



Supporting industry decision-making and hypoallergenicity claims using an experimental setup combining GARD[®]skin Medical Device and Dose-Response protocols

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1. Introduction

Biocompatibility testing is a crucial part of medical device safety assessments both for regulatory acceptance of finished products but also within the developmental pipelines of novel devices. Along with cytotoxicity and irritation, skin sensitization is a key toxicological endpoint that must be assessed for all devices. While testing for the two former endpoints is partly conducted *in vitro*, traditional animal experimentations for skin sensitization testing are still routinely conducted. Thus, validation and implementation of New Approach Methods (NAMs) remains a prioritized goal of the medical device industry.

The GARD[®]skin method (OECD TG 442E) is a NAM for hazard assessment of skin sensitizers based on test chemical exposure of dendritic cell-like cells followed by a genomic readout. Several method adaptations have been proposed in order to address specific needs of various industry segments. Such adaptations include the GARD[®]skin Dose-Response (DR) method, which facilitates quantitative assessment of sensitizing potency, and the GARD[®]skin Medical Device (MD) method, which facilitates testing of solid materials in accordance with ISO 10993 standards.

Here, we describe an experimental setup combining the protocols of above-mentioned GARD[®]skin adaptations, which was utilized to evaluate the relative hypoallergenicity of two sensitive skin dressings: a candidate product "Urigo Peaux Sensibles+" and a marketed comparative product "Urigo Peaux Sensibles".

2. Methods

The GARD[®]skin method (OECD TG 442E) is an *in vitro* assay for assessment of chemical skin sensitizers. The method is based on evaluation of transcriptional patterns of an endpoint-specific genomic biomarker signature in a dendritic cell-like cell line following test chemical exposure. The GARD[®]skin MD method incorporates procedures described in ISO 10993-12, in order to allow for assessment of extracts from solid materials (Figure 1). In short, polar and non-polar solvents are utilized to extract leachables from test materials. Extracts are added to cell cultures at 10% (v/v). (Jenvert et al, 2024)

The GARD[®]skin DR method provides quantitative potency assessment of skin sensitizers. The method evaluates test chemicals in a titrated range of concentrations, in order to investigate the dose-response relationship between the output from the GARD[®]skin prediction algorithm (Decision Values; DV:s) and test chemical concentration (Figure 2). The combined information can be used to derive a quantitative estimation of sensitizing potency, defined as the cDV₀-value, i.e., the least required dose required to elicit a positive response by the prediction model. The cDV₀-value has been confirmed to correlate strongly and statistically significantly with reference values of estimated sensitizing potency, such as LLNA EC3-values and human NESIL values.

In the present study, GARD[®]skin MD and DR protocols were combined in order to compare the relative sensitizing potency of two test materials: A novel wound dressing in development and a comparative product already on the market, labelled as hypoallergenic. The extraction from the solid test items was performed according to ISO 10993-12, but only in a polar vehicle, saline, and with an increased test item to extraction vehicle ratio of 6x. The oversaturated extracts were subsequently subjected to serial dilution facilitating dose-response testing in order to evaluate, assess and compare the test items' relative skin sensitizing potentials.

3. Results

The study outcome showed that none of the two test item extracts induced a positive response at any of the assayed concentrations. Therefore, both were classified as non-sensitizers. (Figure 3).

Furthermore, evaluation of the relative difference between the two test items concluded that there is no statistically significant difference (p=0.05, student's t-test at each respective concentration).

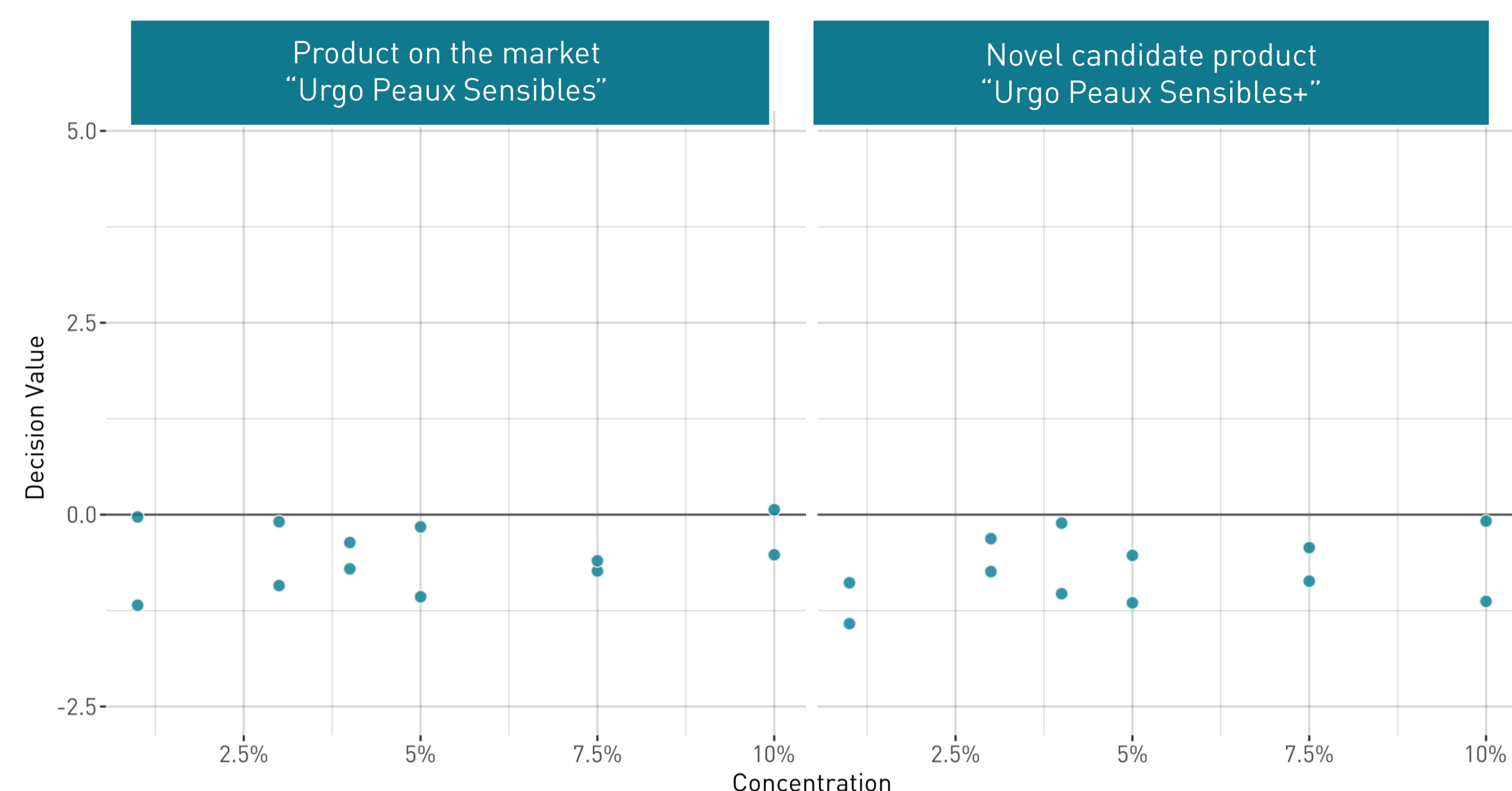
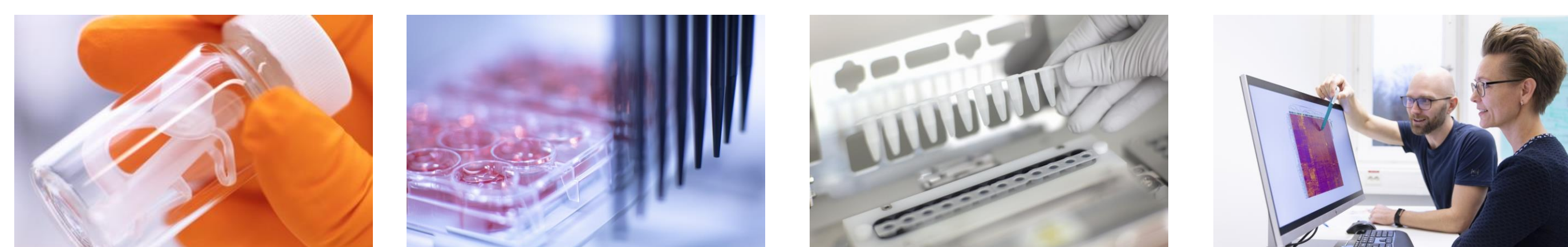


Figure 3. Study results for the two test items evaluated using the combined GARD[®]skin Medical Device and GARD[®]skin Dose-Response protocols. Decision Values (DV)s generated from six tested concentrations are shown for each item, demonstrating no positive response at any concentration.

References:
 OECD 2022, Test No. 442E: In Vitro Skin Sensitization, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing.
 Gradin et al, 2021, Quantitative assessment of sensitizing potency using a dose-response adaptation of GARDskin. Nature Scientific Reports.
 Jenvert, et al., 2024, Evaluation of the applicability of GARDskin to predict skin sensitizers in extracts from medical device materials. Frontiers in Toxicology.

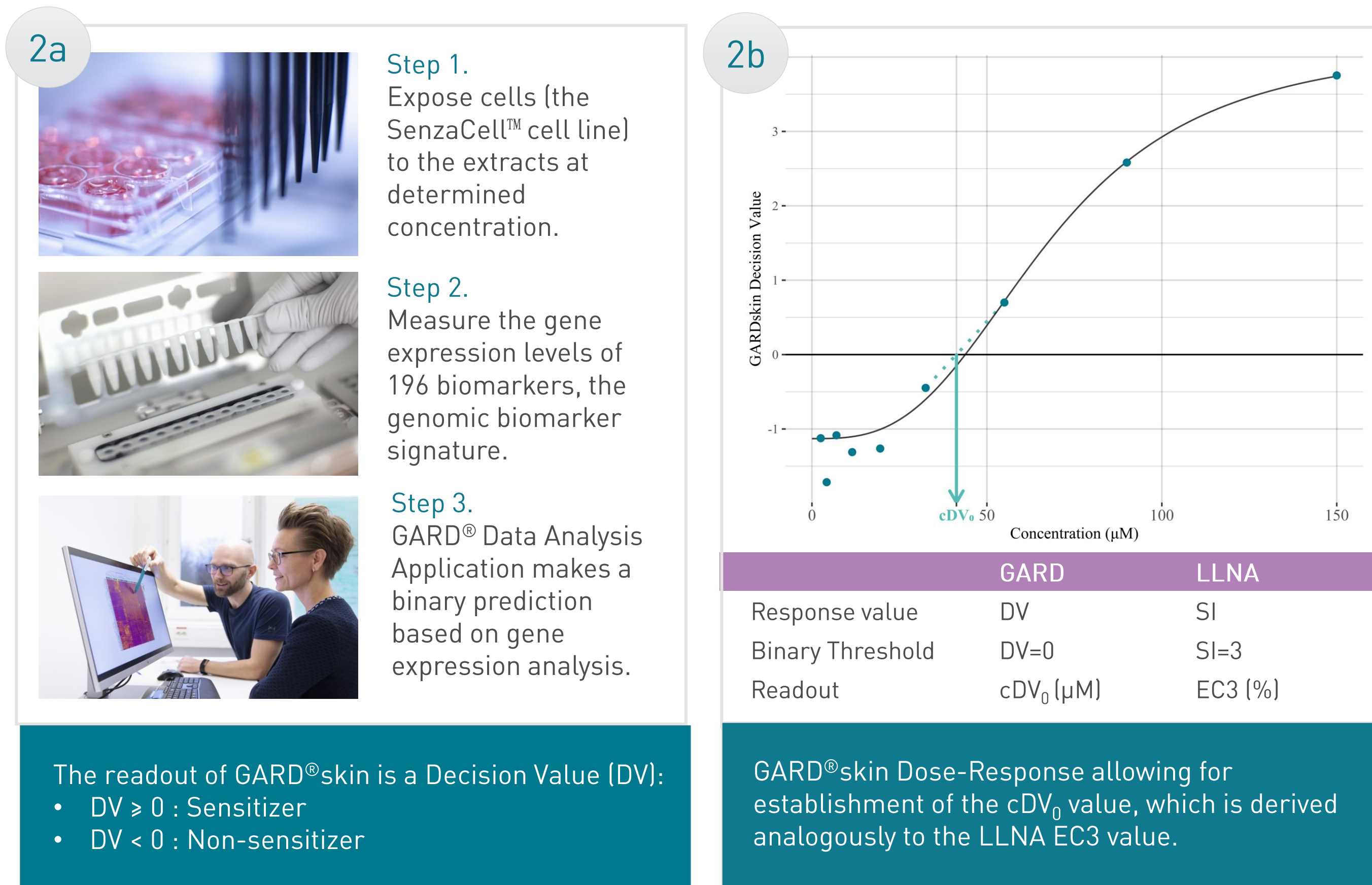
- New Approach Methodologies
- Supporting hypoallergenicity claim
- Medical wound dressings

Skin Sensitization | ISO 10993-10



Step 1. Prepare extracts from test items according to ISO 10993-12:2021.
Step 2. Expose cells (the SenzaCell™ cell line) to the extracts at determined concentration.
Step 3. Measure the gene expression levels of 196 biomarkers, the genomic biomarker signature.
Step 4. GARD[®] Data Analysis Application makes a binary prediction based on gene expression analysis.

Figure 1. Overview of the GARD[®]skin Medical Device assay, adapted from the standard GARD[®]skin method (OECD TG 442E). Testing of the extracts was performed according to the GARD[®]skin Medical Device protocol which combines the extraction protocol as described in ISO 10993-12:2021. The assay generates two Decision Values (DV), one for polar extracts and one for non-polar extracts. A positive value in either indicates skin sensitizing potential of the test item.



The readout of GARD[®]skin is a Decision Value (DV):
 • DV ≥ 0 : Sensitizer
 • DV < 0 : Non-sensitizer

GARD[®]skin Dose-Response allowing for establishment of the cDV₀ value, which is derived analogously to the LLNA EC3 value.

Figure 2. The experiment setup of GARD[®]skin Dose-Response. 2a. shows the standard GARD[®]skin assay in three steps. 2b. Illustrates the dose-response curve generated from GARD[®]skin testing of a test chemical in a titrated range of concentrations.

4. Discussion and Conclusions

In this study the sensitizing potential of two medical device products (wound dressings) was assessed and compared using the combined GARD[®]skin Medical Device and GARD[®]skin Dose-Response protocols.

None of the test item extracts induced a positive response at any of the assayed concentrations and were subsequently classified as non-sensitizers. While this may be considered expected, it is noteworthy that the herein reported study was conducted under extraordinary conditions, following a manifold increase of the material to vehicle ratio during extraction with respect to the extraction procedure otherwise adhering to the standards described in ISO 10993-12.

Taking also into account the demonstrated low limit of detection and proven sensitivity of the GARD[®]skin cell system (Gradin et al., 2021), labelling the novel candidate product as hypoallergenic, or suitable for sensitive use, are supported by the data.

In conclusion, both tested dressings are considered hypoallergenic. These findings illustrate how testing strategies based solely on NAM:s can be integrated in the development of novel devices, providing data for critical decision-making without resorting to animal experimentation for regulatory approval.

This case study demonstrates how NAMs like GARD[®] can be effectively integrated into product development for assessing skin sensitization potential in medical device extracts.

It enables evidence-based decision-making and supports hypoallergenic claims, without the use of animal testing.