Respiratory Sensitization Test

GARD®air

Respiratory sensitization test using genomic biomarkers

Chemical sensitization, also referred to as chemical allergy, is a disease state induced by the human immune system in response to chemical sensitizers. This category of substances produce their harmful effects by triggering a multitude of intricate cellular mechanisms. Sensitization occurs when T-cells learn to recognize a specific chemical sensitizer, presented to them by activated dendritic cells . Following subsequent exposure, T-cells react rapidly to induce a state of inflammation. This in turn leads to disease-associated symptoms, such as itching, blistering and tissue damage in case of skin contact, and coughing, wheezing and asthma-like symptoms in case of inhalation.

Chemical compounds may have intrinsic properties that preferentially lead to sensitization of the skin or the respiratory tract, also referred to as allergic contact dermatitis (ACD) and occupational asthma (OA), respectively (Dearman et al., Journal of Applied Toxicology 2011). This understanding may have an impact on how chemicals are safety tested and labelled for potentially hazardous effects.

Even though the onset of occupational and allergy-induced asthma requires respiratory exposure, the sensitization can occur via either skin or respiratory exposure (Kimber et al., Toxicology 2002). This is because respiratory sensitizers are intrinsically different from skin sensitizers and activate different immunological pathways.

Respiratory sensitizers preferentially induce a Th2-type immune response, as opposed to the Th1 and cytotoxic T-cells, primarily induced by skin sensitizers. It is hypothesized that Dendritic Cells (DC) are involved and that Th2-skewing occurs in association with antigen-presentation, through the co-stimulatory profile exhibited by DCs at the immunological synapse. For these reasons, DCs are the target for our assay development, and form the cellular basis of **GARD**air.

GARD®air

- Safety assessment of respiratory sensitizers
- *In vitro*
- Human dendritic-like cell line
- Genomic biomarker signature monitoring innate immune function, dendritic cell activation and Th2 polarisation
- Bioinformatic model
- Can discriminate between skin and respiratory sensitizers
- ~ 90 % accuracy

The principle of **GARD**air relies on genomic screening of cultivated cells after stimulation by the chemical of interest. The workflow is demonstrated in Figure 1.

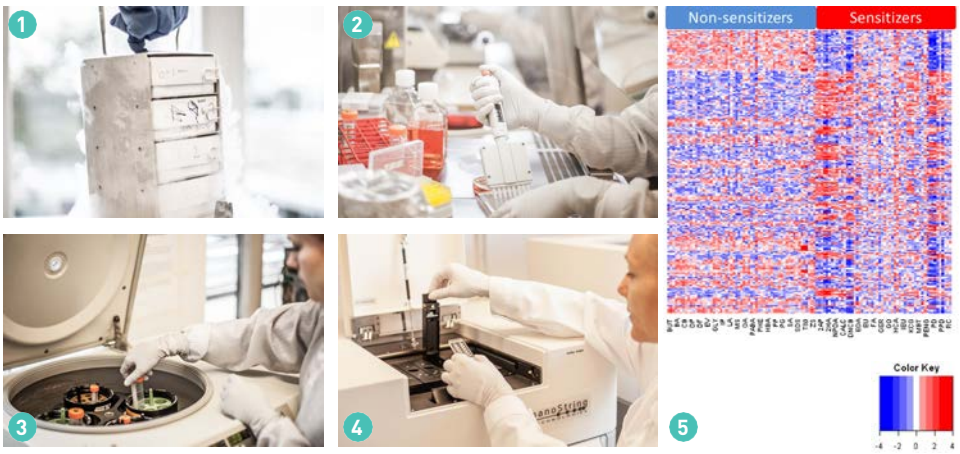

Figure 1. An overview of the **GARD**air assay.
Step 1: A dendritic-like cell line is used as target for exposure of substances to be tested.
Step 2: The cells are exposed to the substance to be tested.
Step 3: The genomic products (transcripts) are isolated for downstream quantification.
Step 4: The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

Test system

GARDair has been developed by using a data driven approach looking at biomarkers that discriminate between respiratory sensitizing and non-sensitizing chemicals when exposed to a human dendritic cell-line (SenzaCells). A biomarker panel, the so-called **Genomic Prediction Signature (GPS)** has been selected using the whole human genome. This GPS consists of numerous biomarkers, linked to bridging of innate and adaptive immune functions, DC activation and skewing towards Th2 type immune responses.

Quality Statement

GARDair **follows a strict quality system specifically developed in-house.**

Accuracy: ~90%

GARDair is an *in vitro* assay based on human cells that provides a unique and reliable way to analyse the respiratory allergenic potential of chemicals.

Human relevant testing

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**air mimics the molecular changes induced in dendritic cells in response to foreign substances. (examples of chemicals tested in Table 1).

Table 1. Prediction results of external test data set using finalized **GARD**air prediction model. False classifications are highlighted – Pink indicates incorrect classification, blue correct. RS: Respiratory Sensitizer, NRS: Non-Respiratory Sensitizer, SS: Skin Sensitizer, FP: False Positive, FN: False Negative.

Chemical name	True group	GARD Prediction
2-Mercaptobenzothiazole	NRS / SS	NRS
4-Hydroxybenzoic acid	NRS	NRS
Benzaldehyde	NRS	NRS
Octanoic acid	NRS	NRS
Chloramine-T hydrate	RS	RS
Cinnamyl alcohol	NRS / SS	NRS
Diethyl phthalate	NRS	NRS
DNCB	NRS / SS	NRS
Eugenol	NRS / SS	NRS
Glycerol	NRS / SS	NRS
Glyoxal	NRS / SS	RS (FP)*
Isoeugenol	NRS / SS	NRS
Isophorone diisocyanate	RS	RS
Phenol	NRS	NRS
Piperazine	RS	RS
PPD	NRS / SS	NRS
Reactive orange 16	RS	RS
Resorcinol	NRS / SS	NRS
Salicylic acid	NRS	RS (FP)*
SDS	NRS	NRS
Chlorobenzene	NRS	NRS
DMSO	NRS	NRS
Maleic anhydride	RS	RS
Phenyl isocyanate (MDI)	RS	RS
Phthalic anhydride	RS	RS
Toluene diisocyanate	RS	NRS (FN)*
Trimelitic anhydride	RS	RS

* Glyoxal – FP (skin sensitizer), Salicylic acid – FP (irritant), TDI – FN (solubility issues)

Final result and report

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

The final report includes a box plot (example in Figure 2) and the specification of the result, i.e. if the chemical is a respiratory sensitizer or not. Result above the threshold (0) corresponds to sensitizers and below to non-sensitizers.

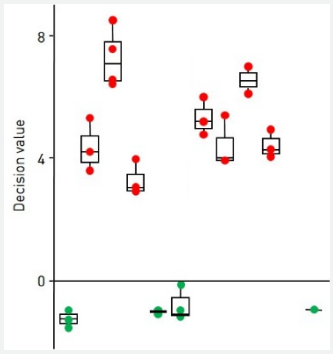

Figure 2. Example of a box plot. Each dot corresponds to a biological replicate. Decision values for NRS (green) and RS (red) are separated by a threshold at 0.

This project has received funding from the European Union´s **Horizon 2020** research and innovation programme under grant agreement No.756014.

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