Genomic Allergen Rapid Detection: *In Vitro* Skin Sensitisation Assay

(GARD™)

The *in vitro* GARD™ assay addresses the third molecular key event of the adverse outcome pathway (AOP) of skin sensitization.[1] The UN GHS (United Nations Globally Harmonized System of Classification and Labelling of Chemicals) defines a skin sensitiser as a substance that will cause an allergic response after skin contact.[2]

The GARD™ assay is a reliable *in vitro* method and is performed in accordance with the DB-Alm Protocol GARD (not published by DB-Alm yet) at Eurofins BioPharma Product Testing Munich GmbH[1, 3] with chemicals, cosmetics or personal care products and pharmaceuticals.

This is one of four test methods (DPRA, KeratinoSens™ and h-CLAT) for the assessment of skin sensitisation potential.

Assessment of skin sensitisation potential with the GARD™

- The third molecular key event of skin sensitisation addresses the activation of dendritic cells (DC), which is typically accompanied by expression of specific cell surface markers, chemokines and cytokines.
- The GARD™ assay can be performed to predict the ability of chemicals to induce skin sensitisation based on the analysis of relative expression levels of a biomarker signature of 196 genes. These biomarkers represent the various activated mechanisms in dendritic cells (DCs) in response to chemicals. The test method uses SenzaCell, a human myeloid leukemia cell line. This cell line works as an *in vitro* model of human dendritic cells and chemical stimulation of the cells can be assessed.
- With this test method transcriptional quantification of the genomic predictors, termed collectively the GARD™ Prediction Signature (GPS), using a Nanostring nCounter technology can be determined. Based on a derived decision value (DV) from a Support Vector Machine (SVM) model chemicals can be predicted to be sensitisers or non-sensitisers. The algorithm of this machine uses known data sets from already done cell stimulations with known chemicals. Using this data set for each new chemical a decision value is created and the mean value is used for classification. A sensitising chemical can be further classified into category 1A or 1B.

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- All experimental steps until the isolation of the RNA are performed at Eurofins. The qualification of the RNA and the Nanostring endpoint measurement are performed in cooperation with SenzaGen AB in Sweden.
- In combination with other complementary information within an Integrated Approach to Testing and Assessment (IATA) or as a stand-alone method, the GARD™ Assay can be used as a reliable *in vitro* method to assess skin sensitising potential of chemicals.

"The GARD™ method mimics the immune system by using human dendritic cells. It predicts the ability of chemical compounds to induce skin sensitisation by measuring changes in the genomic profile of the cells after chemical treatment."

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Procedure

Principles of the GARD™ Assay

Protocol	
Cell line	SenzaCells provided by SenzaGen AB Human myeloid leukemia cell line
Analysis	Cell viability and assessment of genomic biomarkers
Test chemical concentrations	Input finder: 9 concentrations with a minimum of 1 mM Main stimulation: 1 concentration leading to a cell viability of 90 ± 5%
Incubation time	24 h ± 0.5 h at 37 °C and 5% CO_2
Quality controls	Medium control Negative control: 0.1% DMSO Positive control: 75 µM p-phenylene diamine (PPD)
Solvents of test chemical	Dimethylsulfoxid (DMSO) Water
Application	At least three independent performed experiments
Data delivery	Cell viability with FACS analysis Nanostring data analysis using GARD Data Analysis Application (SenzaGen AB)
Classification	Mean DV of biological replicate samples ≥0 = sensitiser Mean DV of biological replicate samples <0 = non-sensitiser

References

1) The OECD (2012), The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. Part 1: Scientific Evidence. Series on Testing and Assessment No. 168

2) UN (2015), United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Sixth revised edition, UN New York and Geneva.

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