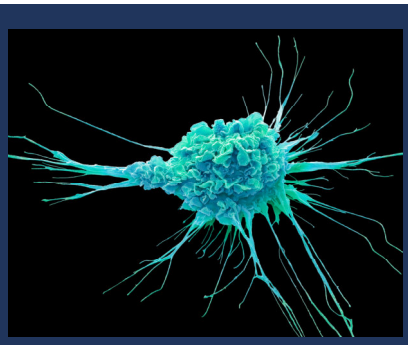
FACTSHEET

HOHENSTEIN

Sensitisation according to DIN EN ISO 10993-10 Annex C

Non-animal test methods for skin sensitisation

The effort to reduce or replace the use of animals in toxicity testing has led to the development of various non-animal methods for risk assessment.



Assays for the testing of skin sensitisation

Direct Peptide Reactivity Assay (DPRA)

Key event 1: Covalent binding to skin proteins

The DPRA is an *in chemico* method that quantifies the reactivity of a test chemical or a product extract through its depletion of synthetic peptides containing cysteine or lysine.

The percentage peptide depletion values for cysteine and lysine are determined and assigned to one of four reactivity classes in a prediction model, whereby the tested product is classified as sensitising or non-sensitising to the skin.

IL-18 RhE Assay

Key event 2: Keratinocyte response

Skin sensitisation is assessed *in vitro* by measuring the basal release of interleukin 18 (IL-18) after application of a product extract to reconstructed human epidermis (RhE). IL-18 is quantified in the culture medium of the RhE using the ELISA method. In parallel, the viability of the cells is measured by MTT test. (The assay can be combined with the test for skin irritation according to DIN EN ISO 10993-23).

U937 CELL LINE ACTIVATION TEST (U-SENS™)

Key event 3: Activation of dendritic cells

U-SENS™ method is an *in vitro* assay that quantifies changes of CD86 cell surface marker expression by flow cytometry on a human histiocytic lymphoma cell line (U937 cells).

This test is particularly suitable for

- Medical devices (independent of the material)
- Textiles in the healthcare sector

Customer benefit

- Proof of conformity of the medical device with the Medical Device Regulation (prerequisite for CE labelling)
- Part of the biological evaluation and risk assessment of your medical device
- Product optimisation
- Consumer safety
- Minimisation of complaints

Test sample requirements

General
- Please send in the entire product for customised samples
- When sending several samples, ensure that the ingredients are not transferred to other samples, i.e. pack separately in plastic bags
- Specify sufficiently precise descriptions (material composition, article number, etc.) of the test sample

Quantity of material
- At least 80 g of the test sample or at least 3 complete products per test

Duration of the test
- Usually 25-30 working days; date confirmation after receipt of test sample

Background

Adverse Outcome Pathway for skin sensitisation

The prediction of skin sensitisation in humans relies on combining several individual tests in experimental strategies, as none of the validated animal-free assays alone can fully reproduce the complex network of underlying mechanisms. The OECD has described the sequential events that lead to a skin sensitising effect. This series of events is referred to as the AOP (Adverse Outcome Pathway).

The AOP for skin sensitisation describes four key events, whereby key events 1 to 3 are described in the risk assessment in accordance with DIN EN ISO 10993-10 Annex C as alternative methods not involving animal testing.

Key event 1 - Covalent binding to skin proteins: the molecular initiating event after penetration of the stratum corneum is the irreversible formation of the hapten-protein complex.

Key event 2 - Keratinocyte response: this key event involves the activation of biochemical pathways in the keratinocytes and includes inflammatory mediator responses as well as gene expression changes associated with cell signalling pathways such as the formation of interleukin (IL-)18.

Key event 3 - Activation of dendritic cells: the detailed biochemical events after formation of the hapten-protein complex have not been fully clarified. Effects at the cellular and tissue levels are also not completely known but involve epidermal responses which include:

1) immune recognition of chemical allergens by keratinocytes, specialized epidermal dendritic cells (i.e. Langerhans cells) and dermal dendritic cells;

2) Cellular responses manifested as expression of specific cell surface markers like e. g. CD86 in U937 cells.

Key event 4 - T-cell proliferation: at the organ level (lymph nodes and skin), the responses are:

1) dendritic cell migration to the lymph node, where the antigen is presented to activate naive T-lymphocytes (T-cells), and

2) differentiation and proliferation of T-cells into allergen-specific effector and memory T-cells.

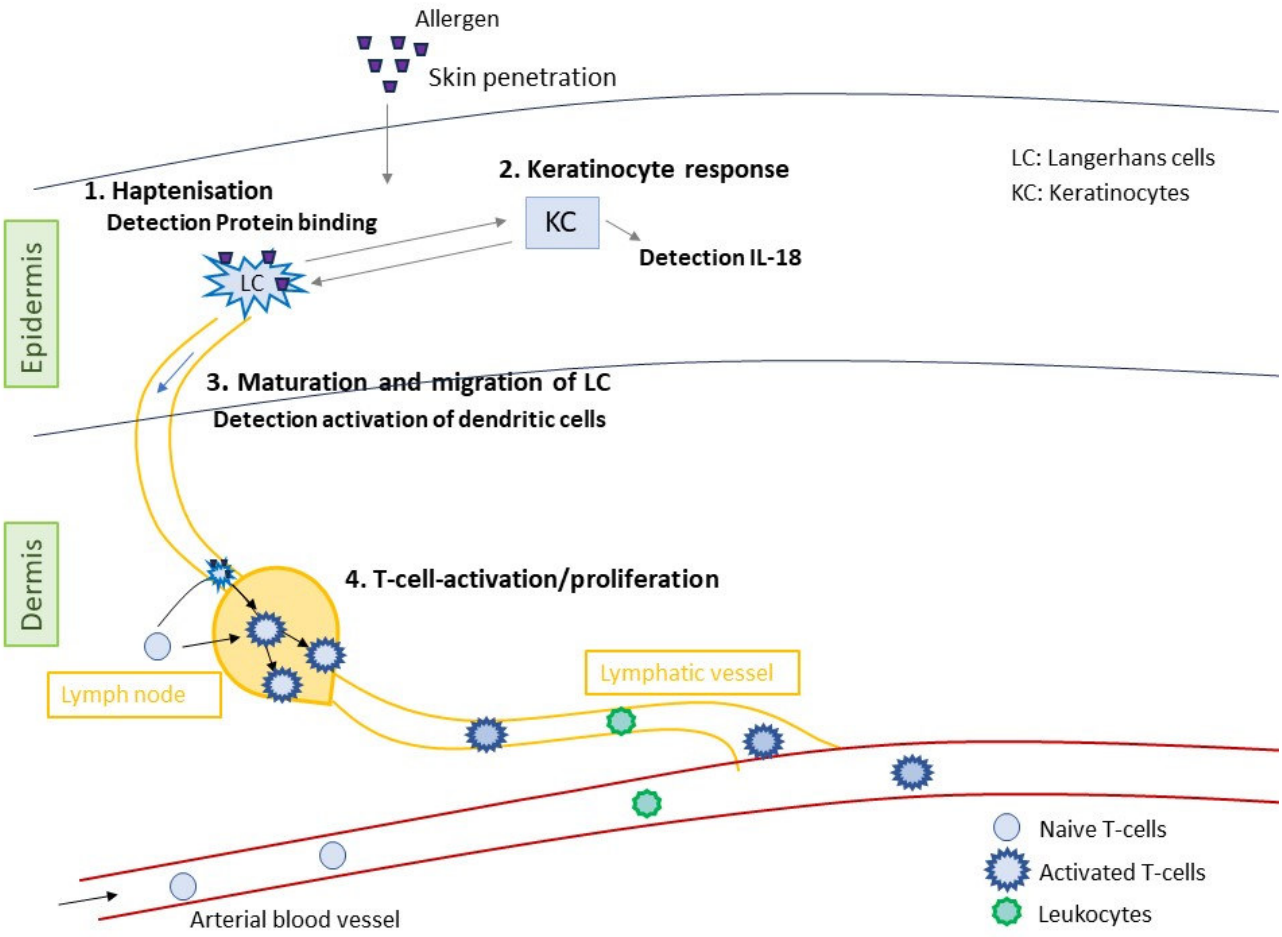


Figure: Key events along the AOP of skin sensitisation