Reliable and Truly Animal-Free Skin Sensitization Testing – Adaption of the In vitro GARD™skin to Animal-Free Conditions

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Introduction

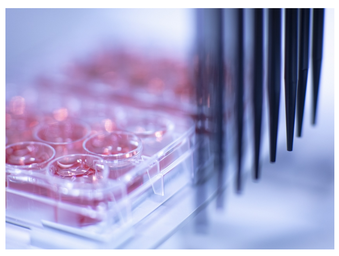
Significant progress has been made in the development and regulatory implementation of New Approach Methodologies (NAMs) for the endpoint of skin sensitization. However, most of these assays still rely on the use of animal-derived products, such as Foetal Calf Serum (FCS) within their standard protocols. Sourcing of FCS is associated with significant animal welfare concerns, and replacement of animal serum is an important step towards truly animal-free sensitization testing.

The GARDskin assay (OECD TG 4.106) represents the next generation of *in vitro* assays for skin sensitization testing based on genomics and machine learning. The aim of this poster is to describe a novel adaption of the GARDskin assay to enable future testing under animal product free (APF) condition by replacing FCS with Human Serum (HS) in the cell maintenance protocols.

Methods

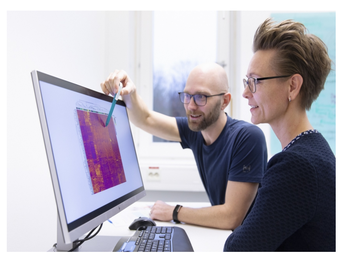
GARDskin was performed according to validated protocols (Figure 1). In short, the cellular rationale for the assay is the human dendritic cell-like cell line SenzaCell. Following chemical exposure of cells to a test chemical, the transcriptional levels of the genes in the GARDskin prediction signature were measured and used as input into the GARDskin classification algorithm, based on a priori defined Support Vector Machines (SVM) prediction model. For final classifications, any test chemical inducing a positive (≥0) mean GARD Decision Value (DV) is classified as a skin sensitizer.

For the purpose of this study, transition to APF cell maintenance protocols were performed on steady-state cells by exchanging FCS with HS (20% (v/v)). The impact of APF cell culture protocols was investigated by monitoring morphology, cell growth and phenotypic profiles of FCS-dependent and HS-dependent cell cultures during a time span of five passages. Following the establishment of an HS dependent cell culture protocol, the animal-product-free version of GARDskin was validated internally using a proficiency set of chemicals (n=11).



Step 1.
The SenzaCell cell line is utilized as an *in vitro* cell system mimicking dendritic cells.

Step 3.
Gene expression analysis is performed in order to evaluate a set of predictive genomic biomarkers.



Step 4.
Gene expression data is analysed using the GARDskin prediction model, allowing for classification of sensitizers.

Readout: Decision Value (DV) > 0 = Sensitizer, Decision Value (DV) < 0 = Non sensitizer

Figure 1. Overview of the GARDskin workflow

Results

SenzaCells adapted well to APF maintenance protocols, with similar morphology (data not shown) and growth rates to cells cultivated in animal derived FCS (Figure 2A). No statistically significant difference was observed between the two cell culture conditions on the average cell surface expression of the cell surface markers CD54, CD86, HLA-DR, or CD80. The expression of CD14 and CD1a was lower in the HS-dependent compared to FCS dependent cell cultures (Figure 2B).

The expression of all markers were within the range observed from historical data for cells cultivated in FCS, and within the acceptance criteria for GARDskin (Table 2). The GARDskin protocol adapted to human serum demonstrated full concordance for hazard classifications to the protocol based on animal-based FCS, with all classifications fully corresponding to available reference data (Figure 2C).

Discussion

The work presented in this study represents our ambition to gradually replace all animal-derived components from the GARDskin protocols with human equivalents to enable completely animal-free testing. Replacement of the animal-derived sera was considered as the most critical component to start with, since its production is associated with significant animal welfare concerns, and early replacement and establishment of a cell bank for future testing could avoid unnecessary use of large volumes of animal sera. Based on the results of this study, SenzaCells adapted well to routine culture in HS and the replacement of animal-derived FCS with a human equivalent did not significantly alter growth rates or morphology of cells. Although minor differences were observed for the phenotypic markers CD14 and CD1a in this study, expression of these surface markers were within the range observed from historical data for cells cultivated in FCS. The animal product-free version of the GARDskin protocol demonstrated full concordance to the protocol based on animal-derived FCS, with all classifications in agreement with available reference data. This work demonstrates the potential to successfully replace the GARDskin cellular maintenance protocols to animal-product-free conditions, thus providing an appealing *in vitro* approach for truly animal-free skin sensitization hazard testing.

References
Johansson H. et al. Validation of the GARDskin Assay for Assessment of Chemical Skin Sensitizers: Ring Trial Results of Predictive Performance and Reproducibility. Toxicological Science. 2019

Conclusion

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- Senzacells™ adapted well to routine culture in human serum with comparable cell growth, morphology, and minimal impact on cellular phenotypes.
- GARD™skin based on HS demonstrated full concordance to protocols based on animal-derived FCS for hazard identification of skin sensitizers.
- This work represents our ambition to gradually replace all animal derived components with human equivalents to enable completely animal-free skin sensitization testing.

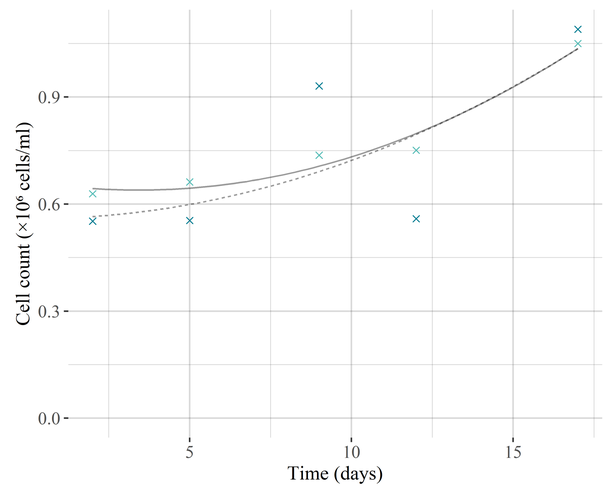

Figure 2A. Cellular growth rates
Cellular growth was monitored for cells cultivated in either HS or FCS during five passages. Second degree polynomials were independently fitted to measurements from the different culturing conditions to capture potential differences in cell growth. No differences were observed for the different culturing conditions.

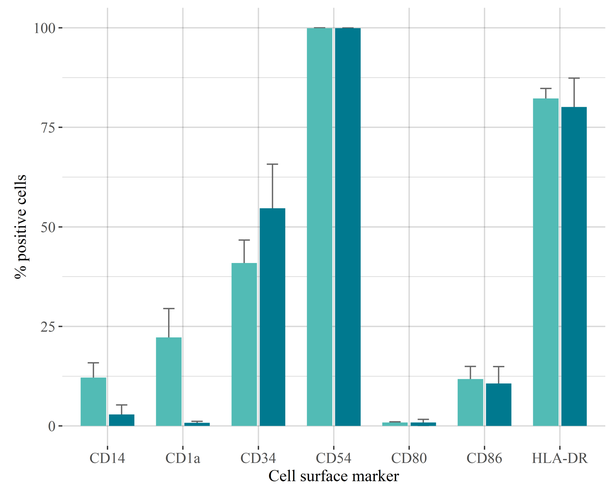

Figure 2B. Comparison of Phenotypic analysis
A set of common myeloid cell maturity markers comprising the QC acceptance criteria in GARDskin was quantified using flow cytometry. No statistical difference were observed for CD34, CD54, CD86, HLA-DR, or CD80. Significant differences were observed for CD14 and CD1a, which were lower in the HS dependent culturing condition.

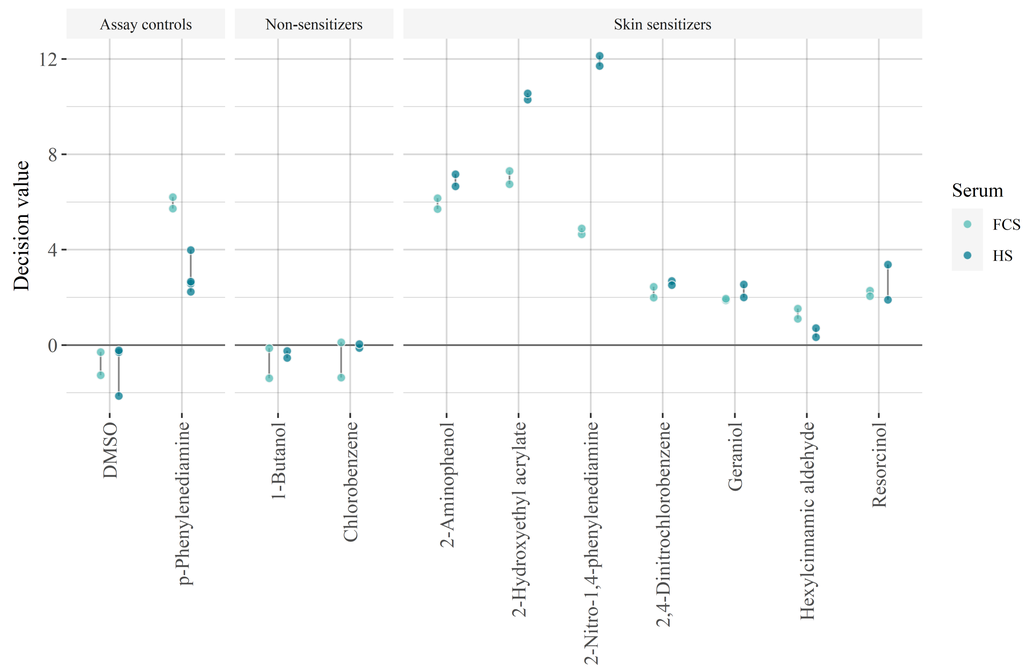

Figure 2C. GARDskin hazard classifications
A proficiency set of chemicals (n=11) was evaluated in the GARD skin assay based on cells cultivated in either HS or FCS using the protocol described in Fig.1. Each chemical was evaluated in biological replicates (n=2). Final classifications were based on mean decision values (n=2) (DV ≥ 0 = skin sensitizer, DV < non-sensitizer). GARD DVs for individual chemicals were highly reproducible between replicate samples in the human serum, and the magnitude of decision values demonstrated good concordance between the different culturing conditions. All chemicals were correctly classified in both culturing conditions.

Table 2. GARDskin acceptance criteria
Summary of the accepted range of expression for the individual phenotypic markers that comprise the acceptance criteria in GARDskin.

Phenotypic marker	Accepted range (%pos cells)
CD86	10-40
CD54	+
HLA-DR	+
CD80	< 10
CD34	+
CD14	+
CD1a	+

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