator
   - Temperature
   - Humidity
   - CO2
   - Cell Growth
   - Plates
   - Manufacturer
   - Cell ID

2. Pipetting
   - Between Row
   - Between Columns
   - User Technique
   - Calibrations during Pipetting
   - Tips
   - Calibration

3. Instrument-specific issues
   - Curve Fitting
   - Non Linearity
   - Stray Light
   - Background
   - Signal

4. Chemical Control
   - Dosing
   - Solvent control
   - Solubility
   - Purity
   - Chemical Compound
   - Prep to Prep
     - Freeze/Thaw Cycle
     - Reagent
     - Kit to Kit
     - Washing Step
     - Nano Particle Interference
     - Blank

5. Assay-specific issues
   - Dosing
   - Contaminants
   - Surface react.
   - Water sol.
     - Agglo/Aggre.
     - Surface "Chemistry"
   - Surface exper. conditions
   - Chemical Composition
   - Specific Surface
   - Particle Size
   - Morphology

6. Nanoparticle-specific issues

What is the purpose of cause and effect analysis?

1. Method to lay out implicit knowledge

2. Systematic approach to identify potential sources of variability in an assay and to highlight key sources of variability

3. Can be used to help design process control experiments, improve plate layout, and with writing a protocol

4. Can be used iteratively to improve assay quality by decreasing variability in key assay steps which decreases the total variability in the assay
Design a new plate format with process control measurements

Plate design includes 8 control measurements in addition to the NP measurement.
Single-laboratory results

Cell pipetting caused highest amount of variability among controls
Interlaboratory comparison with MTS assay

• 5 national metrology institutes were involved in the interlaboratory comparison

• Experimental design:
  • Share two A549 cell lines from ATCC and EMPA
  • Serum from local provider
  • Reagents from local provider
  • Serum and serum-free tests
  • Multiple replicates
  • Share nanoparticles (+ve PS) and chemical control (CdCl₂)
- Looks like harmonization between the laboratories
- No cell line differences
- The serum conditions increases variability
Can the system control measurements identify the cause of the outlier?

- Chemical Process Control - tests overall measurement system

Serum free conditions, variability less than with NP

Differences between cell lines
How sensitive are we to cell seeding variability

- Correlation between no-treatment cells and NP EC50
- If outliers are removed, no strong correlation
- Suggests that within this range of cell seeding variability (OD=1.5-2.5) no big effect on EC50
Impact of cell rinsing for lab A

Changing the rinsing procedure brought lab A results to the interlab consensus values.
What is the purpose of process control measurements?

1. Provide evidence that the measurement process occurred as expected.

2. Should meet specifications before acceptance of the test result.

3. Can be used to identify relative contributions to total variability in assay result. Protocol modifications?

4. Ideal for designing protocols for an interlaboratory comparison

5. Can be used to assess the functioning of different components in a complex assay
Instrument calibration: Process for Determining Analytical Performance of a Widefield Fluorescence Microscope

Series of image pairs acquired over a range of exposure times (example)

The difference between images is used to compute pixel variance (‘noise’)


- Saturation = argmax(σ²_{glass}(t)) {t>0}

Instrument calibration: Establishing Instrument Specifications

By *charting* the **Detection Threshold**, **Saturation**, and **Intensity Response** over time, you can:

- Demonstrate comparability between fluorescence intensity measurements
- Identify changes in the analytical performance of your widefield microscope

*can be used to normalize for day-to-day intensity variations
Potential NIST-ICCVAM interactions

• Current- Protocol evaluation for electrophilic allergen screen assay
  – Cause-and-effect analysis
  – Process control considerations
  – Discussion
  – Potential involvement in interlaboratory comparison
Collaborators

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