# The GARD assay – assessment of skin sensitizing chemicals

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## INTRODUCTION

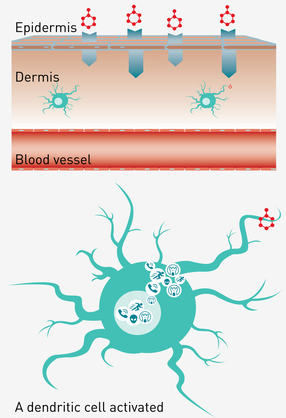
Chemicals have significantly improved our welfare, however severe symptoms can arise in exposed persons. An adverse reaction is Allergic Contact Dermatitis (ACD), that can result after repeated contact. To decrease present chemicals inducing ACD (skin sensitizers), chemicals have to be tested. Previous tests included animals, but EU legislation, OECD test guidelines, TSCA and EPA today prioritize the use and development of alternative methods.

Genomic Allergen Rapid Detection (GARD) is an *in vitro* assay for assessment of chemicals' sensitizing capacity. GARD is based on a human cell line that is exposed to the chemical of interest and the as a gene expression panel is the end-point measurement.

## THE BIOLOGY BEHIND

During skin sensitization dendritic cells (DCs) are key players linking the innate and acquired immune system. Located in dermis, they can get activated by foreign substances penetrating the skin or by molecules secreted by surrounding cells already stimulated by the foreign substance.

Upon activation, their gene expression profile is changed, which is measurable by genomic techniques. The following alteration of the proteome facilitates a multitude of cell modifications. The DCs matures and their migratory capacity increase promoting their escape towards lymph nodes where they ultimately signaling to T cells to proliferate and defense the host against the chemical substance.




A dendritic cell activated by a foreign substance.

## THE GARD PLATFORM

The GARD assay is based of a human myeloid cell similar to DCs, which are immunologically active during sensitization. The GARD protocol include a cell line stimulation of the compound of interest and after incubation the transcripts are harvested. The gene expression is analysed through a bioinformatics multivariate technology (1).

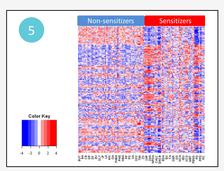
**A brief overview of the GARD process**


Human immunologically relevant cells are used.


The cells are exposed to the substance of interest.


Their genomic products (transcripts) are isolated.


The gene transcripts are quantified.




The readout is processed to assess sensitizing ability.


Final report.

During development of the GARD applications, total gene expression is analysed by microarrays, but after gene signature establishment, assays are transferred to the Nanostring platform (2).

Depending on requested end-point data the GARD platform can be transformed to different applications. Today, GARDskin for skin sensitizers and GARDair for respiratory sensitizers are developed (3).

## GARDskin

Depending on selected end-point gene expression panel measured the GARD platform is flexible. The **GARDskin** assay specifically assess the hazard of skin sensitizers estimating the expression of ~200 genes.

The accuracy has been determined to ~90% analyzing 26 blinded chemicals (4). For comparison the accuracy of LLNA has been estimated to 72%. An example of tested chemicals is provided in table 1.

A method evaluation on further chemicals provided by Cosmetics Europe Skin Task Force has been executed and the results are confirming that accuracies are constantly high (manuscript in preparation).

Table 1. Examples of chemicals tested by GARDskin and LLNA. Blue = correct classification, pink = incorrect classification.

| Chemical | LLNA | GARDskin |
|---|---|---|
| Dinitrochlorobenzene (DNCB) | extreme sensitizer | sensitizer |
| Cinnamal | moderate sensitizer | sensitizer |
| Benzalkoniumchloride | non-sensitizer | sensitizer |
| 7-Hydroxycitronella | weak sensitizer | sensitizer |
| Phenyl Benzoate | weak sensitizer | non-sensitizer |
| DMSO | weak sensitizer | non-sensitizer |
| Xylene | weak sensitizer | non-sensitizer |
| Menthol | non-sensitizer | non-sensitizer |
| Salicylic acid | non-sensitizer | non-sensitizer |
| Sodium lauryl sulphate (SLS) | weak sensitizer | non-sensitizer |

## RESUME

GARDskin

- ✓ *in vitro* model
- ✓ assess skin sensitizers
- ✓ genomic assay
- ✓ end-point measurement of ~200 genes
- ✓ Accuracy: ~90%.

References:
1) Johansson *et al.* BMC Genomics, 2011
2) Forreryd *et al.* BMC Genomics, 2014
3) Forreryd *et al.* PloSOne, 2015
4) Johansson *et al.* Toxicol Sci, 2014