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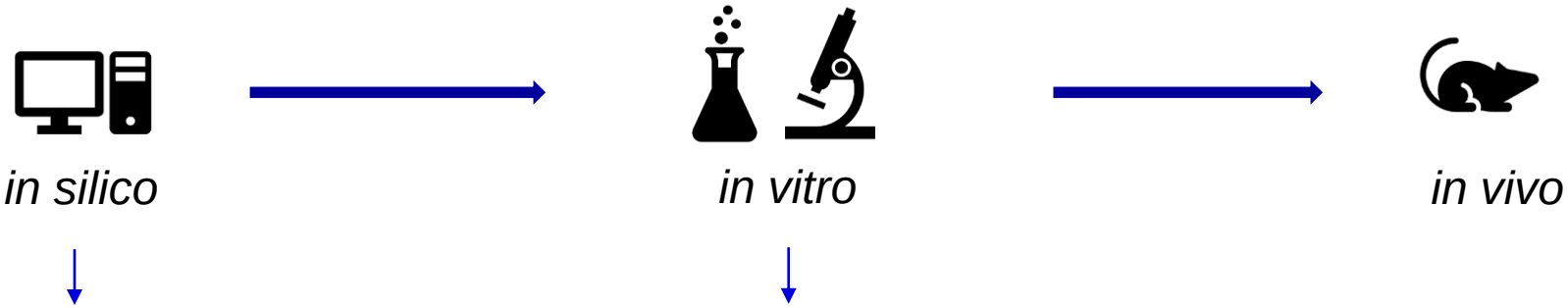
In vitro Toxicity Evaluation

Toxicology Seminar
Autumn 2022

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



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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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 modely

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In vivo x *in vitro* models

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

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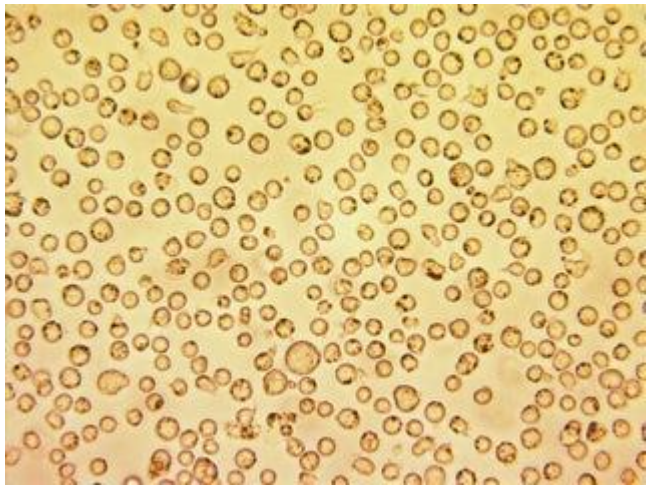
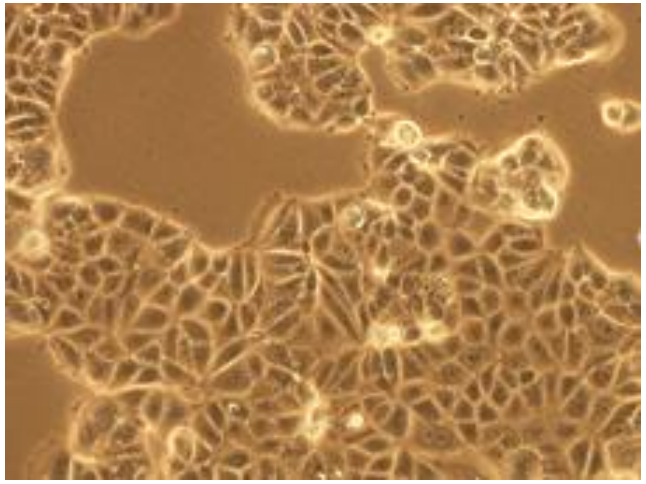
Cell line

- 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)

In suspension x Adherent





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Culture conditions

- !! Sterile conditions !! Risk of contamination
- Laboratory equipment
  - incubator, flowbox etc.

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Culture conditions

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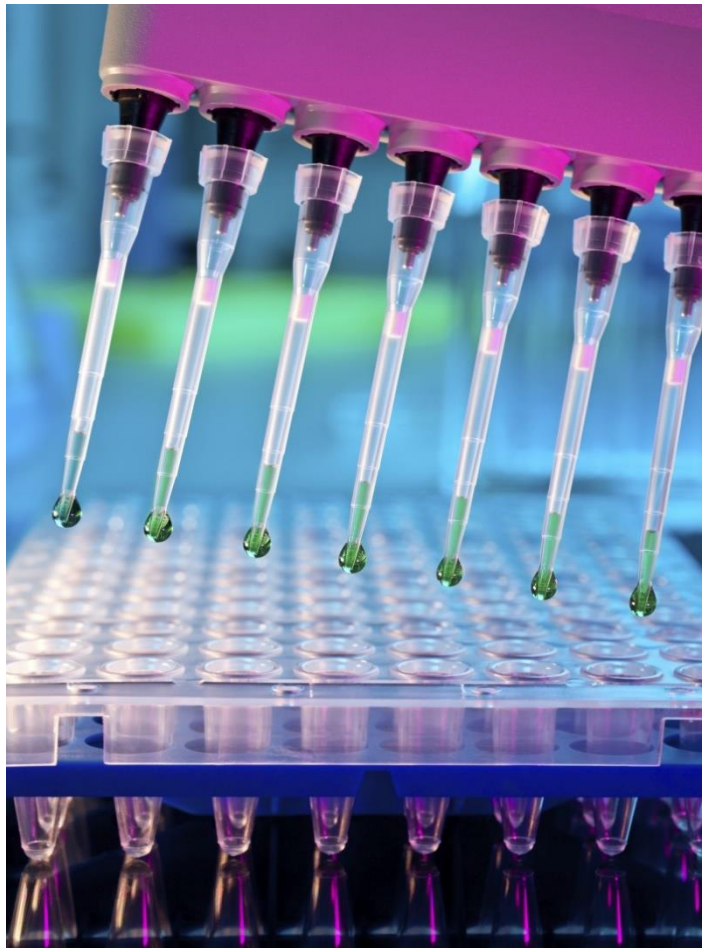
In vitro cell culture model for the evaluation of toxicity

- **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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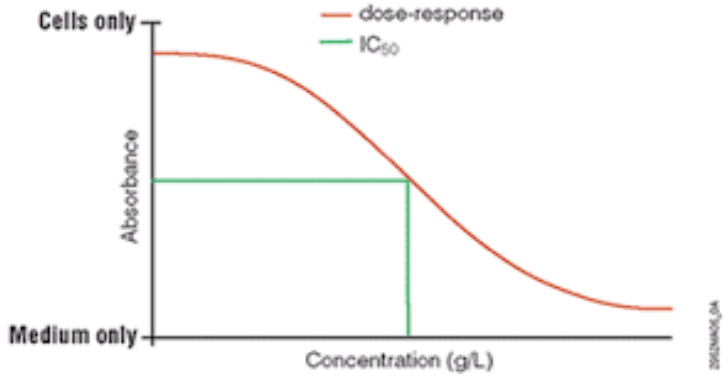
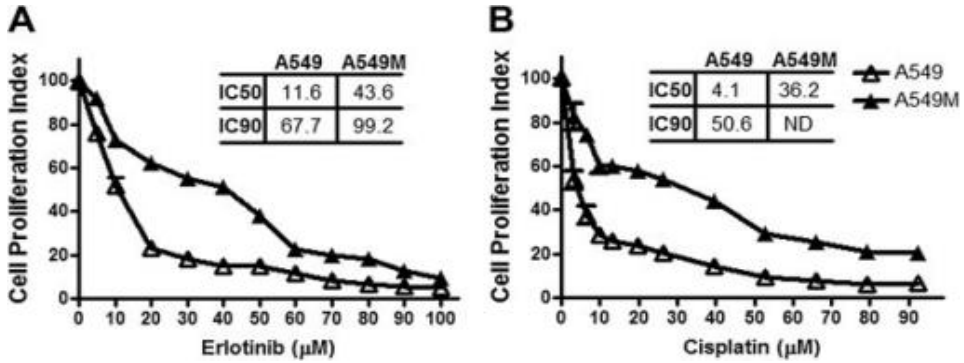
Resulting parameters



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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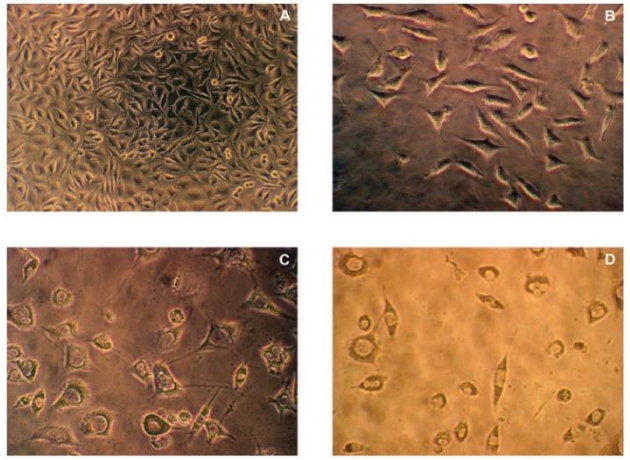
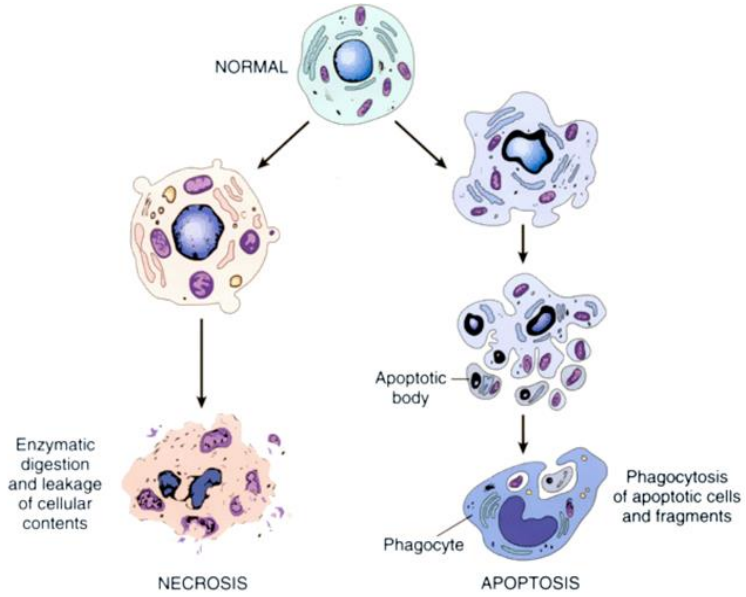
Cytotoxicity

- 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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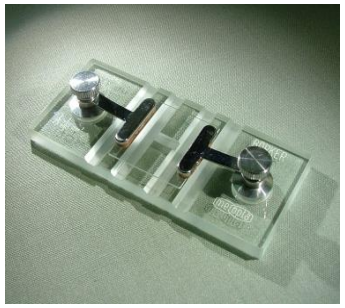
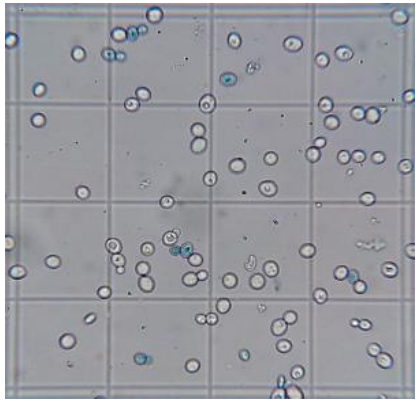
Dye-exclusion test

- Evaluating of the cell viability using intracellular cell dyes e.g. erythrosin B, trypan 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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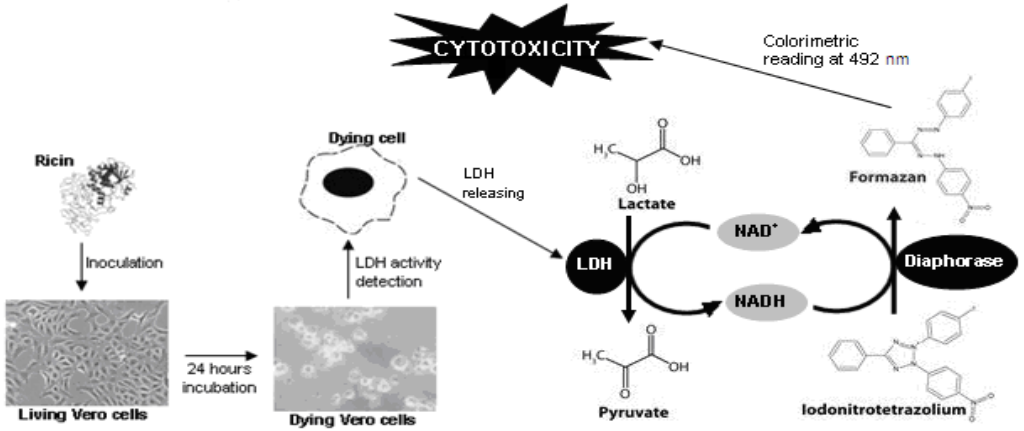
Cell viability

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



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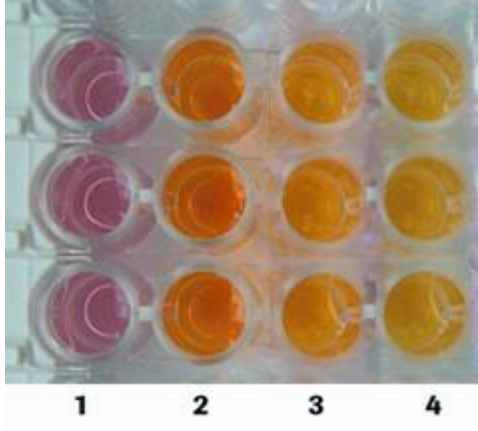
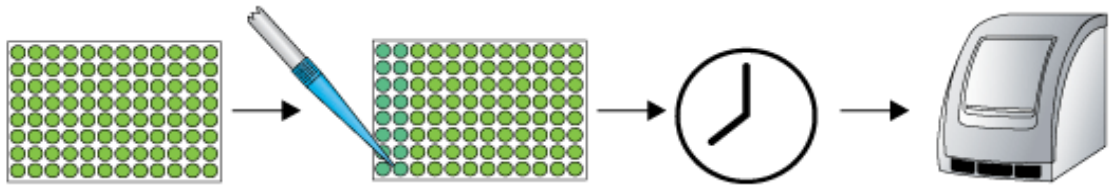
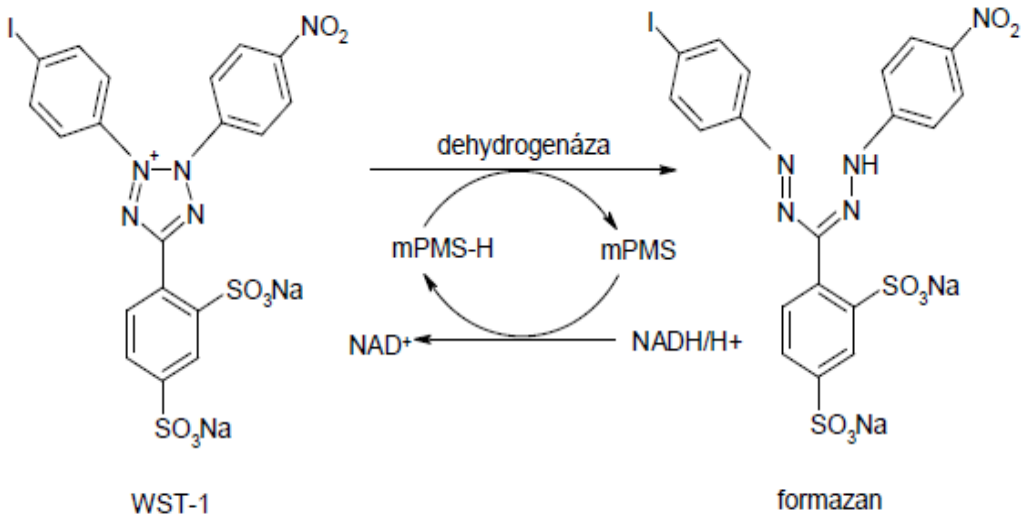
Cell viability and proliferation



- **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)

- Cytotoxicity
- Genotoxicity
- Eye irritation
- Phototoxicity
- Cardiotoxicity
- Nephrotoxicity
- Hepatotoxicity
- Reproductive toxicity
- Ecotoxicity

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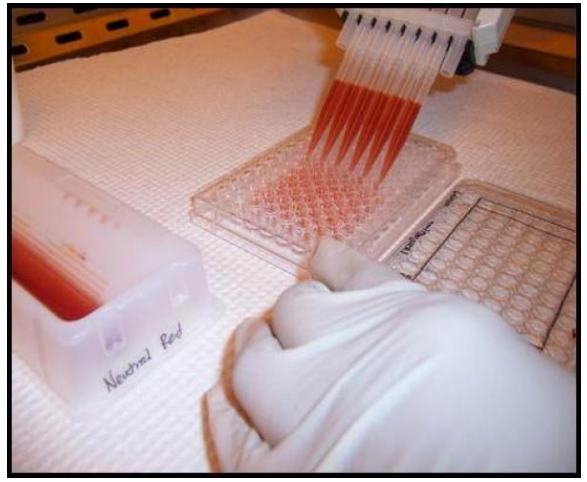
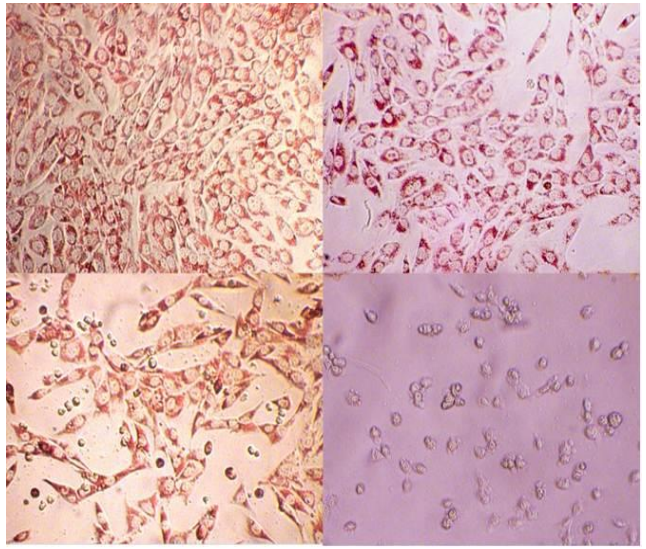
Cytotoxicity

- 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)

<u>Standard testing series:</u>

- 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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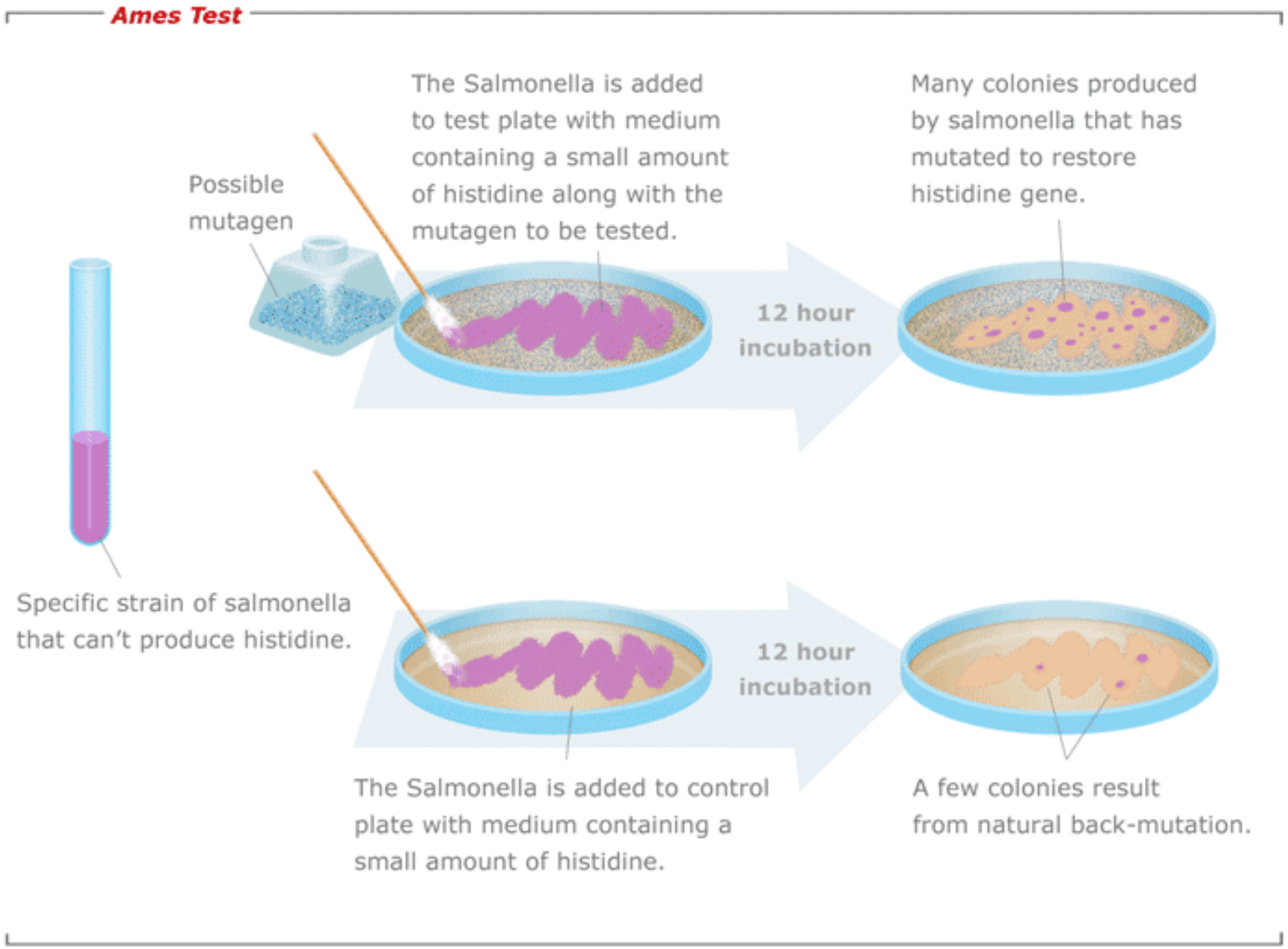
Ames test



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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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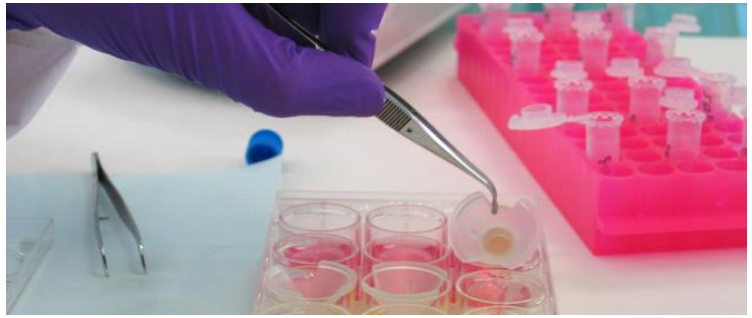
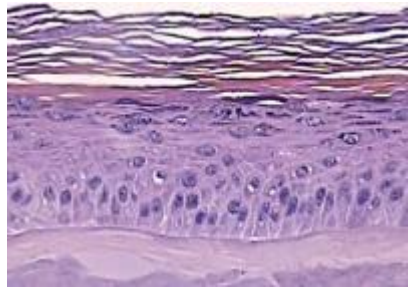
In vitro
Skin Corrosion Test: human skin model

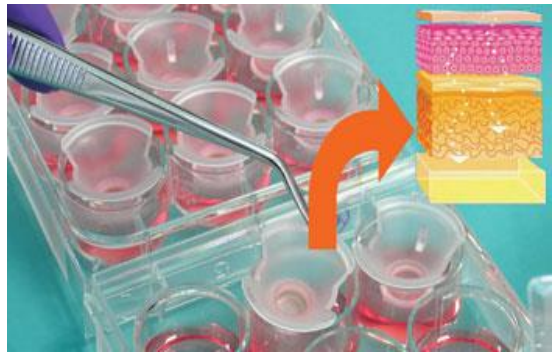
- Episkin, Epiderm, SkinEthic....
- (reconstructed human epidermis – human keratinocytes – with functional stratum corneum)

Principle:

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







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Reproductive toxicity

Embryonic Stem Cell test (EST test)

= evaluation of embryotoxic potencial of tested substances

Principle of the test: determination of inhibited differentiation of embryonic stem cells (ESC) and inhibition of cell proliferation (ESC and 3T3 fibroblasts)

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Thank you for your attention