

Measurement assurance tools and potential application to alternative test methods

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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)

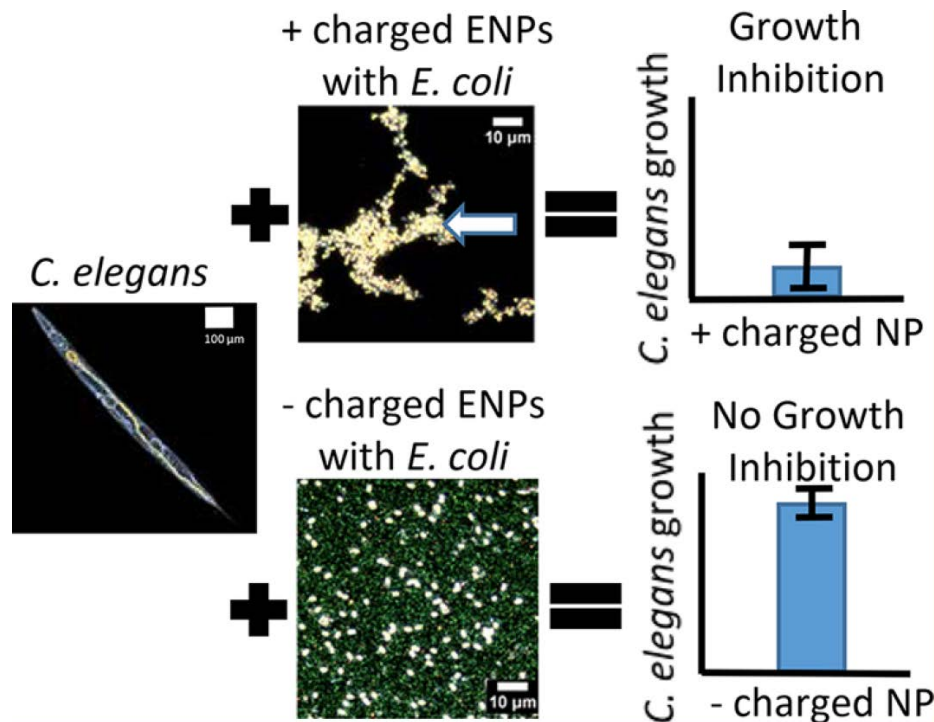
For each assay, we developed a flow chart, cause-and-effect analysis, and control experiments



Workshop paper to be submitted in the next month

Assessing the robustness of an ISO method for *C. elegans* with nanomaterials

- One key topic related to using alternative test methods with nanomaterials is how to assess if the assay is sufficiently robust
- Tested the assay with 15 NPs using different compositions, coatings and sizes
- An artifact was observed for positively charged particles as a result of agglomeration with the food source
- Two orthogonal *C. elegans* assays did not reveal toxicity



Redesigning electrophilic allergen screening assay to increase measurement assurance (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
- Choose positive control concentrations to maximize information about assay performance
- Statistical evaluation of how to determine threshold values and increase confidence in determinations
- Unique considerations for plate reader measurements related to testing a 50% organic solvent/50% phosphate buffer solution