# *In vitro* Toxicity Evaluation
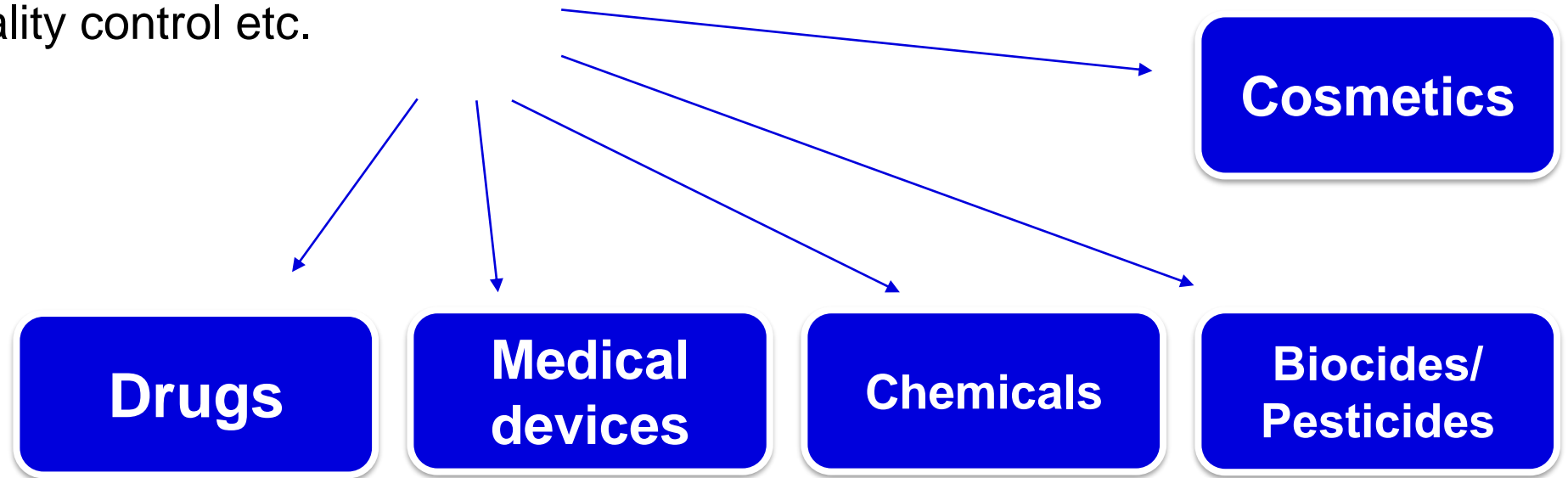
Toxicology Seminar
2021

# Evaluation of toxicity - Purposes
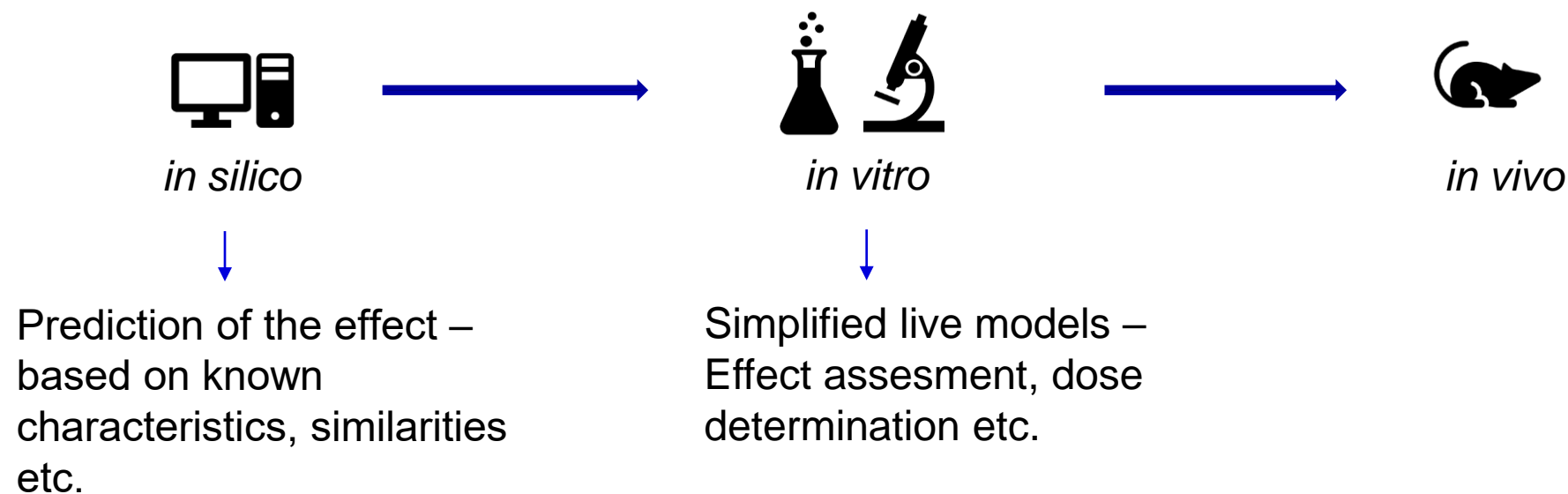
– **Predictive/experimental toxicology**

- ▪ The development, manufacture, quality, effectiveness and safety testing of drugs, foodstuffs and other substances or products

- ▪ The avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality or their effects on people, animals or plants

- ▪ The protection of the natural environment in the interests of the health or welfare of man or animal

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# Evaluation of toxicity

Part of many processes: in the development, registration,

classification, quality control etc.

**Cosmetics**

**Drugs**

**Medical devices**

**Chemicals**

**Biocides/ Pesticides**

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# Position of *in vitro* methods among the processes of toxicity testing



*in silico*           *in vitro*           *in vivo*

Prediction of the effect – based on known characteristics, similarities etc.

Simplified live models – Effect assesment, dose determination etc.

Example of testing strategy – e.g. skin sensitization test

a. *in silico* (structure characteristics, pKa, log P etc.)
b. *in vitro* – human skin models
c. *in vivo* – animal model – usually albino rabitt

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# 3R Principle

**Replacement**

= to replace *in vivo* models by alternative methods e.g. using *in vitro* models

**Reduction**

= to reduce the number of animal models used in the experiments

**Refinement**

= efforts to reduce the painful or stressful procedures

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# *In vitro* models

= living system simplified as compared with the *in vivo* model


Types of *in vitro* models

- Subcelular models (e.g. isolated mitochondria)

- **Cell cultures**

- Tissue cultures

- Isolated organs


- 2D vs. 3D models

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# *In vivo*  x  *in vitro* models

|  | **++** | **--** |
|---|---|---|
| ***In vivo***  | Possible toxicokinetic testing<br><br>Monitoring of the effect of systemic regulation<br><br>In vivo models can not be fully eliminated !! | Financial aspects, time-consuming<br><br>Ethical aspects<br><br>Interindividual differences |
| ***In vitro***  | Testing a larger number of compounds in a short time-period<br><br>Plenty of biological material as a model<br><br>Reproducibility<br><br>Possibility of using human cell cultures<br><br>Determination of organ-specific toxicity (eg. hepatotoxicity, nephrotoxicity etc.) | No information about systemic regulation<br><br>For the replacement of in vivo model validation techniques are required<br><br>The problem with the extrapolation of data<br><br>Not all cell types can be cultured *in vitro*<br>Culturing under non-physiological conditions (culture media, cell lines in the absence of tissue context) |

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# Cell cultures

= population of isolated cells (plant, animal; human cells as well)

Types of cell cultures:

**Primary cell culture**
First culture of isolated cells from tissue (mostly by enzymatic loosening)
Culture is contaminated by other cell types
Most closely related to the physiological condition of the body
Short lifespan (only few days)

**Secondary cell cultures**
Derived from primary cultures
Cultures of normal diploid cells
Viable for 40-50 division

**Cell lines**

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# Cell line

- Permanent cell lines

   (immortalized – they will proliferate "indefinitely")

- Single cell type

- Fully adapted to *in vitro* conditions

- They are derived from **tumor cells** or transformed from normal cells by physical or chemical mutagens


- Sources cell lines:

  - ATCC (American Tissue and Cultures Collection)

  - ECACC (European Collection of Cell Cultures)

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# *In vitro* cell culture model for the evaluation of toxicity

— **One cell type** – homogenous properties e.g

   expression of specific receptors, overexpression of cell

   cycle regulators etc.

— Enable us to study **the molecular basis of the toxic**

   **effect** – how the potential toxic substances affect their

   biological targets

— The results correspond to the effect of the substances

   without any interactions with other cell types or tissues

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# Resulting parameters

| Inhibitory concentration | Lethal concentration | Effective concentration | Toxic concentration |
|:---:|:---:|:---:|:---:|
| $IC_{10}$ | $LC_{10}$ | $EC_{10}$ | $TC_{10}$ |
| $IC_{50}$ | $LC_{50}$ | $EC_{50}$ | $TC_{50}$ |
| $IC_{90}$ | $LC_{90}$ | $EC_{90}$ | $TC_{90}$ |

- Enable us to compare the effect of different substances
- Depend on the time of exposition, selected model etc.
- *Dose-response curve*

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# Cytotoxicity

- It means the response of cells to the effect of toxic substance

- Cytotoxic effect:
  - Changes of cell morphology (augmentation, multinuclear cells, granularity of cell surface etc.)
  - Changes in cell metabolism
  - Inhibiton of proliferation (changes in cell cycle progression etc.)
  - Cell death (apoptosis, necrosis, autophagy etc.)
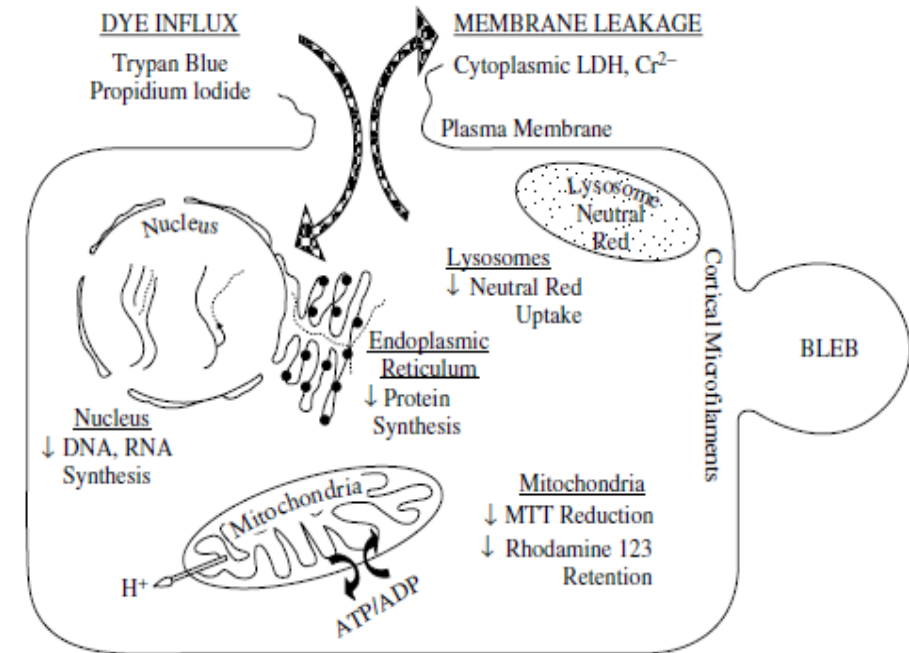
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# Methods for evaluation of cytotoxicity

→ Determination of the **endpoints**

| |
|---|
| Changes in membrane integrity |
| Inhibition of DNA synthesis |
| Deffects in metabolic functions |
| Impaired synthesis of other key cell components |

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# Dye-exclusion test



- Evaluating of the cell viability using intracellular cell dyes e.g. erythrosin B, tryptan blue, neutral red
- Viable (live) cells stay unstained x dead cells are stained
  ⟶ because of the penetration of the dye through impaired cell membrane
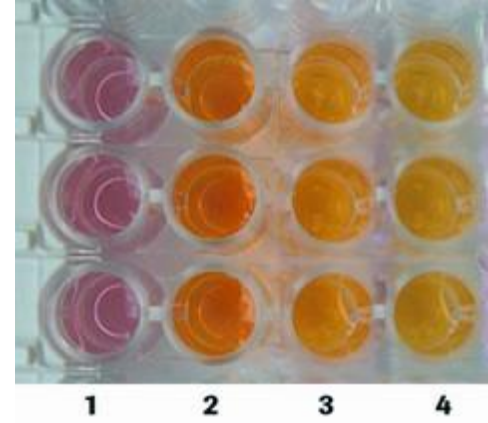- Hemocytometer (e.g. Bürker chamber) + microscope

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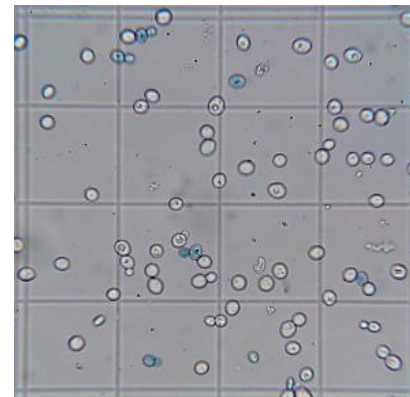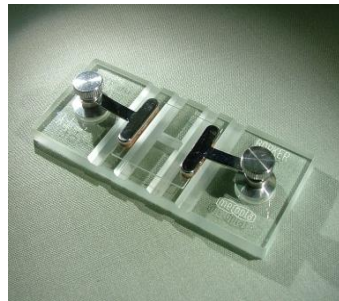
# Cell viability

- Another method:
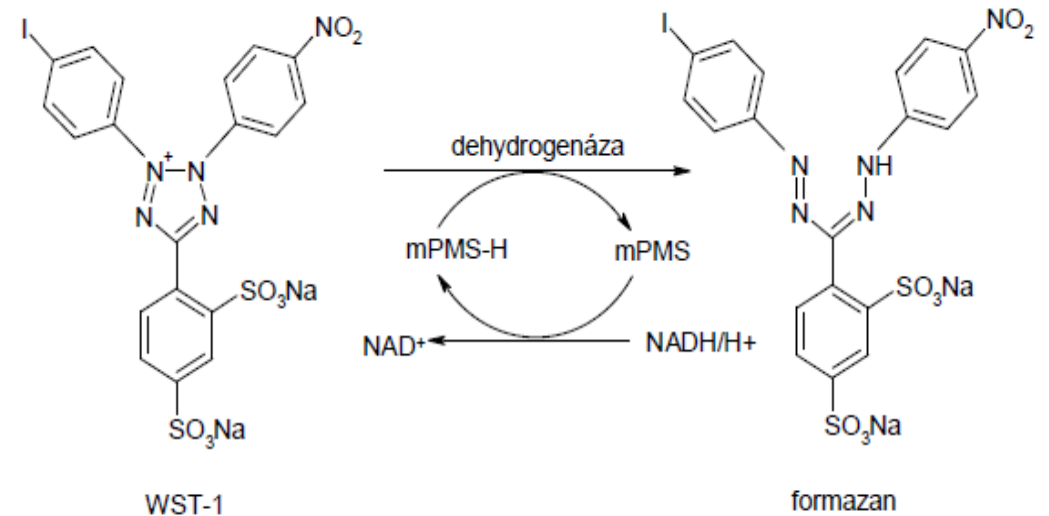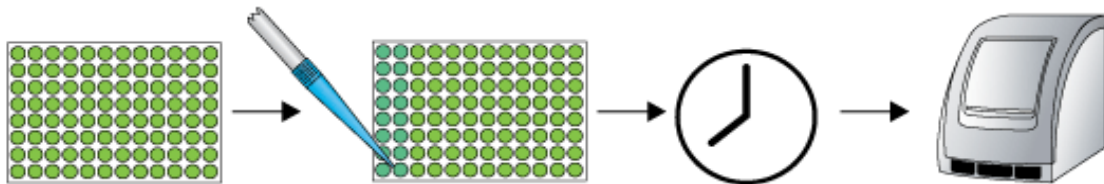  - **Lactate dehydrogenase (LDH) analysis** – impaired membrane integrity, then we can detect LDH extracellularly

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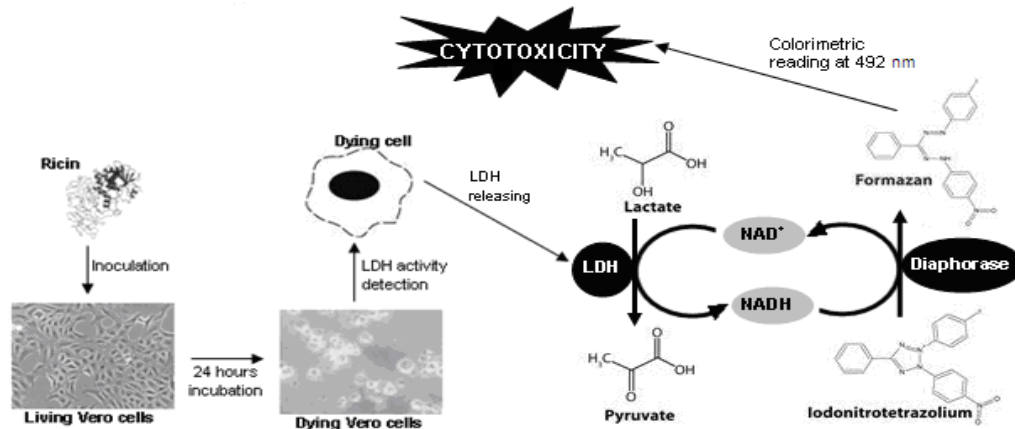
# Cell viability and proliferation



– **Tetrazolium salts**
  – Enzymatic reduction of TS (changes of the color) – changes of the color correlate with the intensity of cell metabolic activity

– MTT; XTT; WST-1 analysis

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# *In vitro* toxicity tests – validated methods

Validated by **OECD**

(Organisation for Economic

Co-operation and

Development)

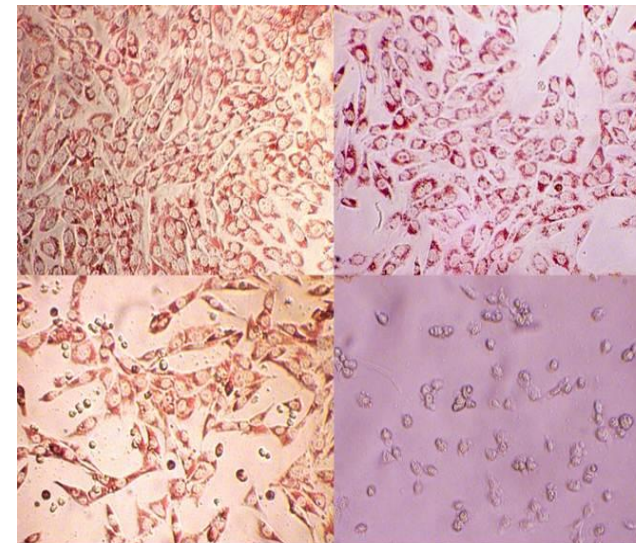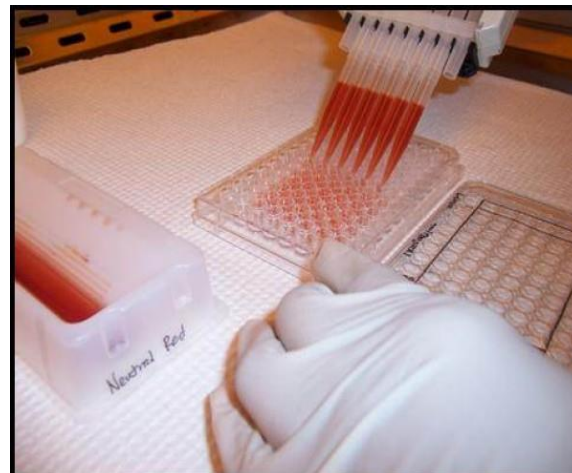  &ndash; Cytotoxicity

  &ndash; Genotoxicity

  &ndash; Eye irritation

  &ndash; Phototoxicity

  &ndash; Cardiotoxicity

  &ndash; Nephrotoxicity

  &ndash; Hepatotoxicity

  &ndash; Reproductive toxicity

  &ndash; Ecotoxicity

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# Cytotoxicity

– E.g. Biological evaluation of medical devices

= Tests for *in vitro* cytotoxicity

– Fibroblast cell line 3T3 - Neutral Red Uptake Test (NRU)

– Incorporation of NR into the lysosomes of live cells – spectrofotometrical determination of changes in color intensity
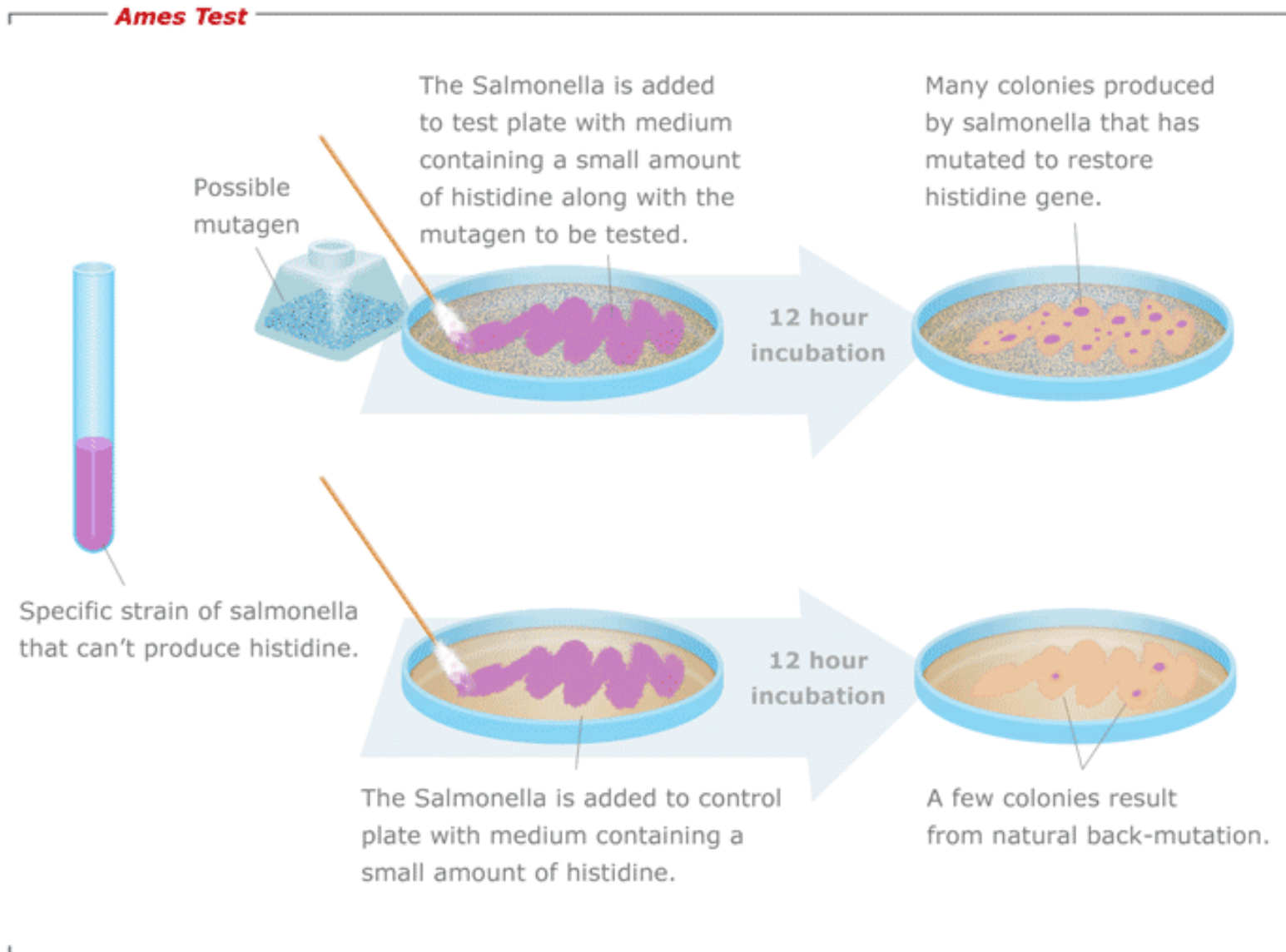
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# Genotoxicity

— Principle:  detection of mutation – DNA damage (changes in genetic information)

Standard testing series:

— The bacterial reverse mutation test **(Ames Test)**

— *In Vitro* Mammalian Chromosome Aberration Test (changes in cell ploidy, detection of polyploidy)

— *In vitro* mammalian cell gene mutation test

— Etc.

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# Ames test



Ames Test

Possible mutagen

The Salmonella is added to test plate with medium containing a small amount of histidine along with the mutagen to be tested.

Many colonies produced by salmonella that has mutated to restore histidine gene.

12 hour incubation

Specific strain of salmonella that can't produce histidine.

The Salmonella is added to control plate with medium containing a small amount of histidine.

A few colonies result from natural back-mutation.

12 hour incubation

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# Eye Irritation/Corrosion

– Methods for testing the effect of potential ocular corrosive or irritant substances

**Examples of *in vitro* test methods:**

– **BCOP** (Bovine Corneal Opacity and Permeability)

– **ICE** (isolated chicken eye) test

– **HET-CAM** (Hen´s Egg Test - Chorioallantoic Membrane)

  – Fertilized eggs – substances are applied on the membrane – changes are detected
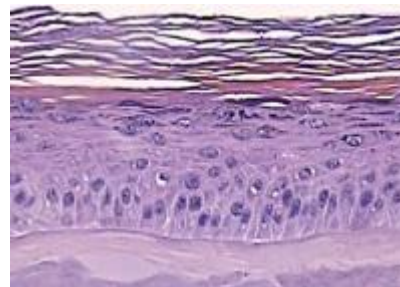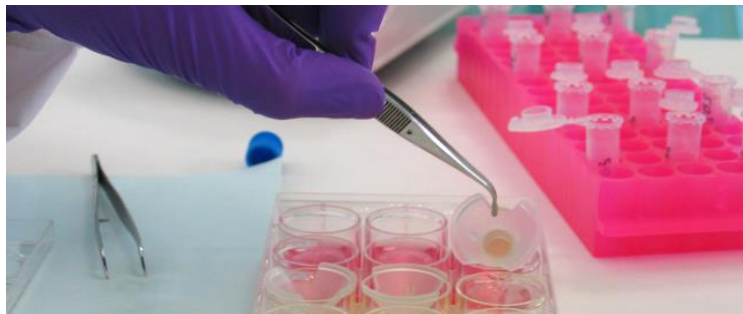  – e.g. hemorrage, koagulation, lysis …

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## *In vitro*
## Skin Corrosion Test: human skin model

– Episkin, Epiderm, SkinEthic....

– (reconstructed human epidermis – human keratinocytes – with functional stratum corneum)

Principle:

– Application of tested substance on the surface of human skin model - MTT analysis – detection of changes in cell viability

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# Thank you for your attention