Skin Sensitization Test

GARDskin™ – 200 genomic biomarkers

[OECD TGP 4.106]

Allergic contact dermatitis is a widespread disease with a prevalence of ~20 % in the western world. The symptomatic rash is caused by an immune response towards foreign substances in direct contact with skin. In order to decrease exposure to harmful substances, testing of chemicals is of high importance. Previously, animal models have been utilized, but today alternative methods are recommended by OECD. Further, widespread bans of animal testing on cosmetics demand alternative methods.

The method described here, **GARD**skin, is designed to assess if chemicals are skin sensitizers, *i.e.* can induce skin sensitization. It is a genomic test measuring changes in gene expression of 200 genes relevant to skin sensitization adverse outcome pathways (AOP). These pathways are defined on the basis that they are activated by chemicals, resulting in biological effects. The high amount of data points measured by **GARD**skin gives a specific and high resolution result compared to tests measuring few data points.

The assay is developed at Lund University, Sweden, and has been thoroughly validated in-house with chemicals blindly provided by the cosmetic industry (Johansson *et al.*, Tox Sci 2014). The accuracy of the test is ~90 % which can be compared to the gold standard LLNA accuracy of 72 % (examples of chemicals tested in Table 1).

Chemical	LLNA	GARD
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

Table 1. Examples of chemicals tested and the classification according to LLNA and **GARD**. Pink indicates incorrect classification, blue correct.

The principle of **GARD**skin relies on genomic screening of cultivated cells after stimulation by the chemical of interest. The workflow can be followed in Figure 1.

Figure 1. An overview of the **GARD**skin process.
Step 1: A human immunologically relevant cell line is used as target for exposure of substances to be tested.
Step 2: The cells are exposed to the substance to be tested.
Step 3: Their genomic products (transcripts) are isolated for downstream quantification.
Step 4: The gene transcripts are quantified using the multiplex NanoString technology.
Step 5: The readout is processed using advanced computer algorithms to assess a chemical's ability to induce an allergic reaction.

GARDskin™

- Skin sensitization test
- *In vitro*
- Human cell line
- Genomic testing
- Bioinformatic model of ~200 genes
- Straight forward conclusive report (Y/N) with personal feedback
- Pathway analysis can be implemented
- Indication of potency

Quality Statement

GARDskin **follows a strict quality system specifically developed in house.**

The test is approved for the OECD Test Guideline Program (TGP no. 4.106).

Accuracy: ~90 %

GARDskin enables a robust way to meet the demands for 3R with reliable outcome as the test is performed *in vitro* on human cells.

Test system

The cell line used is of human myeloid origin with characteristics similar to dendritic cells. *In vivo*, dendritic cells connect the innate and adaptive immune system by transferring signals, from *e.g.* a local point of chemical exposure, to T cells located in the lymph nodes, that subsequently become activated. **GARD**skin mimics the molecular changes induced in dendritic cells in response to foreign substances.

Protocol

GARD Input Finder

- Cells are confirmed to have the correct phenotype and not to be activated.
- Cells are stimulated with the chemical of interest diluted in nine concentrations. Incubation for 24 hours.
- Viability of cells is measured by flow cytometry analysis of propidium iodide stained cells.
- The concentration of chemical inducing a relative viability of 90 % is selected for the final stimulation.

GARD Main Stimulation

- The selected concentration of chemical is used to stimulate cells. Incubation for 24 hours.
- Benchmark chemicals with known sensitizing properties are used to calibrate the result.
- RNA (the product of gene expression) is harvested.
- The stimulations are performed on triplicate cell batches.

Endpoint Measurment

- The NanoString platform is applied to measure the gene expression of the predictive signature (~200 genes).
- The RNA is prepared according to the supplier's instructions. The NanoString method relies on colour coded probes, specifically designed for **GARD**skin, that are hybridized to the RNA and counted.

Data Analysis

- Each sample is given a decision value as either sensitizer or non-sensitizer, based on SVM classifications.

Final result and report

The gene expression data from 200 genes are analysed by an in-house developed application based on a bioinformatic model.

The final report includes a box plot (example in Fig. 2) and the specification of the result, *i.e.* if the chemical is a sensitizer or not (Y/N). Result above the threshold (0) corresponds to sensitizers and below to non-sensitizers. Pathway analysis is available upon request.

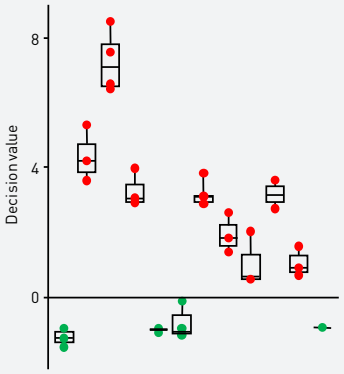

Figure 2.
Example of box plot results. Each dot corresponds to a biological replicate and each box to a tested chemical. The decision values for non-sensitizers (green) and sensitizers (red) are separated by a threshold at 0.

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