Extended solvent selection for *in vitro* sensitization testing using GARD®

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Introduction

The GARD®skin assay is an *in vitro* assay developed for the assessment of skin sensitizers. It is based on SenzaCells™, a human dendritic-like cell line, and a biomarker signature analyzed by a prediction model including pattern recognition and machine learning.

During the development of the GARD®skin platform, two solvents were used: DMSO (0.1%) and water. To increase the applicability domain of GARD®skin and the possibility to dissolve certain test items, for e.g. hard to dissolve substances and UVCBs, we here show a broader range of solvents compatible with GARD®skin. Also, use of higher concentrations of the tested solvents were explored for the possibility to increase test item concentrations.

Concluding highlights

GARD®skin compatible solvents:

- Acetone
- DMF
- DMF/Glycerol
- DMSO
- Ethanol
- Glycerol
- Isopropanol

Increased applicability domain

Results

At certain solvent concentrations (Table 1), the phenotypic surface markers and viability do not change (Figure 1).

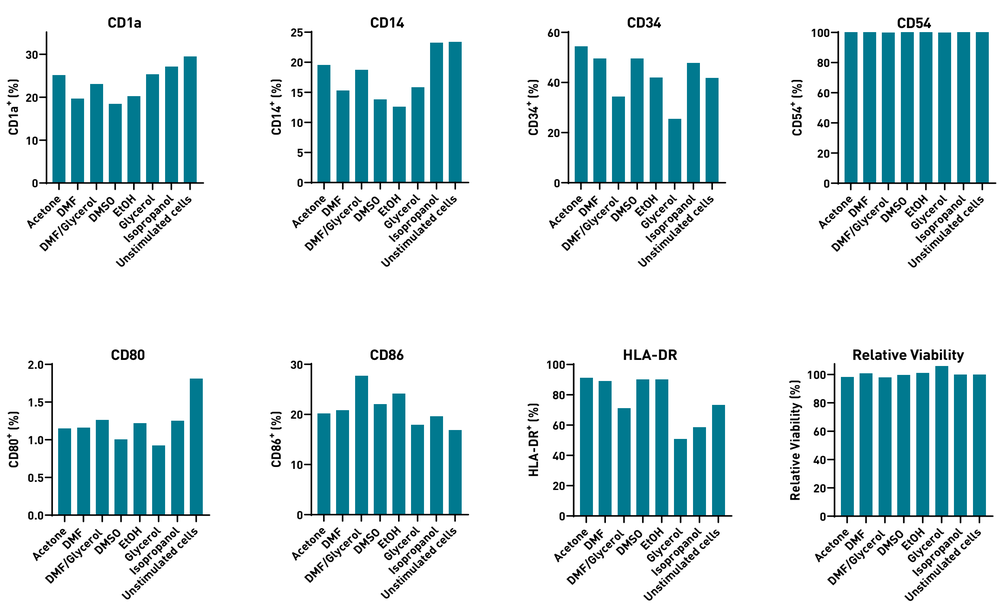


Figure 1. The phenotype markers and viability 24 h after stimulation at the concentrations not generating phenotypic changes.

The biomarker gene expression was explored using principal component analysis (PCA). The solvents clustered together with unstimulated cells, indicating that the gene expression was not affected by the solvents. Further, the non-spiked solvents and the solvents spiked with PPD cluster separately, both in PCA (Figure 2) and heatmap analysis (Figure 3).

Figure 2. PCA plot. n≥3. Green oval circles non-spiked. Pink oval circles PPD spiked samples Isop=Isopropanol, Glyc=Glycerol, D/G=DMF/Glycerol, EtOH=Ethanol, Pos Ctrl=positive control, Neg Ctrl= negative control, Unstim=unstimulated cells.

The selected max solvent concentrations do not induce false positive predictions with GARD®skin. Also, we show that SenzaCells™ are activated when the solvents are spiked with a sensitizer; GARD®skin predictions are positive (Table 1).

Table 1. The max in-well concentrations and the GARD®skin predictions.

Solvent	Max conc. (%)	GARD®skin prediction of non-spiked solvent	GARD®skin prediction of solvent w. PPD
Acetone	1	Non-sensitizer	Sensitizer
DMF	0.25	Non-sensitizer	Sensitizer
DMF/Glycerol	0.25	Non-sensitizer	Sensitizer
DMSO	0.5	Non-sensitizer	Sensitizer
Ethanol	1	Non-sensitizer	Sensitizer
Glycerol	1	Non-sensitizer	Sensitizer
Isopropanol	0.25	Non-sensitizer	Sensitizer

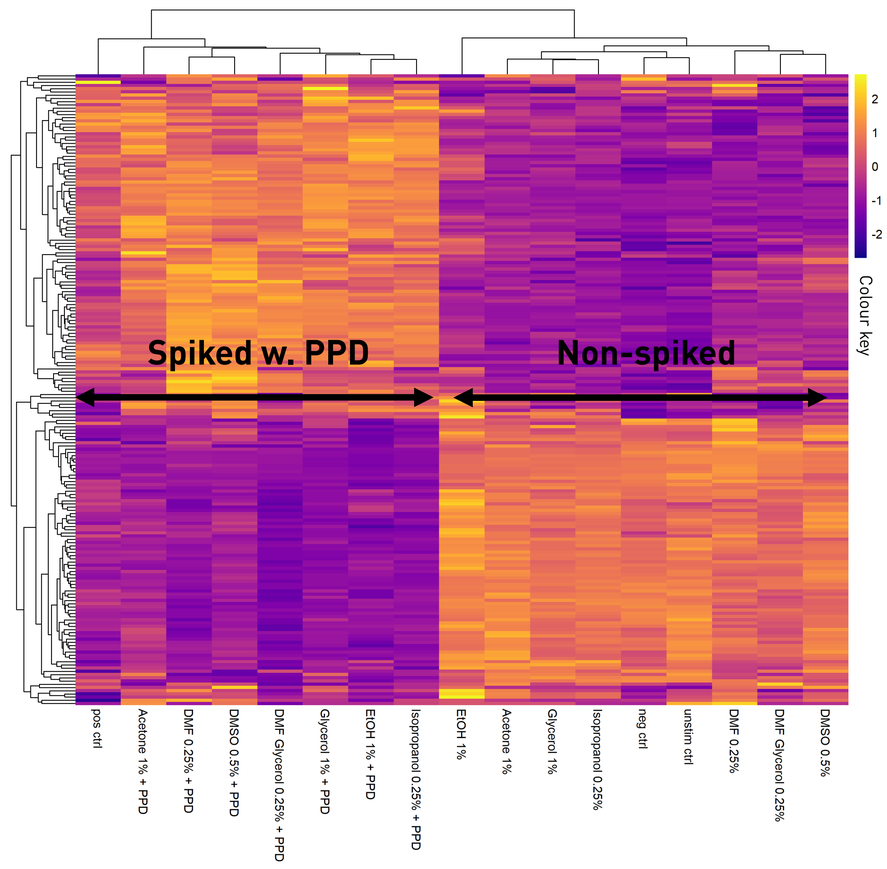
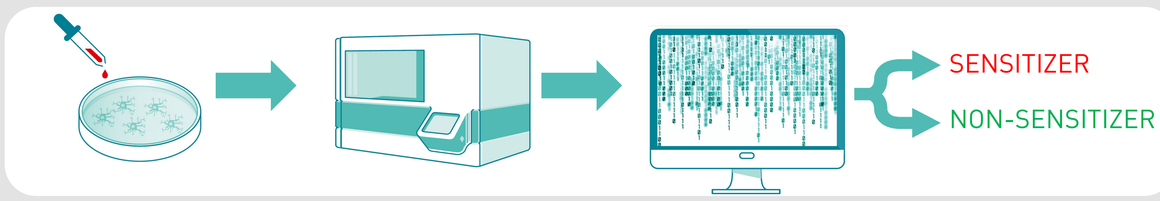


Figure 3. Heat map of the data. The solvents spiked with PPD and non-spiked solvents cluster separately. pos ctrl=positive control, neg ctrl=negative control, unstim ctrl=unstimulated cells.

Together, this shows that the additional solvents listed in this study are compatible with GARD®skin and do not introduce false positives at the used concentrations.

The GARD® platform



In brief, SenzaCells™ are stimulated at a test item concentration generating 90% relative viability (RV90). After 24 h incubation, the transcripts (RNAs) are isolated and quantified with the NanoString technology. A fixed formula derived from machine learning is applied to the measured transcript levels to calculate the prediction.

References: Johansson *et al.* ALTEX, 2017, Johansson *et al.* BMC Genomics, 2011, Forreryd *et al.* BMC Genomics, 2014, Johansson *et al.* Toxicol Sci, 2014.

Material and Methods

Solvent selection: Acetone, Ethanol (EtOH), Dimethylformamide (DMF), DMF/Glycerol (v/v: 4/1) Dimethyl sulfoxide (DMSO), Glycerol and Isopropanol.

Cell stimulations: SenzaCells™ were stimulated with the solvents (0.1% - 2%). The next day, flow cytometry analysis was used to measure the expression CD86, CD54, HLA-DR, CD80, CD34, CD14 and CD1a or cell viability (Propidium Iodide). Solvent concentrations eliciting no change in phenotype or viability, and predicted as non-sensitizers, were selected for a second round of stimulations spiked with PPD as a positive control.

Gene expression analysis: The GARD®skin gene expression signature was analysed using R and the GARD®skin application (GDAA).