

# Tierversuchsfrei: Nachweis der Biokompatibilität in vitro

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# Tentamus – who we are

1.600	Employees worldwide
1.200.000	Samples worldwide/year
15.000	Audits worldwide/year
40	Laboratories worldwide

## Analytics and consultancy in the fields of :

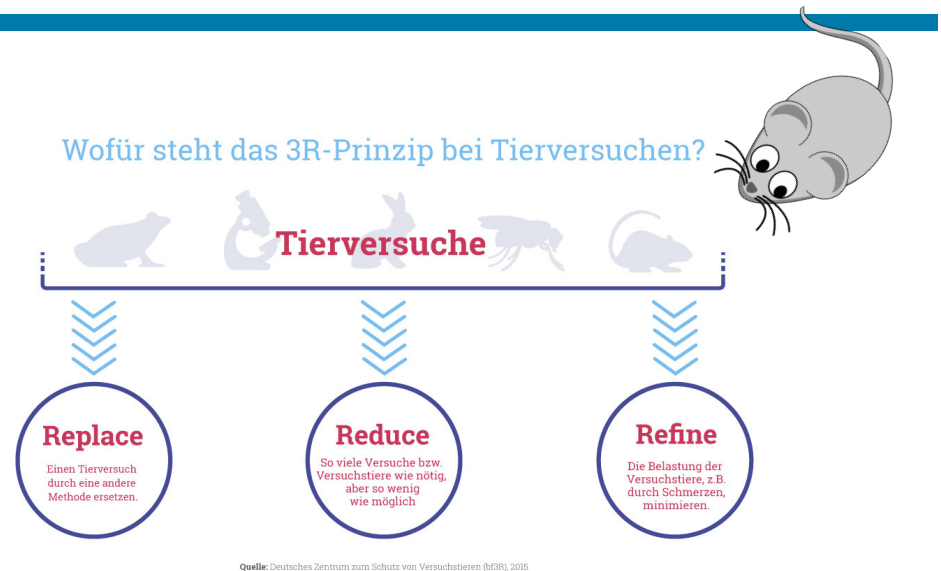
- Pharmaceuticals
- Medicals
- Cosmetics
- Environment
- R&D



# General Intention

## In Vitro we trust!

- Increased need for alternatives to animal testing
- Animal experiments are ethically problematic and expensive and
- In vitro methods offer reliable and validated results which diminishes the need for animals and saves costs
- According EURL-ECVAM concept (3R): Replace, Reduce, Refine



# MicroMol – short history

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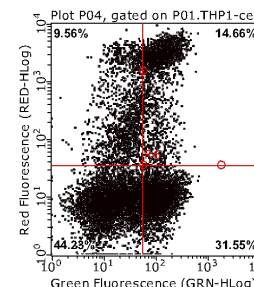
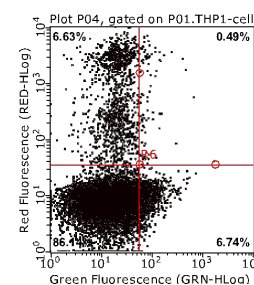
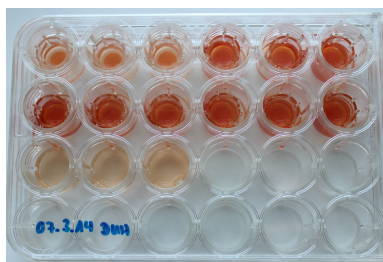
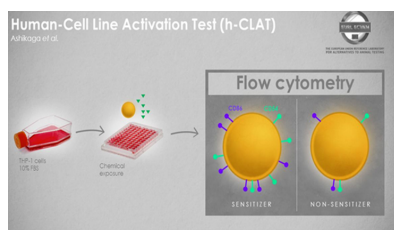
- Founded in 1996
- Spin off from the TH Karlsruhe
- Traditional focus: Microbiology / R&D / Food Analytics
- Research Lab → Contract Research Organization
- Additional issues: Cell- / Immune Analytics / Biocomp
- ISO 17025 accredited / GMP certified
- GLP is on the way (Q1 / 2019)
- 2018 → joins the Tentamus Group





# Biocompatibility

- Biocompatibility is defined as the ability of a material to have no undesirable or systemic effects in the recipient (humans / animals)
- Biocompatibility testings are usually performed according to international guidelines (ISO 10993 / OECD / Eupharm. / USP)
- Based on these guidelines tests are classified according to product application (skin, eye, body..)
- MicroMol offers a broad range of biocompatibility testings according to approved guidelines (ISO10993 / Eu.Pharm / OECD) for **medical devices, cosmetics and chemicals**



## Applications

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- **Medical Devices:** skull pins, surgical forceps, medical plastics, dental material, medical tapes
  - **Cosmetics:** shampoo, creams
  - **Chemicals** (cleaning agents, pharmaceuticals, more...)
  - **Clothes** / uniforms with skin contact
  - **Disinfectants** (corrosion)
  - **Articles of daily use**
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# Biocompatibility Tests - Overview

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- **Skin Analytics**
    - Cytotoxicity in vitro (DIN ISO 10993-5)
    - Skin irritation (DIN ISO 10993-10 / OECD TG 439)
    - Skin sensitization (DIN ISO 10993-10 / OECD TG 442d/e)
    - Skin corrosion (OECD TG 431)
  - **Eye irritation / corrosion**
    - Fluorescein leakage test (OECD TG 460)
    - Human corneal epithelium test (OEC TG 492)
  - **Genotoxicity**
    - AMES Test (DIN ISO 10993-10 / OECD TG 471)
    - Micronucleus Test (OECD TG 487)
    - Mammalian cell gene mutation test (OECD 476)
  - **Pyrogen analytics**
    - Monocyte activation test (MAT) (EP 2.6.30 / USP 151)
    - Endotoxin (LAL-Test) (EP 2.6.14 / USP 85)
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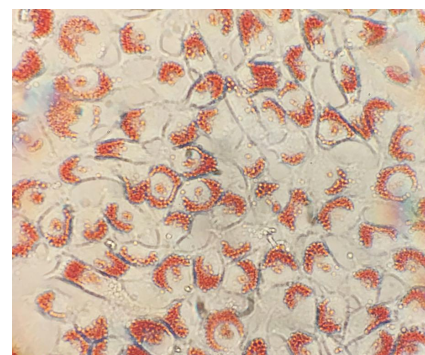
# Cytotoxicity in vitro (ISO 10993-5)

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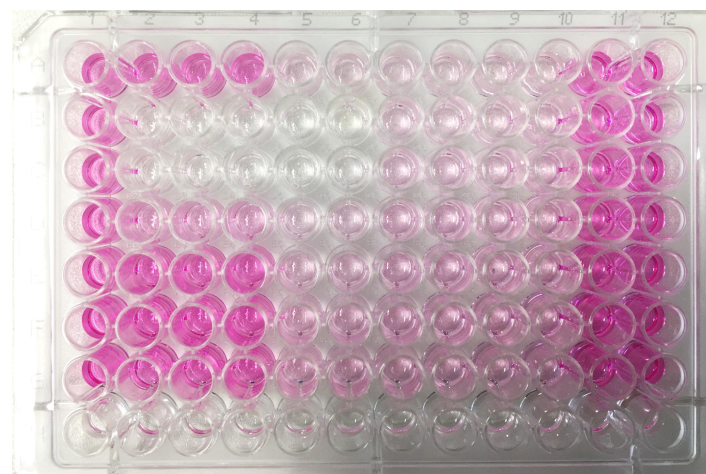
# Cytotoxicity

Basal test to evaluate the cytotoxic effect of chemicals, medical devices, pharmaceuticals and articles of daily use. Measures Cell viability / proliferation upon sample confrontation.

- Various platforms / cell types (e.g. L929 fibroblasts)
- ISO 10993-5, Annex A: **Neutral Red**
- ISO 10993-5, Annex C: **XTT**
- Classification due to grade of cytotoxicity



*Neutral red inclusions*



*Neutral read out plate*

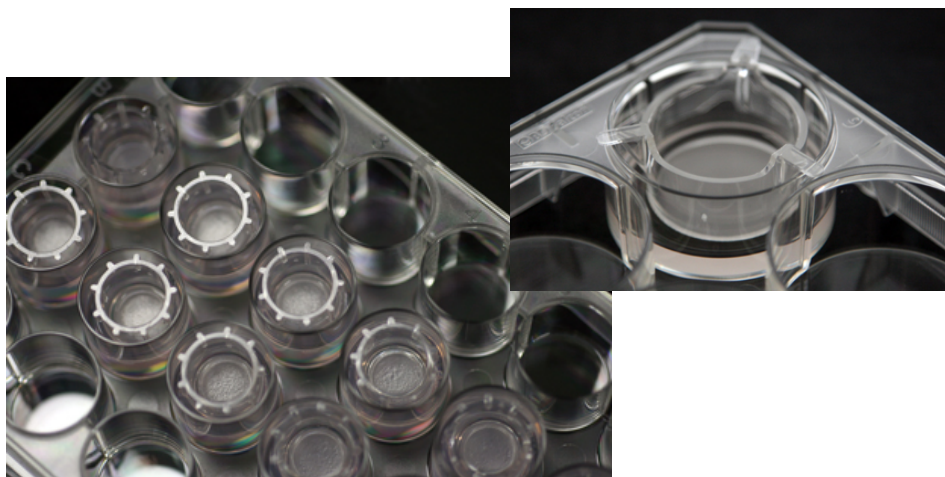
# Skin and Eye irritation / corrosion

(ISO 10993-10 / OECD 439 / 431 / 460 / 492)

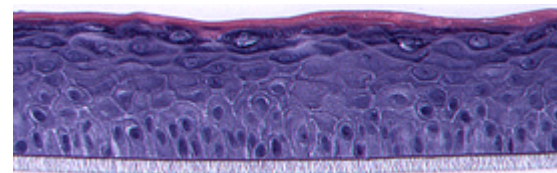
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# Skin and Eye irritation / corrosion

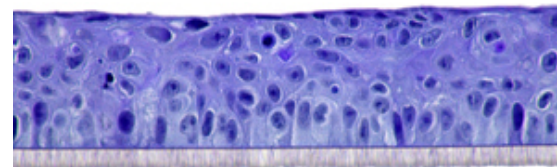
- 3D EpiSkin model: reconstructed human epidermis (RHE) from normal human keratinocytes and reconstructed human cornea-like epithelium
- Testing of medical devices, chemicals and cosmetics to classify their irritant or corrosive potential
- Read out: viability staining (MTT)
- Distinguishes irritant / corrosive vs non-irritant /-corrosive substances



<http://www.episkin.com>



reconstructed human Epidermis (in **vitro skin irritation / corrosion**)



reconstructed human Cornea-like Epithelium (in **vitro eye irritation**)

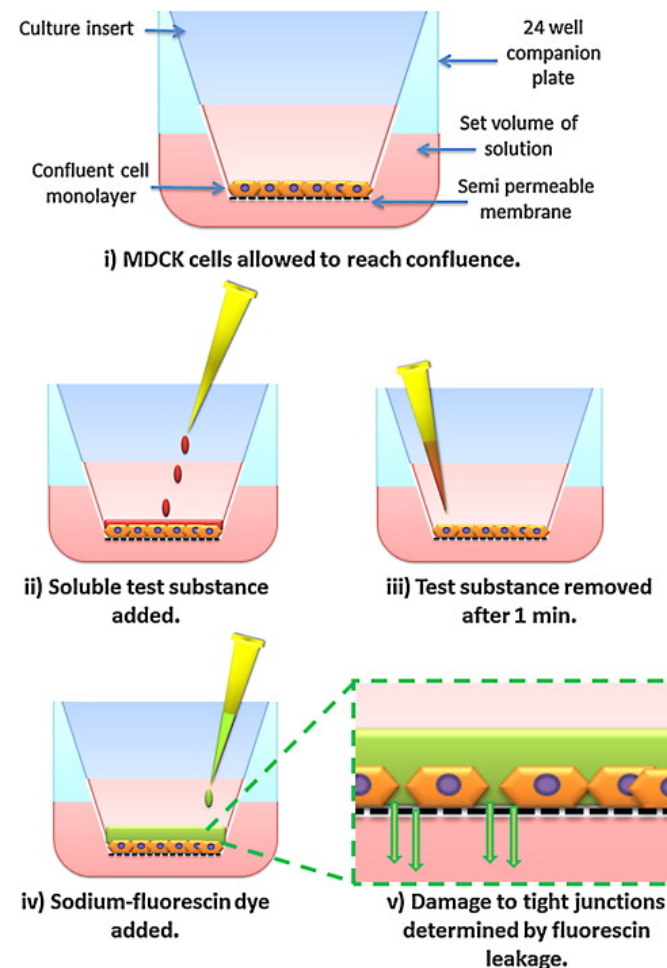
<http://www.episkin.com>



# Eye irritation / corrosion

## OECD 460: Fluorescein leakage test

- Method for Identifying Ocular Corrosives and Severe Irritants
- MDCK cells simulate eye epithelium
- form a very dense epithel-like cell layer
- Upon contact with irritative/corrosive substances loss of cell integrity
- Enables fluorescent dye to pass cell layer



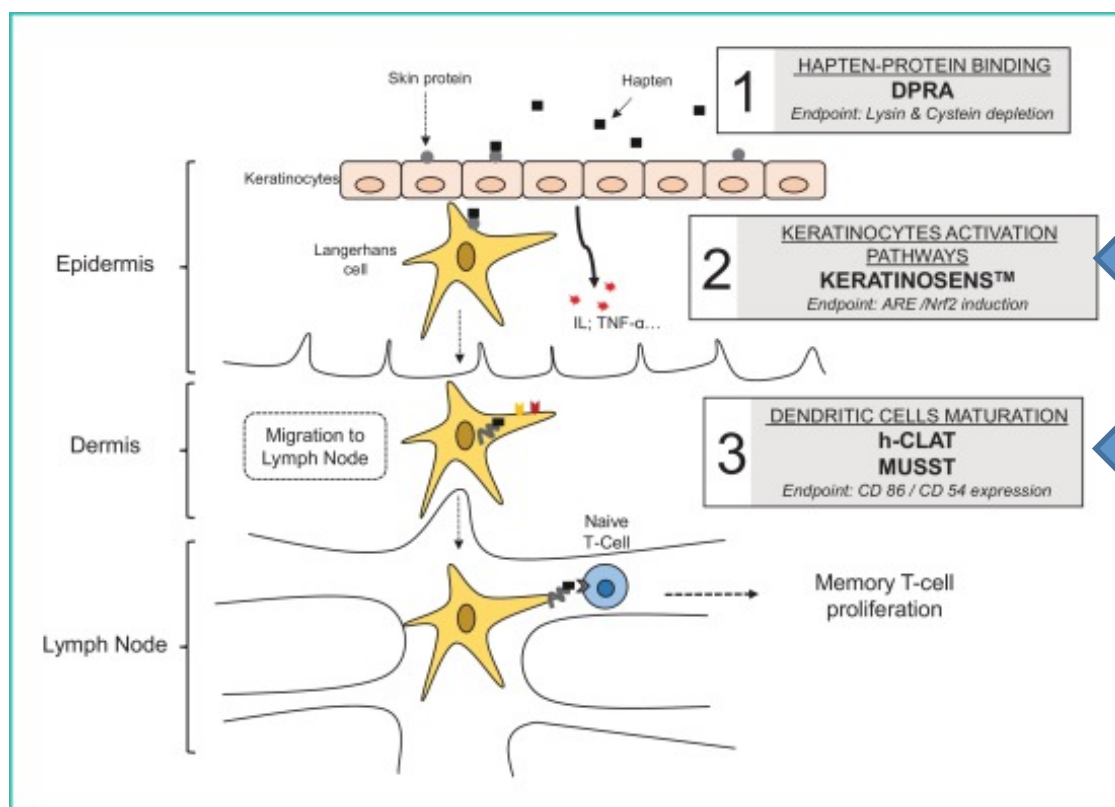
# Skin sensitization

(ISO 10093-10: OECD TG442d/e)

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# Skin analytics

## Mechanism of skin sensitisation – allergic contact dermatitis (ACD)



1st key event  
(Hapten binding)

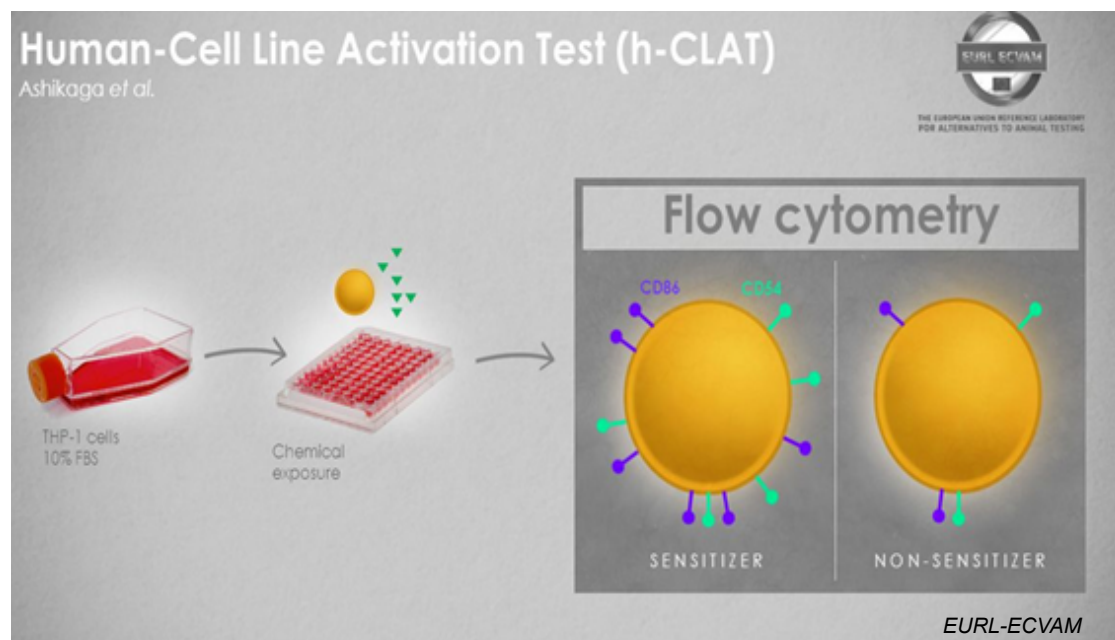
2nd key event  
(Keratinocyte activation)

3rd key event  
(activation of dendritic cells)

4th key event  
(T-cell activation)

# h-CLAT test

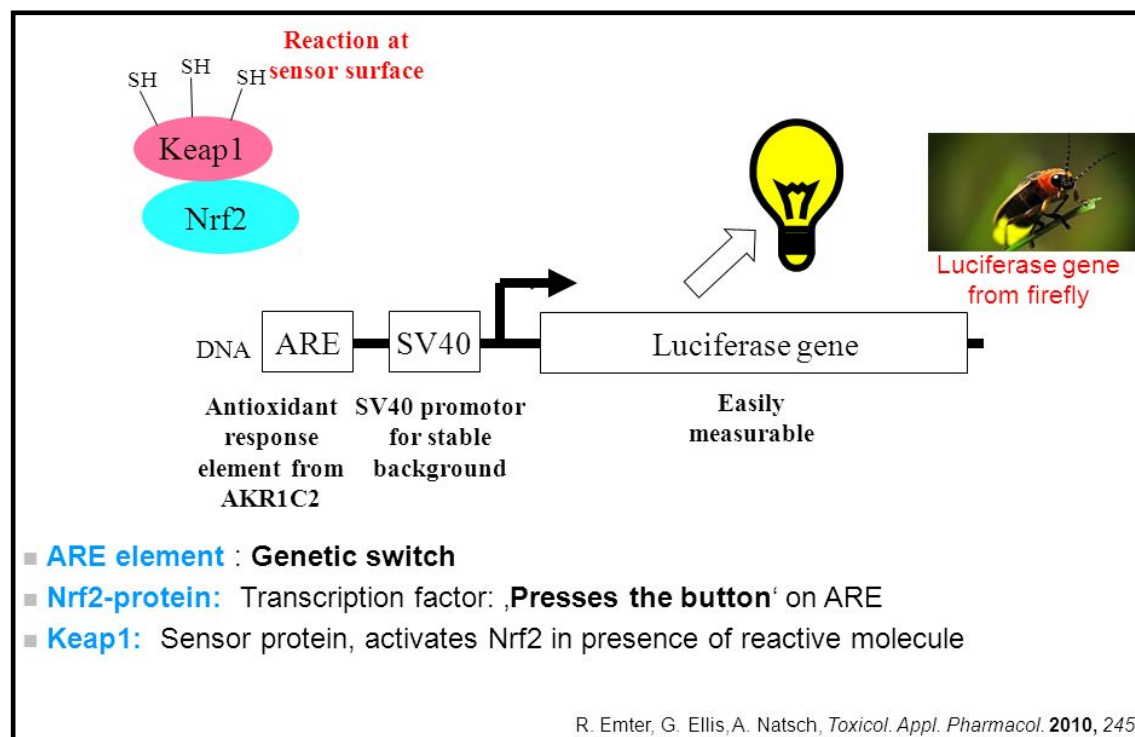
- Human cell line activation test: ISO 10993-10 (OECD TG 442e)



- Monocytic cell line **THP-1** (mimics DC activation, 3rd key event)
- Upregulation of inflammatory markers (CD54 / CD86) upon confrontation
- Read out: Flow cytometry
- Distinguishes sensitizers from non sensitizers

# KeratinoSens™ assay

- Genetically modified HaCat cells (human Keratinocytes, KeratinoSens™)
- Addresses the 2nd key event in skin sensitisation
- Induction of ARE (Antioxidant responsive Element) by NRF2
- Induction of Luciferase gene → light emission that can be measured



# Pyrogen analytics

(EP 2.6.30 / 2.6.14 / USP <85> and <151>)

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# Pyrogens, Endotoxins

- Pyrogens are substances causing a rise in body temperature (inflammation / fever) due to TLR (Toll-like receptors) activation
- **Endotoxins** (like lipopolysaccharides (LPS) produced by Gram-negative bacteria)
- **Non –Endotoxins** like:
  - Teichonic acids
  - Yeast and Mold
  - Viral and fungal proteins
  - Metals and compounds in elastomers
  - Rubber compounds

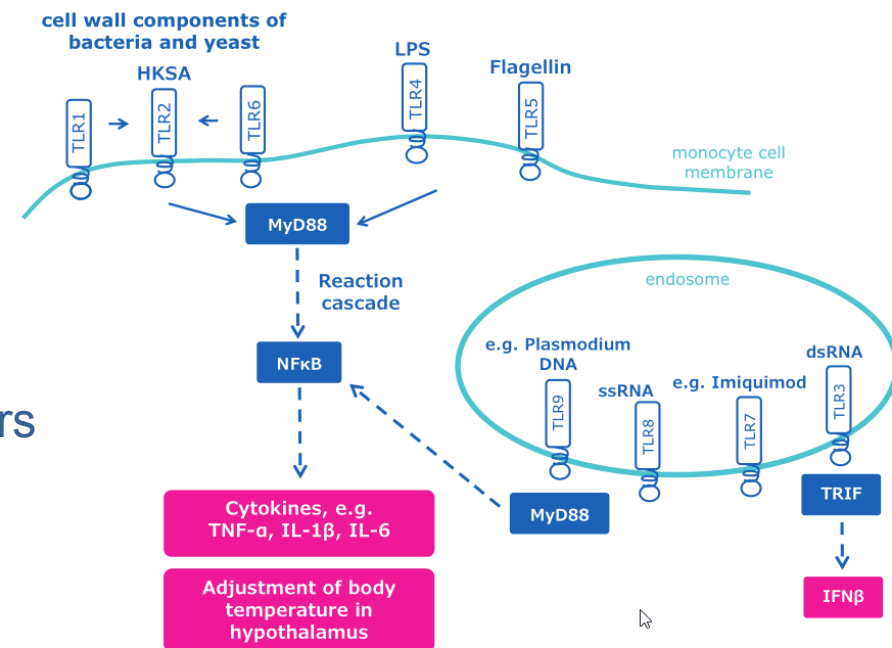
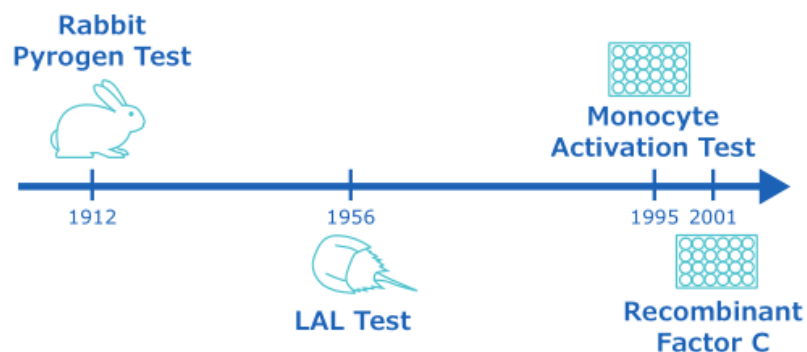





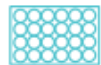
Figure 1. TLR signaling pathways

Source: Merck Millipore



# Pyrogen Tests



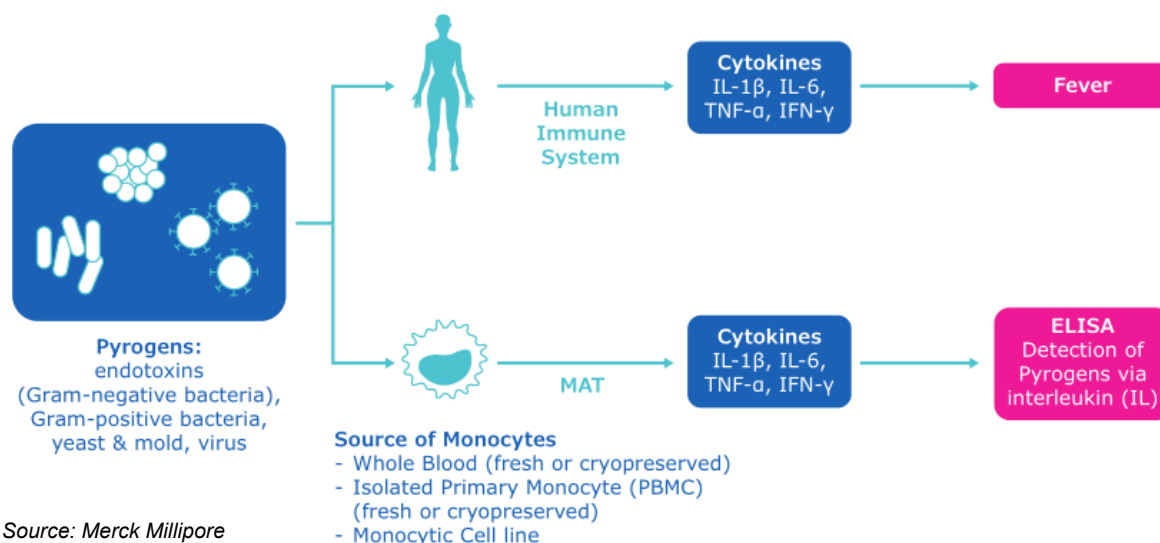
	Test Type	Animal based?
Endotoxins	 <b>Bacterial Endotoxin Tests (BET) or Limulus Amebocyte Lysate (LAL)</b> EP 2.6.14, USP 85 <b>Principle:</b> use of immune response of the horseshoe crab against invasion of Gram negative bacteria	Yes
	 <b>Recombinant Factor C (rFC)</b> In July 2016 in the EP, FDA Q&A June 2012 <b>Principle:</b> based on a rFC, genetically engineered protein, which is activated by endotoxin to produce a fluorescent end product which is quantifiable.	No
Pyrogens	 <b>Pyrogen Test (Rabbit Pyrogen Test: RPT)</b> EP 2.6.8, USP151 <b>Principle:</b> rectal measurement of the body temperature after injection of the product	Yes
	 <b>Monocyte-activation Tests (MAT)</b> EP 2.6.30, FDA Q&A June 2012 <b>Principle:</b> Monocytes activated by pyrogens produce cytokines/interleukins (IL) that are detected in an immunological assay (ELISA)	No

Source: Merck Millipore

**We focus on the monocyte activation test which is independant from animal sources and detects a broad range of pyrogens!**

## Monocyte activation test (MAT-assay)

- Pharm Eu 2.6.30; USP <151>
- Monocyte based cytokine release upon pyrogen contact
- IL-1b, IL-6, TNF- $\alpha$ , more
- **Reactivity not restricted to LPS**
- Sources: Human blood, PBMCs, Monocytic cell lines
- Validated commercial kit available → cost extensive
- Micromol offers a validated, EP 2.6.30 compliant protocol → **cost effective**



Source: Merck Millipore

# Genotoxicity

(DIN ISO 10993-3 / OECD TG 471 / 487)

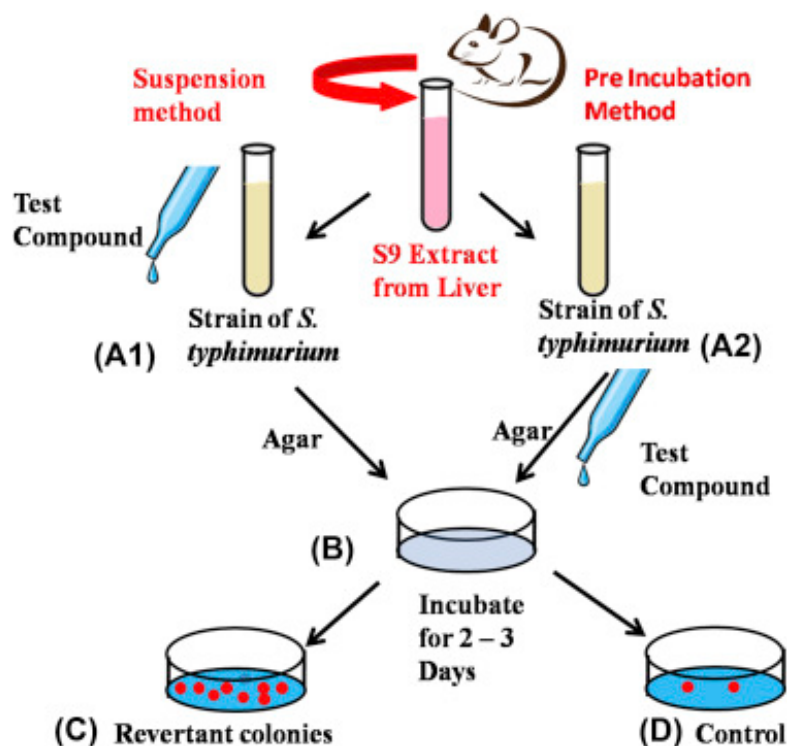
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# Ames test (OECD TG 471)

## Micronucleus Test (OECD 487)

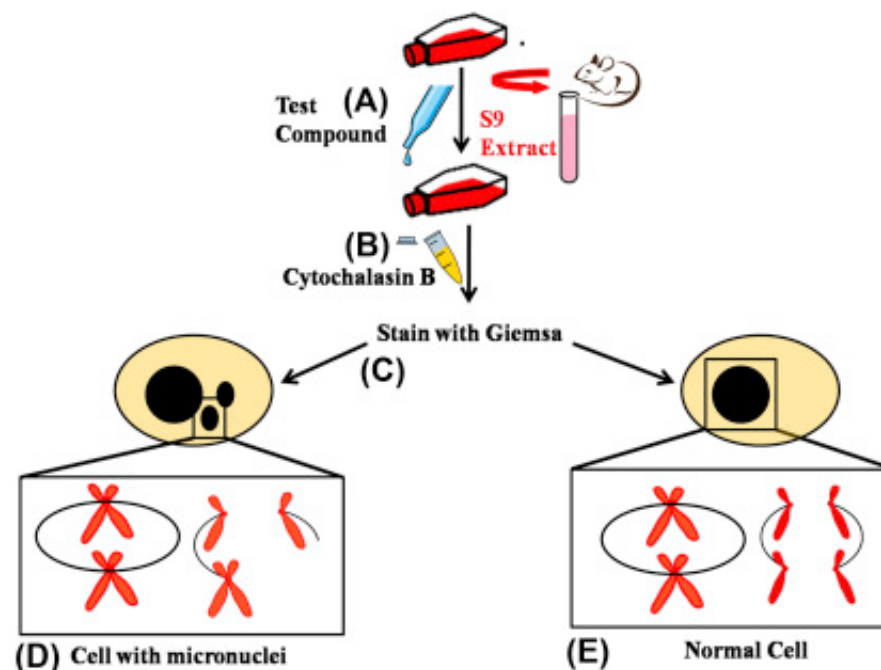
### Ames Test

- Prokaryotic system
- Detects mutagens
- His- Mutant
- Determination of His<sup>+</sup> revertants



### Micronucleus Test

- Eucaryotic system
- Detects chromosome damage (loss (aneugens) or breakage (clastogens))
- Quantification of cells containing micronuclei



Thanks for your attention !

Tentamus - Labs for Life

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