The GARD™ Skin Assay: A New *In Vitro* Testing Strategy for Skin Sensitization

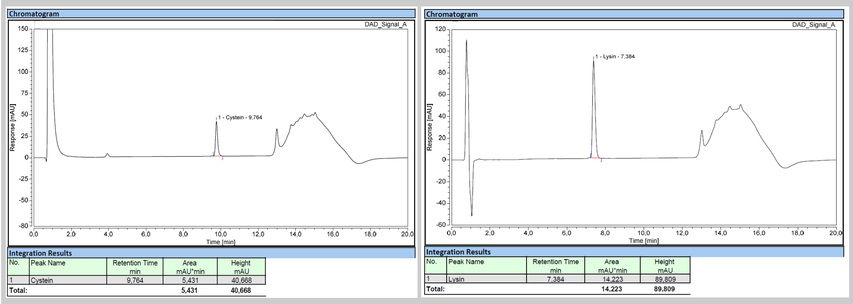
E. Schmidt, V. Zuckerstätter, H. Gehrke | Eurofins BioPharma Product Testing Munich GmbH

INTRODUCTION

A skin sensitiser refers to a substance that will lead to an allergic response following skin contact as defined by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS). The potential to induce skin sensitisation is an important consideration included in procedures for the safe handling, packaging and transports of chemicals.
The assessment of skin sensitisation typically involves the use of laboratory animals. Classical methods comprise the Magnusson Kligman Guinea Pig Maximisation Test, the Buehler Test (TG 406) as well as the local lymph node assay, in its radioactive and non-radioactive form (TG 429, TG 442A/B). In order to replace *in vivo* experiments validation studies on alternative, mechanistically based *in chemico* and *in vitro* test methods on skin sensitisation were conducted under the auspices of ECVAM and have been considered scientifically valid for the evaluation of the skin sensitisation hazard of chemicals.
Genomic Allergen Rapid Detection (GARD™) is an *in vitro* assay designed to predict the ability of chemical substances to induce skin sensitisation based on the analysis of the relative expression levels of a biomarker signature of 196 genes using a human myeloid leukaemia cell line called SenzaCells. The GARD™ assay is based on chemical stimulation of the SenzaCells, acting as an *in vitro* model of human Dendritic Cells (DCs). The readout of the assay is a transcriptional quantification of the genomic predictors, collectively termed the GARD™ Prediction Signature (GPS), using Nanostring nCounter technology.

The Problem

KE1: DPRA



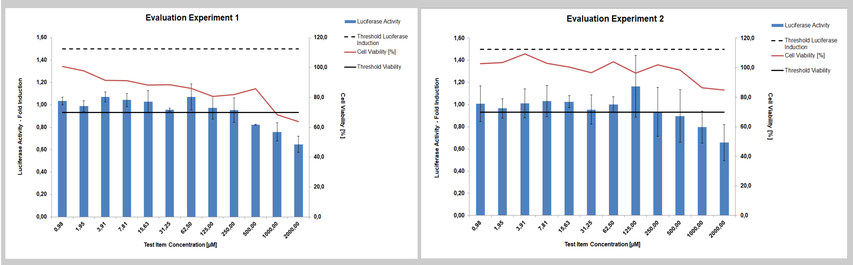
Reference control cysteine: Ø 15.6 mAU
Cysteine sample: Ø 5.43 mAU
Depletion of 65.2% for cysteine

Reference control lysine: Ø 14.3 mAU
Lysine sample: Ø 14.2 mAU
Depletion of 0% for lysine

Overall: 65.2% : 2 = 32.6%

Positive

KE2: KeratinoSens™

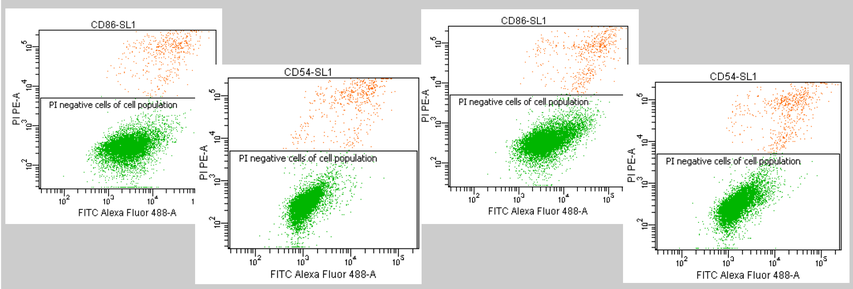


Main experiment 1:
PC: induction over 1.5, no cytotoxicity
Test item: no induction over 1.5, cytotoxicity in the highest two concentrations

Main experiment 2:
PC: induction over 1.5, no cytotoxicity
Test item: no induction over 1.5, no cytotoxicity

Negative

KE3: h-CLAT



Main experiment 1:
PC: expression CD86 and CD54 over threshold
Test item: expression CD86 and CD54 under threshold

Main experiment 2:
PC: expression CD86 and CD54 over threshold
Test item: expression CD86 and CD54 under threshold

But: log P_{ow} over 3.5

Inconclusive

Overall prediction: Impossible

The alternative solution is the GARD™ assay, with potency predicition as a further benefit

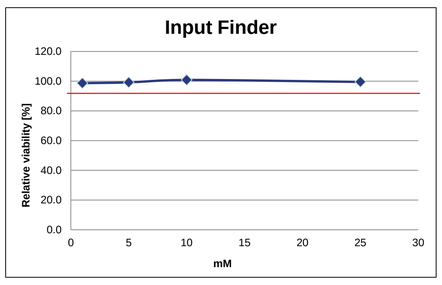
Solution: the GARD™ Assay

The GARD™ skin assay analyses on a genomic level, if a substance is a sensitiser or not. The GARD™ skin assay has further the unique advantage to predict the potency of a skin sensitiser and cassify it into 1A or 1B.

The princeple of GARD™ Assay:
Requirements: The molecular weight of a test material has to be specified.
GARD™ *Assay has been validated with DMSO and water as possible solvents.*

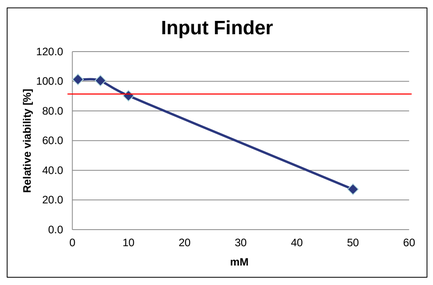
Input finder – Evaluation of cytotoxicity

Test Item 1



No cytotoxicity: the highest soluble concentration for the main stimulation

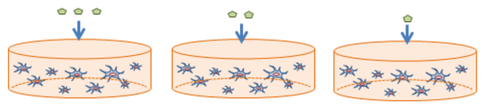
Test Item 2



Cytotoxicity: The concentration with a relative viability of 90% ± 5% for the main stimulation

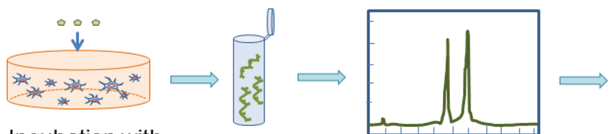
GARD Input Finder - Determination of the test substance concentration, where the cells react and 90% ± 5% survive (RV)

Test substance



Grown SenzaCells are incubated with different concentration of test substance

GARD Main Stimulation: Three independent experiments on the different batches of cells



Incubation with the test substance of determined concentration

RNA isolation

RNA quality check with Bioanalyzer

RNA hybridysation with 196 genes selected for sensitisation.
After hybridisation RNA-samples are prepared on a cartridge by an nCounter Prepstation

Data analysis with nCounter Digital Analyser

RNA quality check and data analysis in collaboration with SenzaGen AB

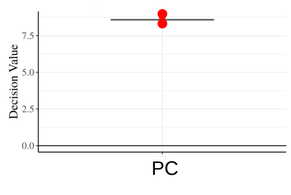
Cytotoxicity main stimulations

		Medium Control	DMSO Control	Positive Control**	Test Item 1	Medium Control	DMSO Control	Positive Control**	Test Item 2
Concentration (mM)		-	-	75	25	-	-	75	15
Main Stimulation 1	Cell viability [%]	93.6	95.6	85.4	95.5	95.7	97.0	83.5	84.7
	RV [%]*	100.0	102.2	91.3	102.1	100.0	101.4	87.3	88.6
Main Stimulation 2	Cell viability [%]	95.7	97.0	83.5	95.7	95.7	97.0	83.5	84.9
	RV [%]*	100.0	101.4	87.3	100.0	100.0	101.4	87.3	88.7
Main Stimulation 3	Cell viability [%]	96.1	95.1	85.5	95.3	95.3	94.9	82.7	82.6
	RV [%]*	100.0	99.0	88.9	99.1	99.1	98.7	86.0	86.0

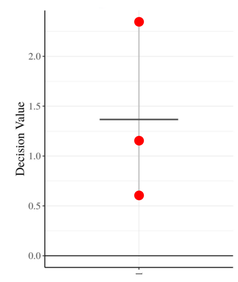
* all toxicity criteria are fulfilled for RNA-isolation; ** p-phenylenediamine

Nanostring analysis; prediction • negative / • positive

5. Potency: analysis of a different RNA-code set; prediction • 1A/ • 1B



	PC	Test Item 2
Main stimulation	Decision value	Decision value
1	8.36	2.34
2	8.69	1.15
3	8.72	0.60
Mean	8.69	1.15
	Prediction	
	1A	**1A**



CONCLUSION

The DPRA, KeratinoSens™ and h-CLAT are well known sensitization assays which address three different key events of the AOP. The GARD™ skin assay is a new procedure that analyses the sensitization potential based on almost 200 human genes. If a substance is a skin sensitiser with the GARD™ skin assay you have the benefit of measuring the potency on top with a different code set to make a 1A or 1B classification.

The GARD™ skin assay is especially for products that have a high log P_{ow} (h-CLAT > 3.5, KeratinoSens™ > 7) because in those cases the classical sensitization tests are inconclusive if negative and there is no option for a replacing test method. Therefore, the GARD™ skin assay is not only an excellent alternative of the sensitization methods for these cases but it can furthermore predict the potency of a skin sensitiser, a unique feature, which makes it a testing method needed in the future.

OECD Guidelines for Testing of Chemicals, number 442C "*In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA)" (adopted: June 18, 2019); OECD Guidelines for Testing of Chemicals, number 442D "*In Vitro* Skin Sensitisation Assays Addressing the AOP Key Event on Keratinocyte Activation" (adopted: June 25, 2018); OECD Guidelines for Testing of Chemicals, No. 442E: "*In Vitro* Skin Sensitisation assays addressing the Key Event on activation of dendritic cells on the Adverse Outcome Pathway for Skin Sensitisation" (adopted June 25, 2018); OECD Guidelines for Testing of Chemicals, number 429 "The Local Lymph Node Assay" (adopted July 22, 2010). Organisation for Economic Cooperation and Development, Paris; Internal requirements from SenzaGen AB (DB-Alm Protocol GARD – Genomic Allergen Rapid Detection (GARDskin) not yet published.