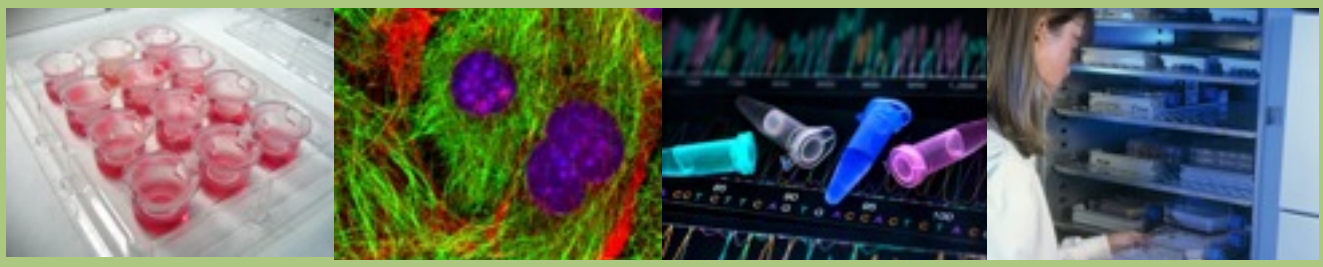
SENS-IS : in vitro sensitization quantification



The SENS-IS assay is based on the monitoring of the quantitative over expression of specific biomarkers after application of chemicals, mixture, natural extracts or finished products onto 3D reconstituted epidermis

SENS-IS ASSAY METHOD

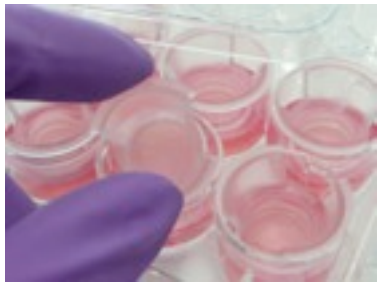
1-chemical application on Episkin

Human 3D reconstructed epidermis (Episkin®) are exposed for 15 min to 30µl of 50, 10, 1, 0,1 % test chemicals in PBS, olive oil or DMS0.

2- Washing

After 15min exposure, the Episkin® are rinsed. This step is very important to avoid non specific irritation.

3- Post-incubation and sampling



After 6 hours of post-incubation, the samples are harvested and frozen in liquid nitrogen before tissue lysing and RNA extraction.

4-Tissue lysing and cDNA preparation

The tissues are mechanically disrupted using a tissue lyser (Qiagen).
RNA extraction and cDNA preparation is done with classical methods.

5 - R T - P C R quantification

Quantification by RT-PCR of 62 biomarkers classified into 3 groups : irritation, ARE and SENS-IS genes

6-Results analysis

Assay validation after analysis of :
negative controls (PBS, Olive oil, DMSO)
irritant control (5% SLS)
two sensitizer controls (50% HCA, 1%TNBS)

Irritation : positive response if at least 7/24 genes are significantly induced

Sensitization : a molecule is classified as positive if at least:
- 7/17 genes in ARE genes group and/or
- 7/21genes in SENS-IS genes group are significantly induced

Potency assessment :
positive at 0,1% : extreme
positive at 1% : strong
positive at 10% : moderate
positive at 50% : weak

ImmunoSearch

SKIN SENSITIZATION

Allergic contact dermatitis resulting from skin sensitization is an important occupational and environmental health problem. Many hundreds of chemicals are known to cause skin sensitization, and allergic contact dermatitis is the most common manifestation of immunotoxicity in humans. It is important, therefore, that skin sensitization hazards/risks of new chemicals and products be evaluated accurately.

The need for alternative testing

There are strong scientific, ethical, and legislative reasons why it is important to ensure that opportunities to Reduce, Refine, and Replace the use of experimental animals in research and investigative studies are exploited quickly and effectively. However there are a number of requirements for an alternative non-animal test to replace the mice used within the LLNA that represent integrated biological models that incorporate, in a fully coordinated and physiologically relevant way, all the events and processes that are needed for the acquisition of sensitization .

A test based on a 3D reconstituted epidermis

To address that complexity, the SENS-IS assay use 3D reconstituted epidermis to better take into account the step of skin penetration of products wih different solubility or physical state. Accordingly, **SENS-IS can quantitate not only pure chemicals but also natural products, mixtures and finished products.**

Moreover, it is estimated currently that approximately 25% of contact allergens must be activated by air oxidation, or within the skin, to acquire the chemical reactivity necessary to associate with proteins. The use of an epidermis ensure that skin metabolism of chemicals is taken into account when assessing the sensitizing potency.

A genomic signature to assess skin sensitization

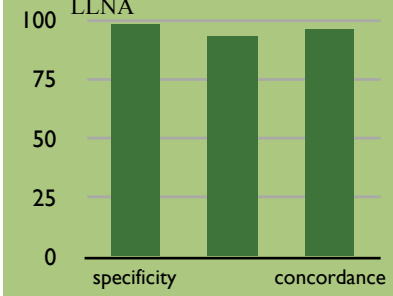
To adress all the aspects of complexity of skin sensitization and to take into account the variety of different types of chemical sensitizers SENS-IS uses a protected set of 62 biomarkers split into 3 groups. A group of 24 genes to measure skin irritation and détermine the minimal irritating dose. A group of 17 genes involve in the REDOX detoxification pathways (ARE genes) and a group of 21 genes involve in inflammation, cell trafficking and tissue homeostasie (SENS-IS genes). The groups of genes where selected so that at a given concentration, a product is positive if it induces more than 7 genes in any of the groups.

Regulatory acceptance is underway.

SENS-IS is already used as the preferred in vitro alternative test for sensitization by major cosmetic companies. In a first tiered evaluation made by Cosmetic Europe on 10 blinded products over 15 different assays, SENS-IS was the only one able to correctly classified both for danger and potency the 10 chemicals. SENS-IS is currently under evaluation by ECVAM on studies assessing within lab reproducibility, between lab transferability and reproducibility

Stastistical analysis

SENS-IS Performance for the prediction of 140 chemicals as sensitizers as compared to LLNA



SENS-IS potency class concordance with LLNA (non sensitizer, weak, moderate strong and extreme) was 131/140 chemicals :

93%

ABOUT IMMUNOSEARCH

ImmunoSearch is a leading European Research company specialized in immunotoxicology and developing alternative toxicology tests to address skin and eye irritation/sensitization

Based in Sophia Antipolis/Grasse in France ImmunoSearch scientists regroup expertise in immunology, genomic analysis and skin biology.

We are at the forefront in providing test for measuring both efficacy and toxicological potential of cosmetic and chemical ingredients.

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