# Extended applicability domain with new solvent selection for the GARD®platform

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## Introduction

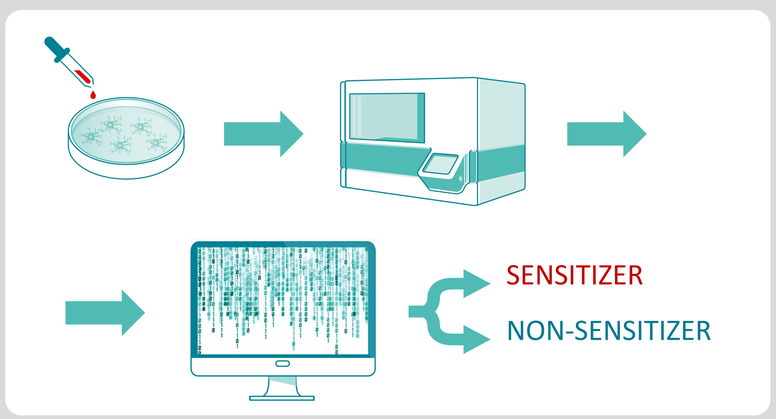
The Genomic Allergen Rapid Detection (GARD®) assay is a state of the art *in vitro* assay developed for the assessment of skin sensitizers. It is based on gene expression analysis of SenzaCells, a human myeloid cell line, after stimulation by the test item.

During the development of the GARD® platform, two solvents were used; DMSO (0.1%) and Water. To increase the applicability domain of GARD® and the solubility of certain test items, for e.g. Medical Device extracts and UVCBs, we here show a broader range of solvents compatible with GARD®.

## Conclusions

Here, we show that the GARD® platform is compatible with the following solvents:

- Acetone
- Ethanol
- Glycerol
- Super refined olive oil
- DMF
- DMSO
- Water
- Isopropanol




## The GARD®platform

**Figure 1.** The dendritic cells (DCs) are sentinels of the immune system during sensitization. Upon stimulation their gene expression is altered and the cells become activated. This is reflected by the GARD® platform which is based on SenzaCells, a human myeloid cell line similar to DCs. The assay protocol includes stimulation of the cells by the test item. Prior to the main stimulation, after which the transcripts are harvested, a screen for the appropriate test item concentration is performed. This concentration is defined as giving ~90% relative viability (RV90) or to a max concentration of 500 µM. The NanoString technology is used for the transcript quantification and bioinformatic multivariate technologies involving pattern recognition are used for gene expression analysis.

## Objectives

To dissolve substances to be tested by the GARD® protocols, DMSO (0.1%) or Water have previously been used. To broaden the applicability domain of GARD® we wanted to test a broader range of solvents and/or a higher solvent concentration. Also, we wanted to explore the possibility to use highly non-polar solvents, as this is requested by regulatory authorities for Medical Devices extracts (ISO 10993).

## Material and methods

To examine the maximum limit concentration of Acetone, Dimethylformamide (DMF), DMSO, Ethanol, Glycerol and Isopropanol, cells were stimulated with the solvents in a range between 0.1% and 5%. After stimulation, the cells were analysed by flow cytometry after staining with CD86 antibodies or PI (Propidium Iodide) and analysed by flow cytometry. Cells with an increased expression of CD86 and/or high PI signal were regarded as activated and/or less viable, respectively.

To explore the possibility to use oils as extraction vehicles in combination with the GARD® platform, three oils were selected: Sesame oil, Super refined sesame oil and Super refined olive oil. Oils are highly non-polar and not compatible with conventional aqueous cell system. To circumvent this a trans well system was used. Furthermore, the oil was spiked with a sensitizer, 2-hydroxyethylacrylate (2-HA), for verification of the system.

Finally, the RNA was harvested from the selected stimulations and the GARD®skin prediction signature consisting of ~200 genes analysed.

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

## Results and discussion

All solvents were found to be non-sensitizing at the concentrations selected for GARD®skin gene expression analysis (Table 1 and Figure 2). Therefore, those can be considered as compatible with the platform, not introducing background effects.

**Table 1.** The solvent concentrations not generating cytotoxicity and/or increase in CD86 expression.

| Solvent | concentration (%) |
|---|---|
| Acetone | 1 |
| DMF | 0.25 |
| DMSO | 0.25 |
| Ethanol | 0.5 |
| Glycerol | 2 |
| Isopropanol | 0.25 |

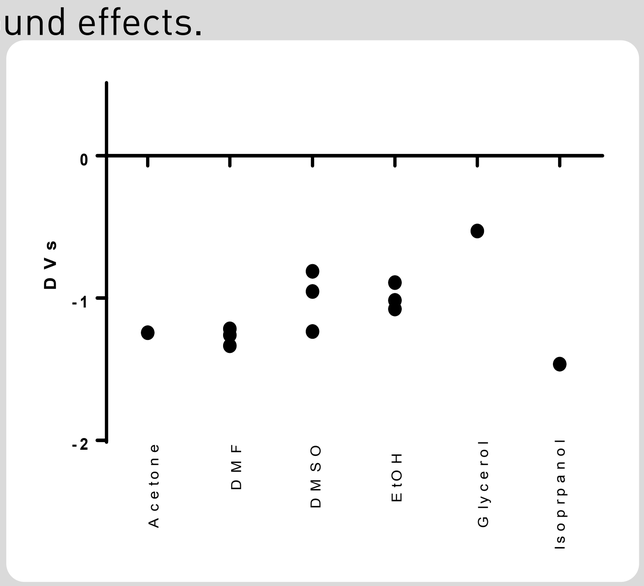


**Figure 2.** The GARD®skin decision values (DVs) generated by the bioinformatic gene expression analysis after solvent cell stimulation. DVs < 1 = non-sensitizers, DVs > 1 = sensitizers

Notably, it was found that the DMSO concentration could be increased from 0.1 to 0.25%, introducing a possibility to test poorly soluble substances.

The oils extracts did not induce cell cytotoxicity (PI staining), but we found that all of them introduced minor sensitizing effects (positive Decision Values, DVs, Figure 3). However, the super refined oils had very low (DVs), especially the olive oil. This makes it an extraction vehicle candidate as it can be used for relative sensitization assessment. Further, spiking the oil with the sensitizer 2-HA, gave the expected cytotoxicity, RV90 was found, and the GARD®skin DV shows clearly that the substance is a sensitizer.

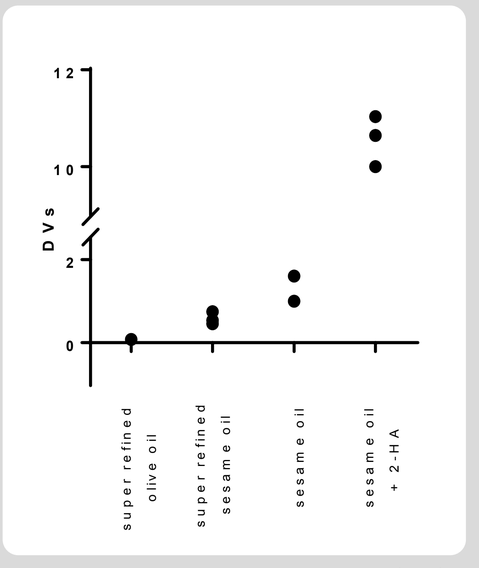


**Figure 3.** The GARDskin decision values (DVs) generated by the bioinformatic gene expression analysis after oil cell stimulations. DVs < 1 = non-sensitizers, DVs > 1 = sensitizers

Interestingly, the unrefined sesame oil showed a higher DV compared to the other oils. This may be explained by the fact that it is known to introduce sensitization in sensitive individuals.