CASE STUDY: Applying GLPs to New, Non-Animal Methodologies

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Institute for In Vitro Sciences
Advancing Science & Animal Welfare Together

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MISSION

To increase the **use** and **acceptance** of non-animal test methods worldwide.

VISION

A day when methods based on **humane** human-specific responses will be used by industry and regulatory agencies to determine the **safety** and **efficacy** of substances. This vision fuels the **passion** we have for our organization, driving us to provide **innovative solutions** for industry and regulatory agencies.

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What Are In-Vitro Methods?

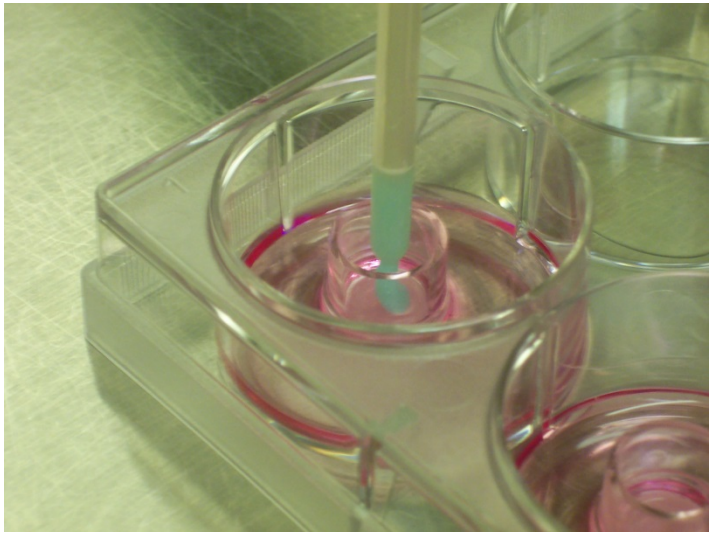
Image provided by Do Something.Org

In vivo safety testing

- "in the living body"
- testing of ingredients or products on animals or human subjects

In vitro safety testing

- "in glass"
- testing that does not require the use or sacrifice of living animals
- instead cells or tissues from humans or animals are used



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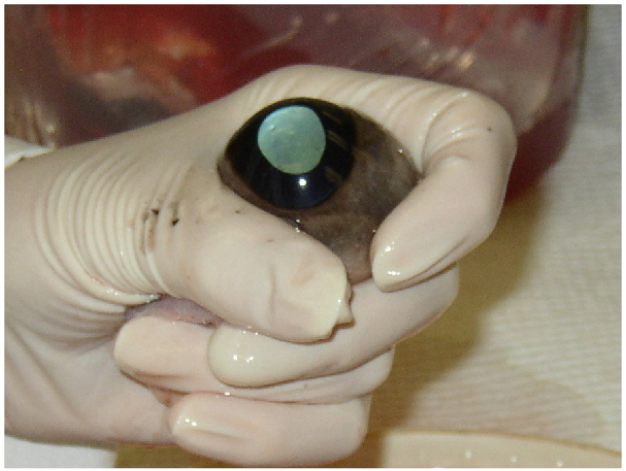
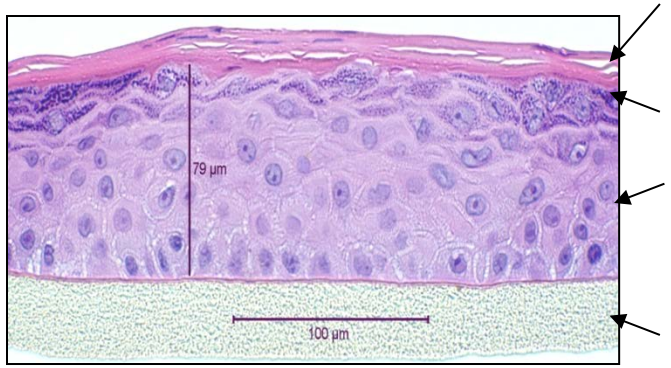
Types of Alternative Methods

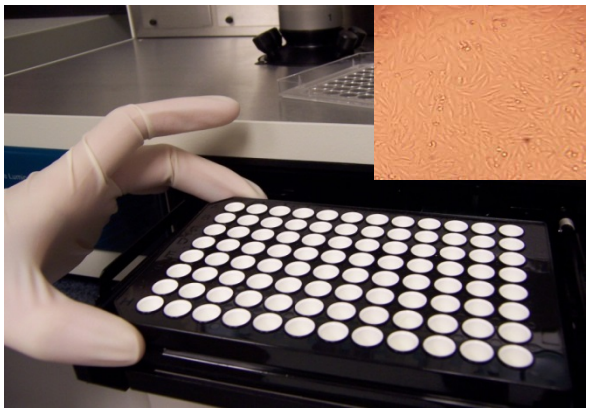


Organotypic Models

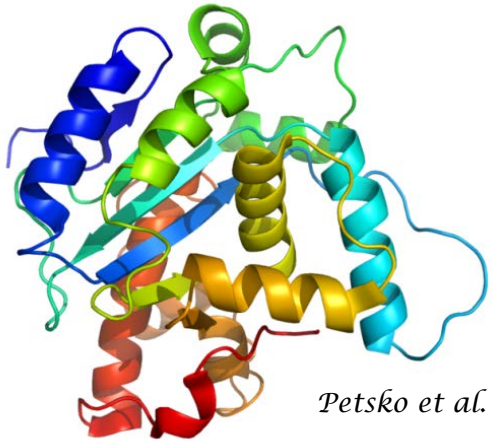


Courtesy of MatTek Corporation

Reconstructed Human Tissue Models



96-well cell-based assays



***In Chemico* Assays**

The Complete Assay Package

Test Method =

Test System (Cells, Tissue constructs) +

Exposure Conditions (media, volume & time etc) +

Endpoint(s) (Measures of the Action) +

Prediction Model (Calibration Curve)

Validation evaluates all of these components of the test method as a single package

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Facility Design

Section 58.41 General –

Each testing facility shall be of suitable size and construction to facilitate the proper conduct of nonclinical laboratory studies. It shall be designed so that there is a degree of separation that will prevent any function or activity from having an adverse effect on the study.

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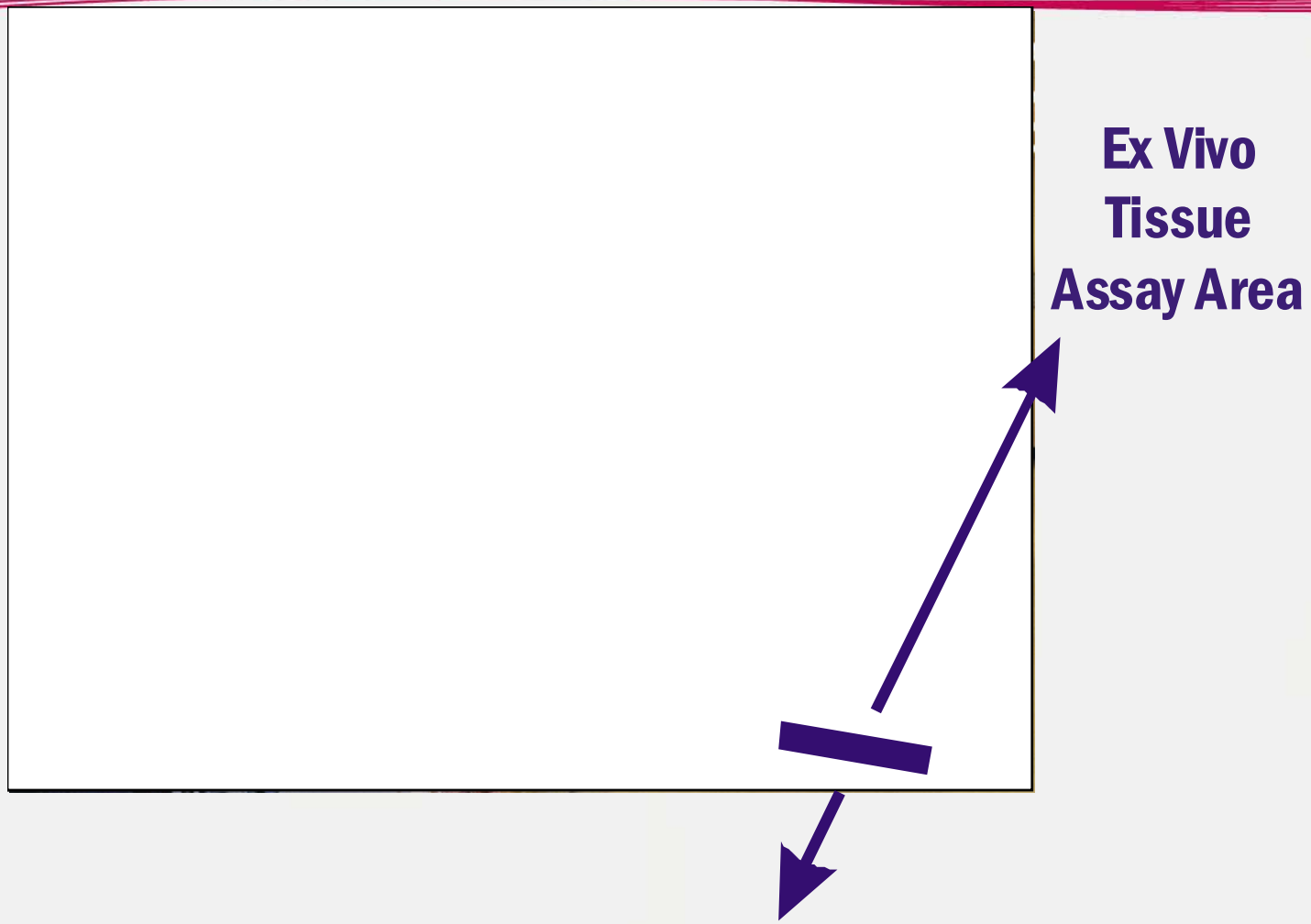
Separation of Activities



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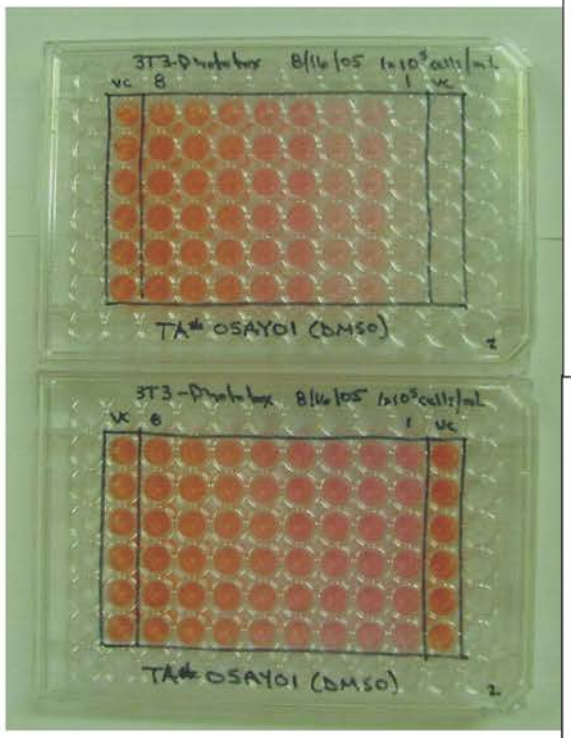
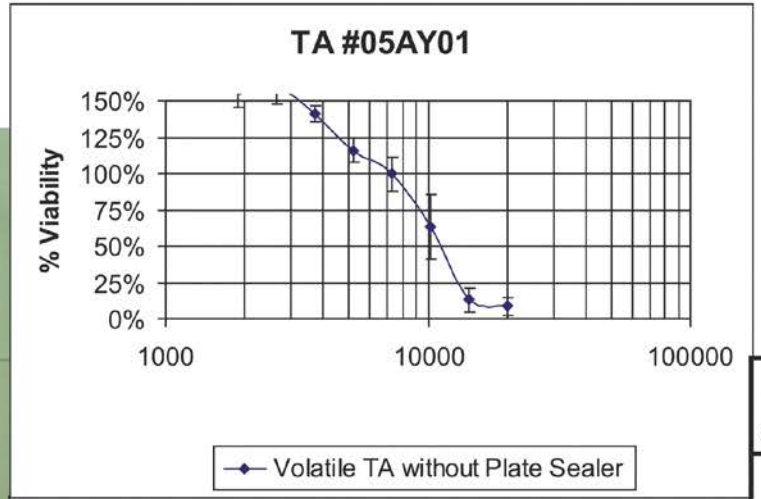
Separation of Doses

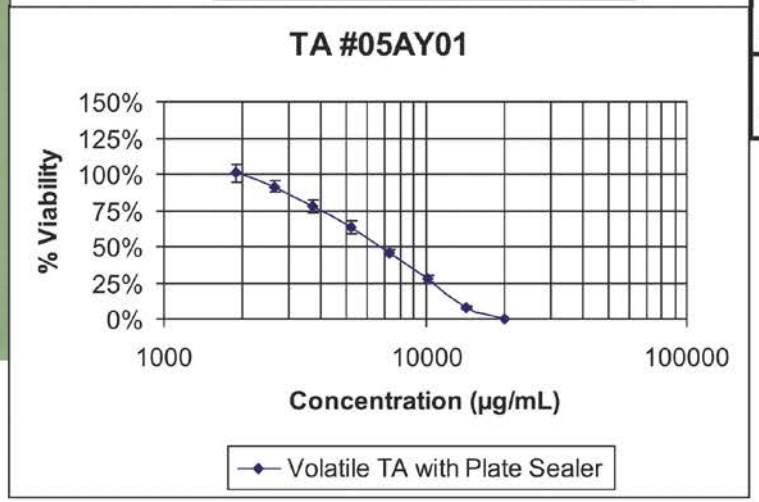
Example of Neutral Red Uptake Assay on Sample 05AY01

(With and Without the Use of a Plate Sealer)



VC = Vehicle Control
Dose ID 1= Most concentrated dose
Dose ID 8 = Least concentrated dose





	Without Plate Sealer	With Plate Sealer
Left Column Vehicle Control Corrected OD_{550}	0.359	0.333
Right Column Vehicle Control Corrected OD_{550}	0.063	0.301
Mean Vehicle Control Corrected OD_{550}	0.211	0.317

Training

Section 58.29(a) –

Each individual engaged in the conduct of or responsible for the supervision of a nonclinical laboratory study shall have education, training, and experience, or combination thereof, to enable that individual to perform the assigned functions.

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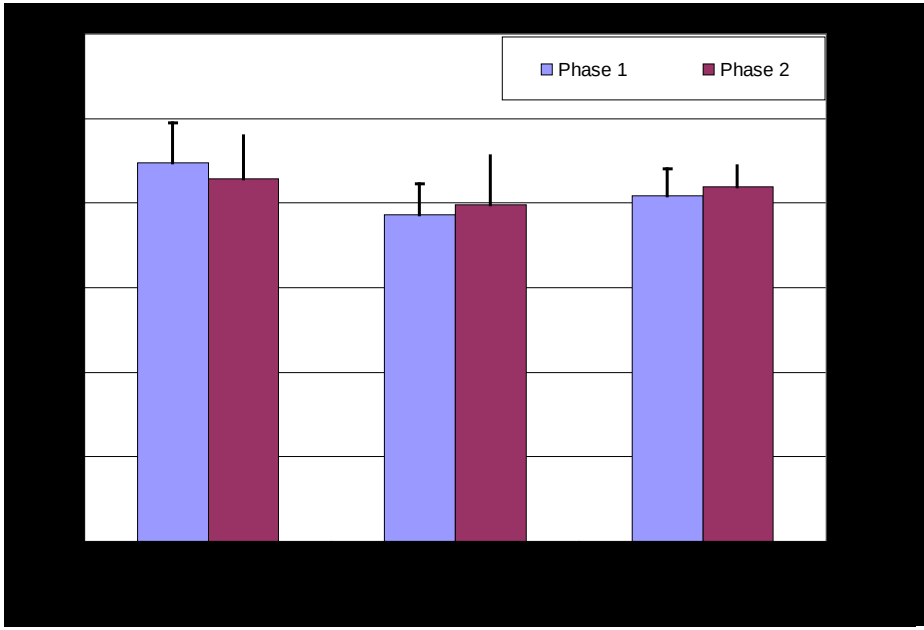
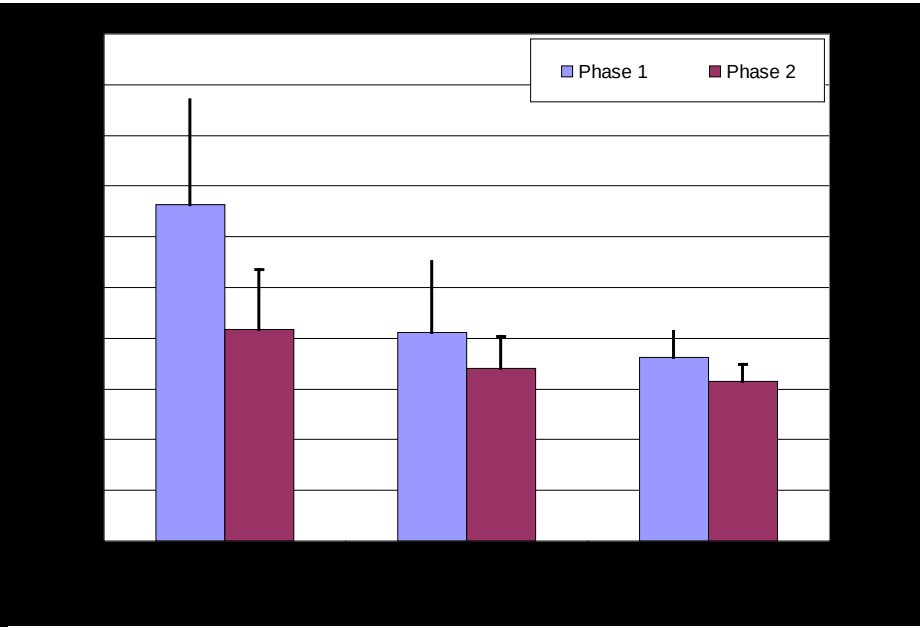
The Importance of Training





Negative Assay Controls for In Vitro Studies

58.3(c) ***Control article*** means any food additive, color additive, drug, biological product, electronic product, medical device for human use, or any article other than a test article, feed, or water that is administered to the test system in the course of a nonclinical laboratory study **for the purpose of establishing a basis for comparison with the test article.**

OECD GLP Advisory Document 14 - Control, negative: Separate part of a test system treated with an item for which it is known that the test system should not respond; the negative control provides evidence that the test system is not responsive under the actual conditions of the assay.

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Using Assay Controls - Negative

- Measure the response of the test system over the course of the study
- Expected to cause little or no change in the test system
- Follow the protocol procedures

Positive Assay Controls for In Vitro Studies

58.3(c) ***Control article*** means any food additive, color additive, drug, biological product, electronic product, medical device for human use, or any article other than a test article, feed, or water that is administered to the test system in the course of a nonclinical laboratory study **for the purpose of establishing a basis for comparison with the test article.**

OECD GLP Advisory Document 14 - Control, positive: Separate part of the test system treated with an item the response to which is known for the test system; the positive control provides evidence that the test system is responsive under the actual conditions of the assay.

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Using Assay Controls - Positive

- Evaluate the performance of the test system.
- Selected for their ability to detect test system changes in either direction of response
- Historical database
- Acceptance criteria

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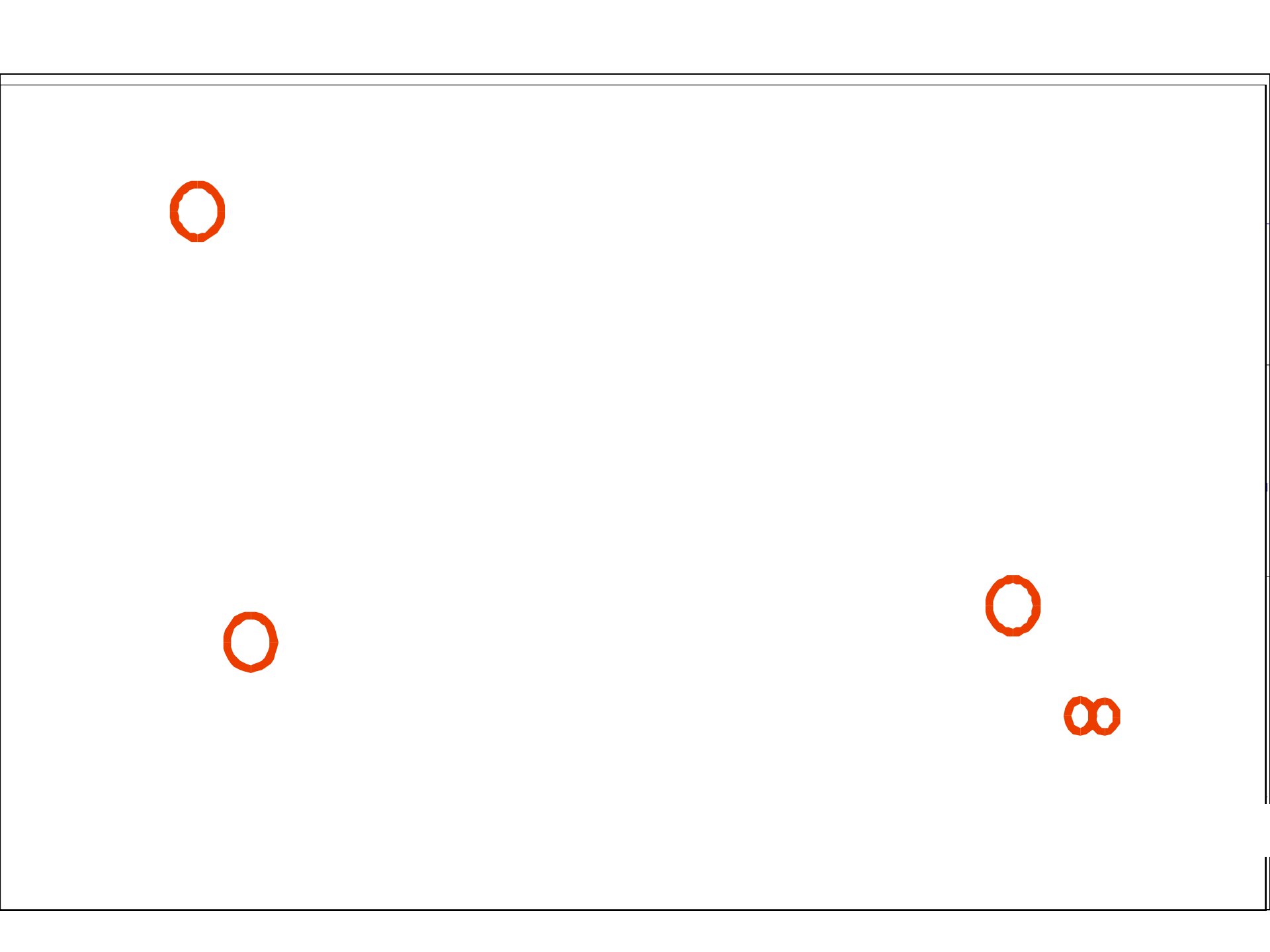

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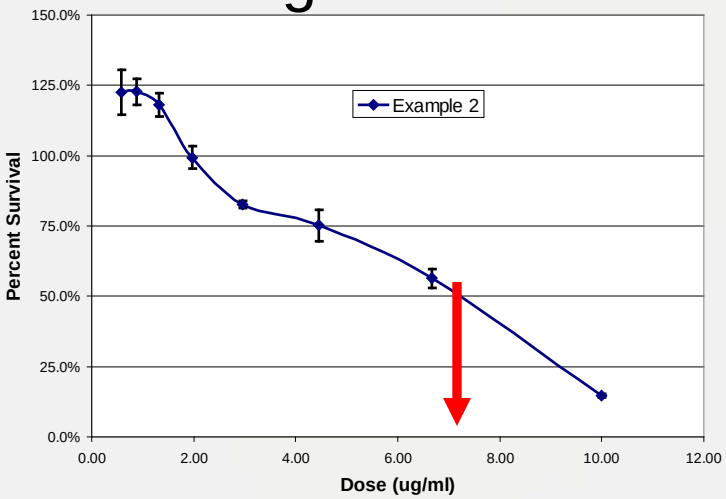
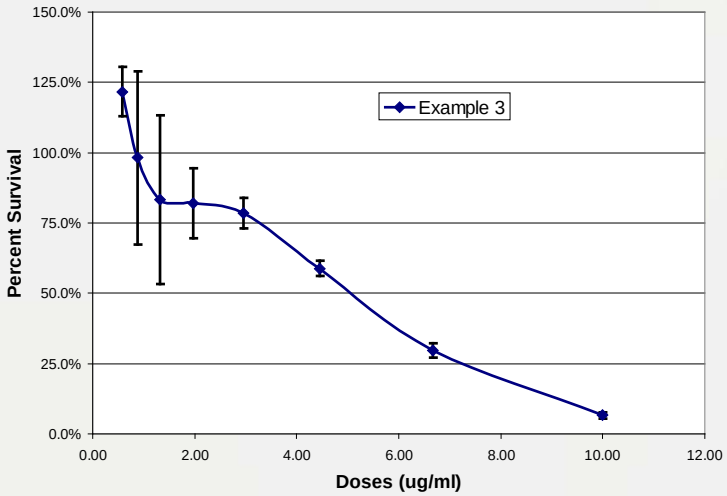
NHEK Cell Cytotoxicity

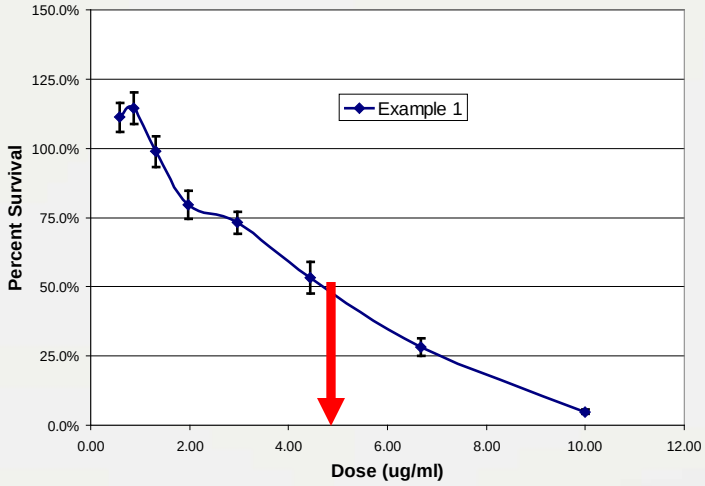
Right Shift



Variable



Historical Positive Control Average



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Defined Acceptance Criteria

- Used to determine the acceptability of a trial (run) of the assay
- Often based on the values from the concurrent assay controls
- Assay control values compared to historical assay control values
- Acceptable range of responses predetermined from the historical values
- Acceptable range should relate to the required precision of the assay and the functional range of assay end point values

Using Control or Reference Substances

Often called Benchmarks in *in vitro* work

- Match the chemical/product type of the unknown(s)
- Set upper and/or lower limits of response
- *In vivo* database available

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How to Choose a Benchmark

- Similar in chemical composition
- Similar in product type
- Benchmark choice depends on the test article, not the assay.

Example:

Test	Test Article	Benchmark
NRU	Shampoos	Baby Shampoo
NRU	Eye Drops	Eye Drops

* In both cases the positive control is SLS

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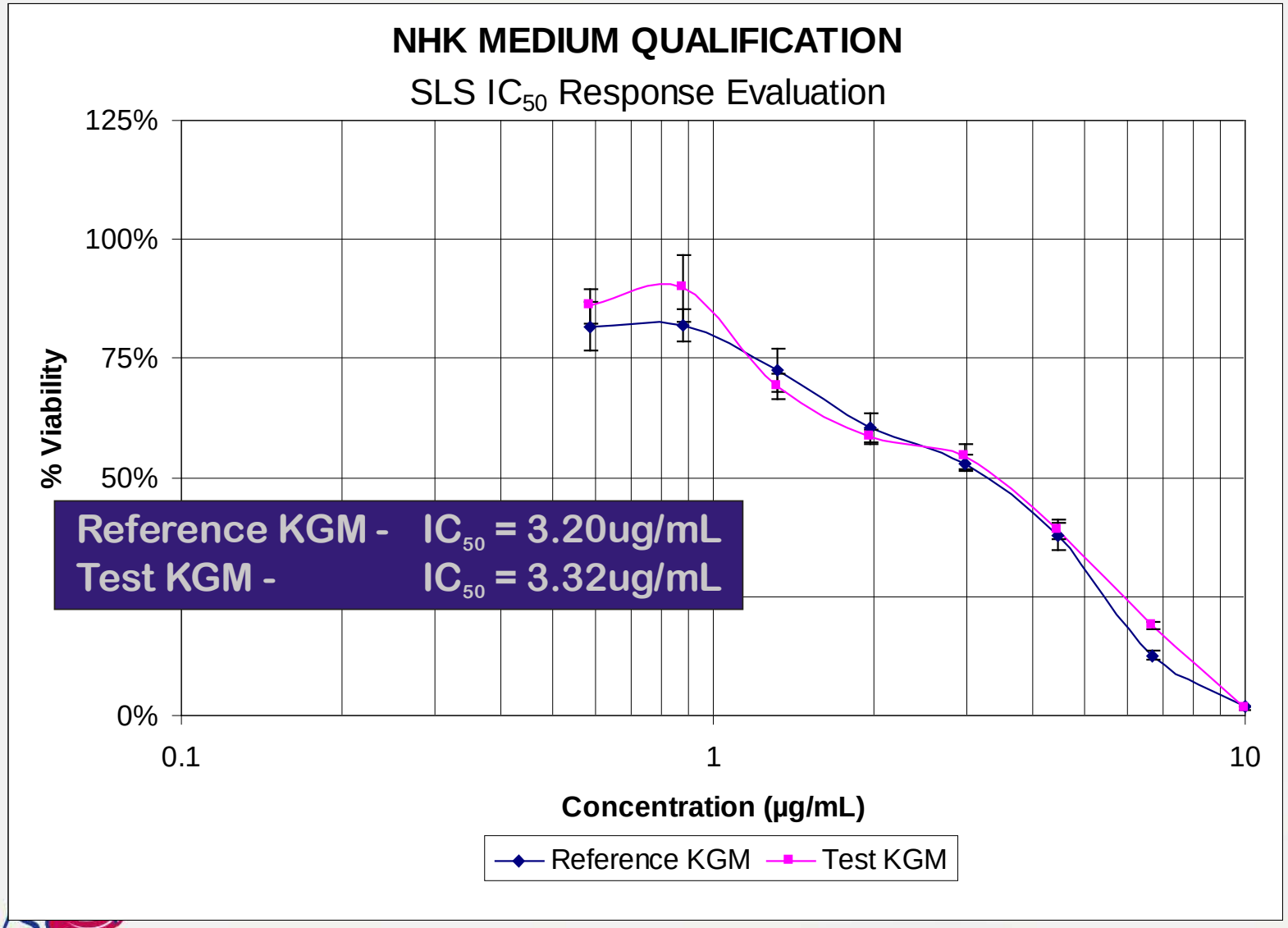
Media and Reagent Qualification



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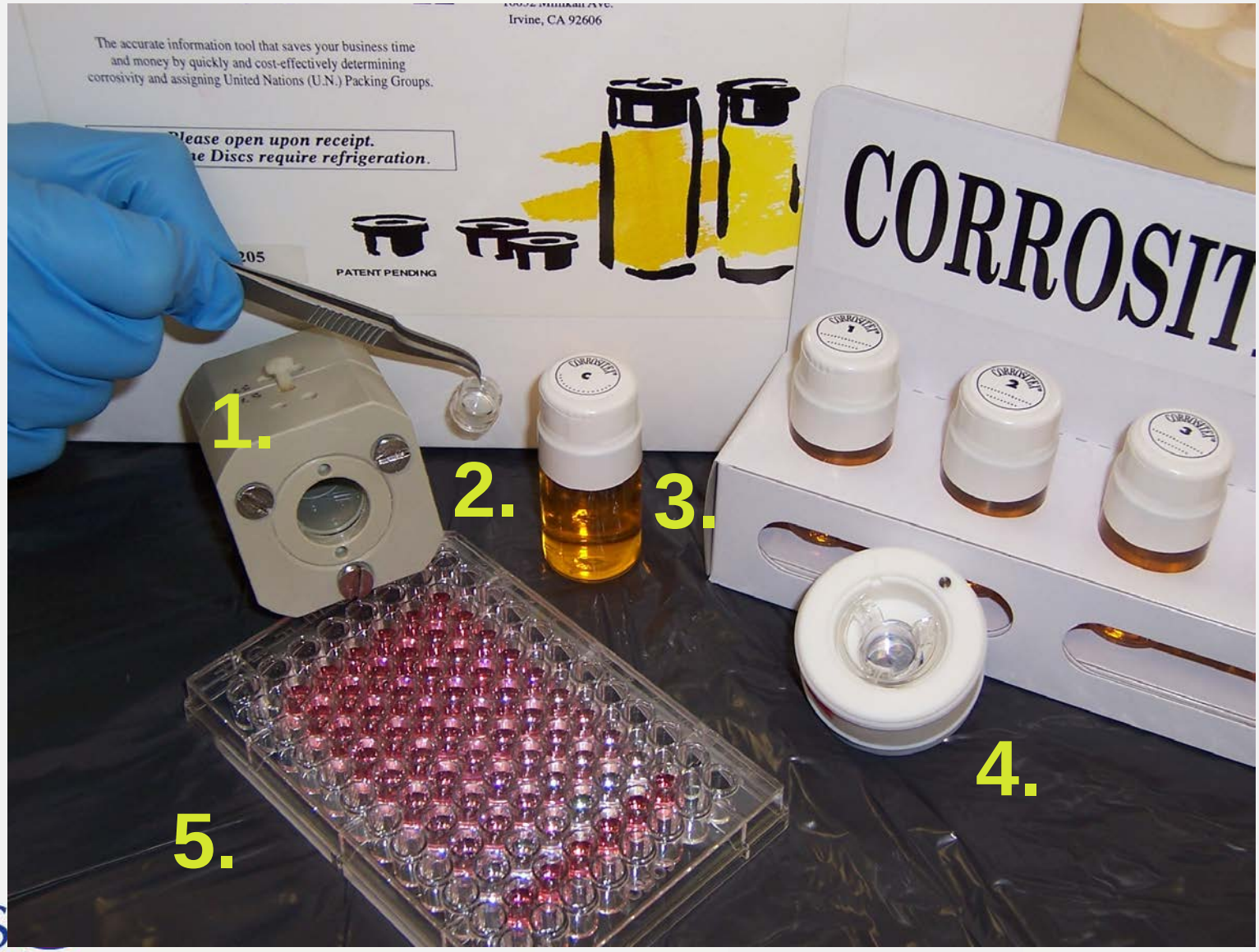
Examples of *In Vitro* Test Systems



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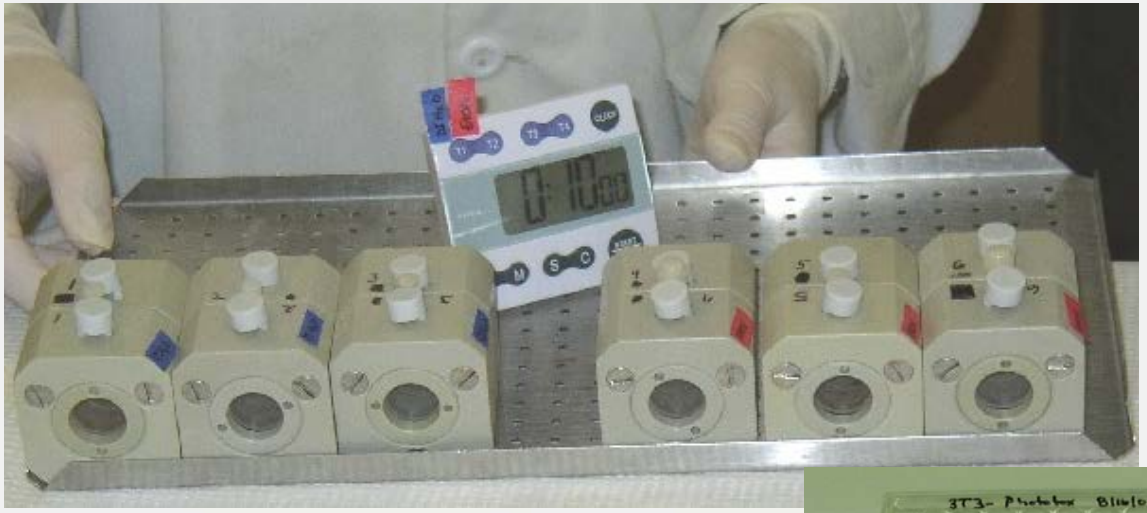
Test System Identification



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Test System Considerations

- Justification for use
- Show that each lot is performing within normal limits
- Assign normal limits dependent on the assay endpoints

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Cell Considerations

- Source – of purchase and of cells
- Identification of test system
  - Mycoplasma testing
  - Maintenance at levels under complete confluency (i.e. prevent differentiation)
- In house cell line log records

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Basic Quality Control of Cells and Reagents

- Purchase from reputable sources
- Verify species and tissue of origin (HeLa?)
- Verify state of differentiation (if appropriate)
- Segregate medium and reagents
- Test for mycoplasma and other bacterial/fungal contamination

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Mycoplasma Infected Cells

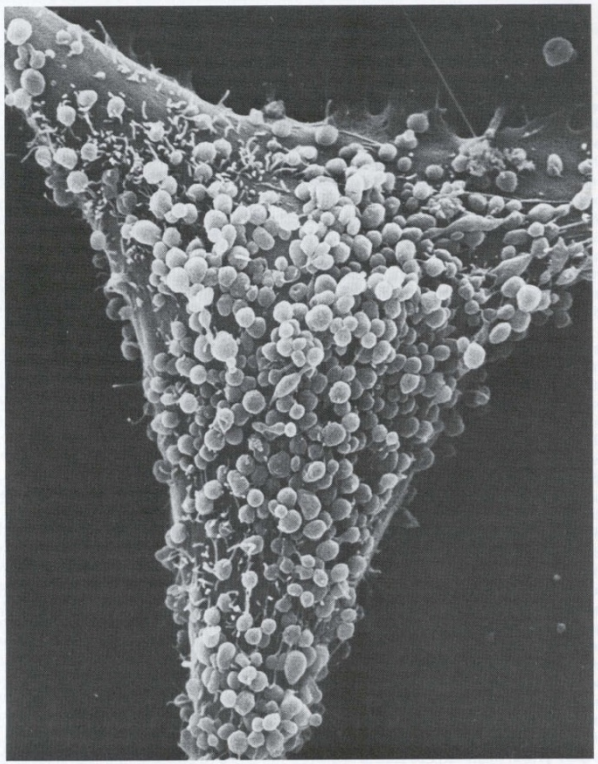
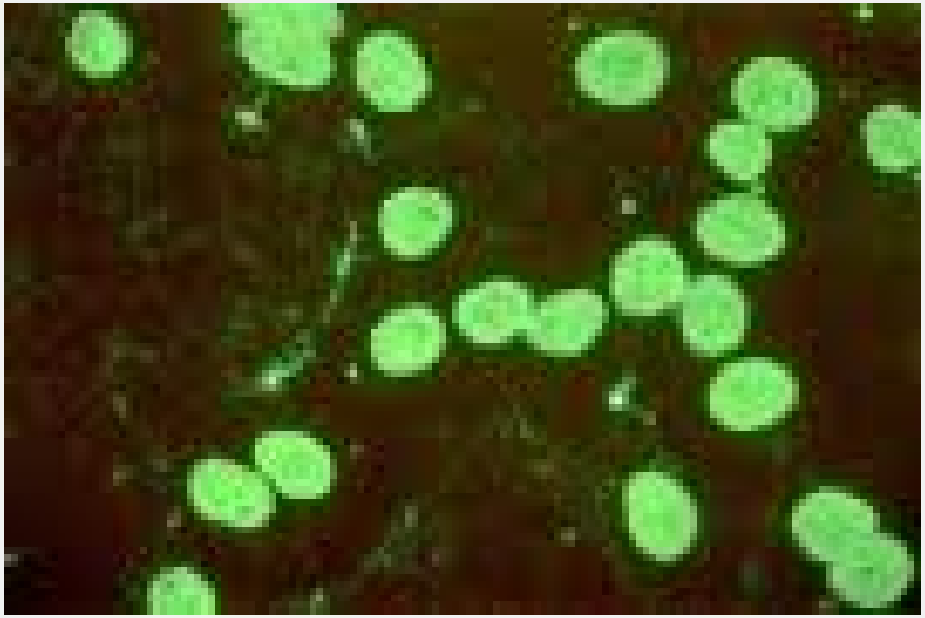


FIG. 1. Mouse tumor A-9 cell line infected with *Mycoplasma hyorhinis*. Photo courtesy of David M. Phillips, Population Council.



McGarrity G.J., et al (1984) Cytogenetic effects of Mycoplasma infection of cell cultures: A review. In Vitro 20:1-18.

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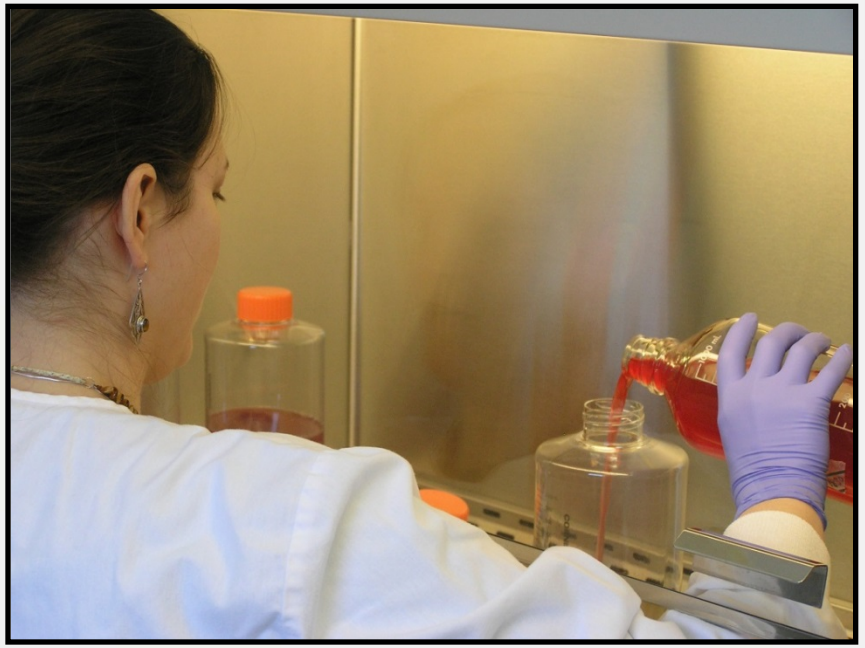
Sources of Mycoplasma in the Cell Culture Laboratory

- Cell Culture Reagents
  - Serum
  - Media
  - Trypsin
  - Growth factors
- Personnel
- Equipment
- Donor Tissue
- Contaminated cultures in the laboratory



3D Tissue Construct Considerations

- Functional Characterization – required performance standards for assay
- Shipping Validation

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Rational for Instituting an *In Vitro* Test System Supplier Qualification Program

- Users of the test systems are responsible for ensuring that they are of a suitable quality to assure that the data received are reliable and reproducible.

- The *in vitro* testing community itself is the most useful body to insist that suppliers provide a defined state of quality

- Decisions are made based on the data gathered from these systems

- A great deal of time and money is invested in developing *in vitro* methods utilizing these test systems. Test system quality should remain reliable and consistent.

- A potential source of assay variation can be controlled through consistent test system manufacturing practices.

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Future Test Systems?

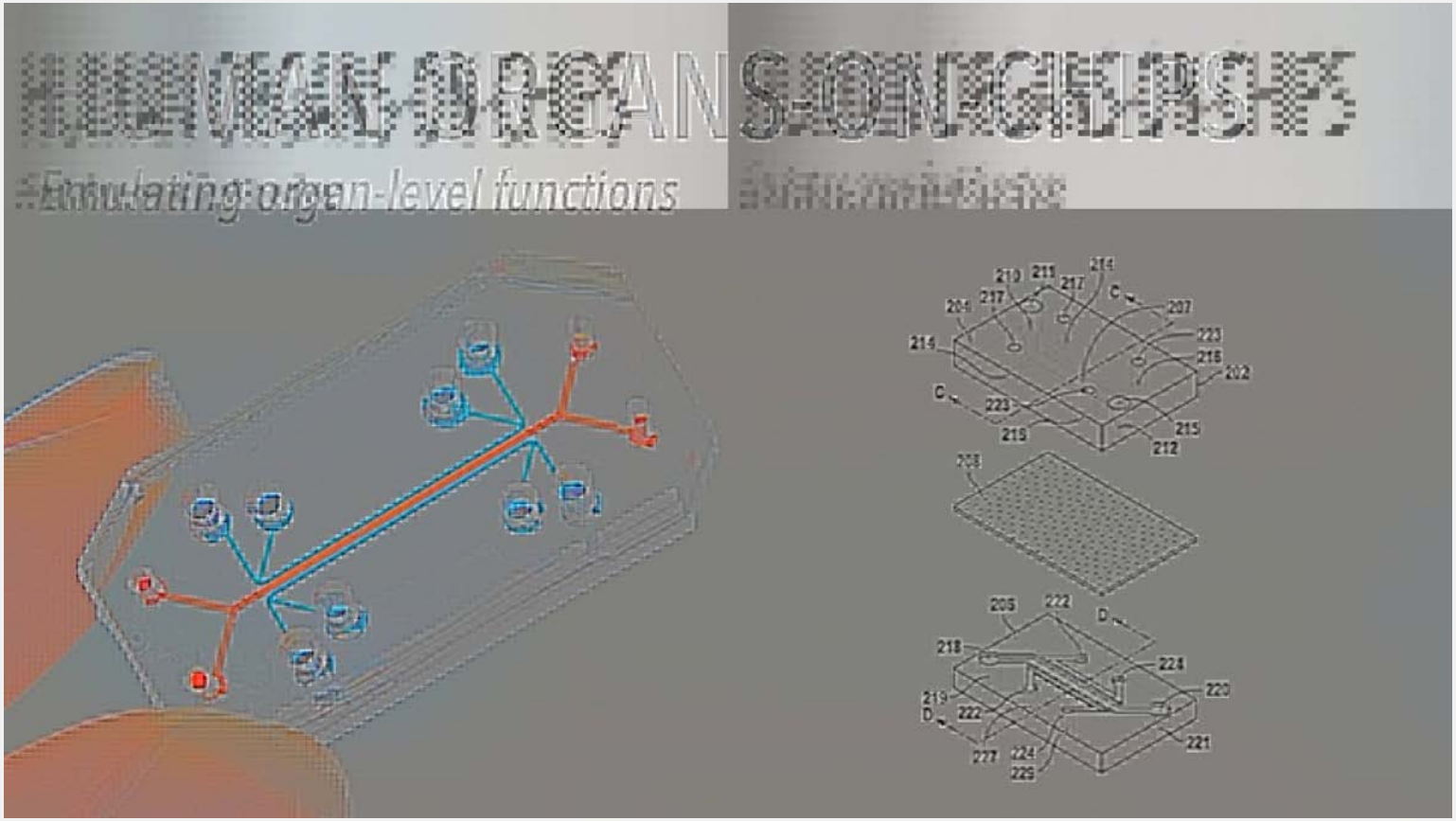

Image from Harvard's Wyss Institute website

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Future Test Systems

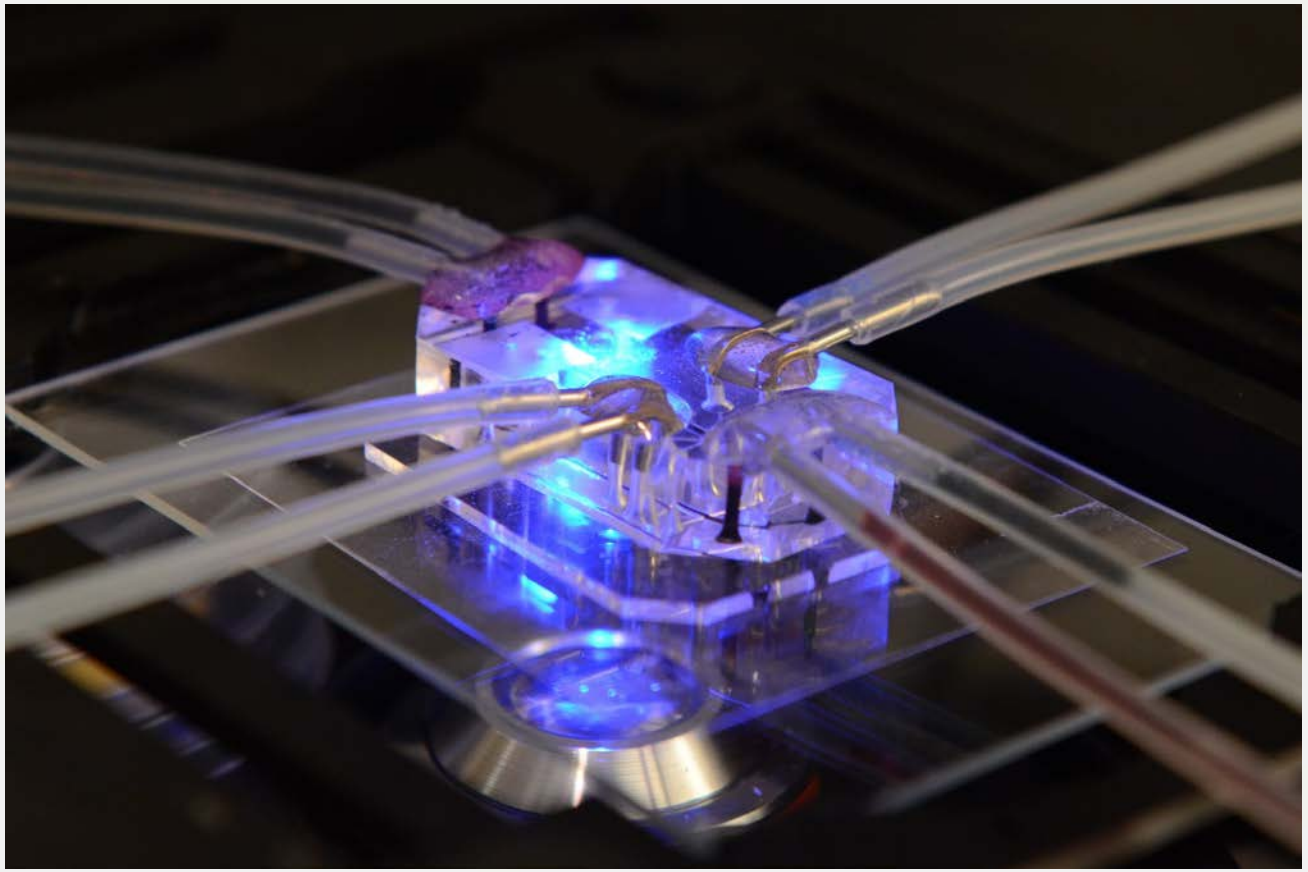

Image from Harvard's Wyss Institute website

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Future Test Systems

What about multiple cell types in the same platform for analysis together?

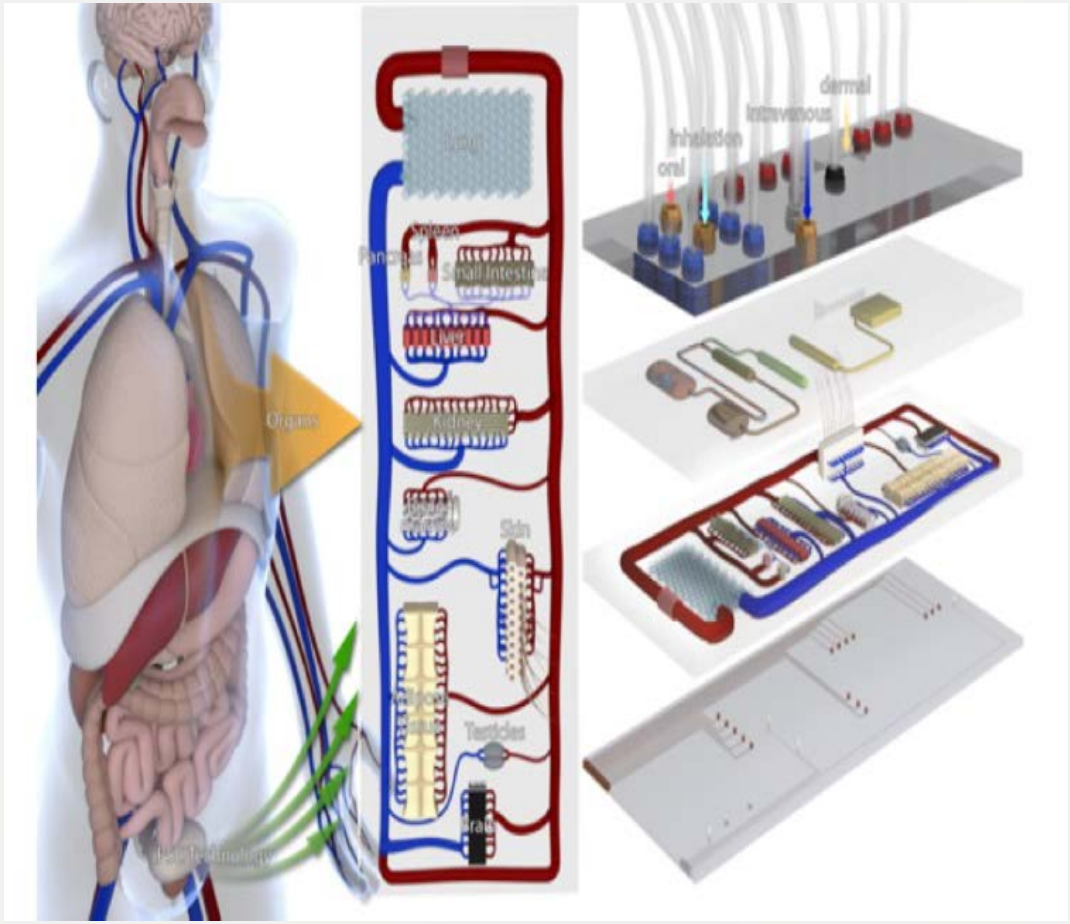

Image from TissUse GmbH website

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Definition of Test System

58.3(i) *Test system* means any animal, plant, microorganism, or subparts thereof to which the test or control article is administered or added for study. *Test system* also includes appropriate groups or components of the system not treated with the test or control articles.

Suggestion - *Test system* means any animal, plant, microorganism, or subparts thereof **presented in its finalized platform or assay conditions** to which the test or control article is administered or added for study. *Test system* also includes appropriate groups or components of the system not treated with the test or control articles.

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Responsibilities of the Users

- Using a defined study protocol and utilizing the test system correctly
- Training the technical staff
- Documenting each stage in the study's execution
- Establishing and following defined acceptance criteria
- Employing concurrent controls and benchmarks

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Thank You

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