# Applicability domain of the GARD™skin Medical Device test for *in vitro* skin sensitization testing of medical devices.

Joshua Schmidt[1], Ron Brown[2] and Rose-Marie Jenvert[3]
[1]SenzaGen Inc., Raleigh, NC, USA, [2]Risk Science Consortium LLC, Arnold, MD, USA, [3]SenzaGen AB, Lund, Sweden.

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## Conclusions

- The chemical space of compounds tested in GARD closely approximates the chemical space of compounds known to be released from medical device materials.
- GARDskin is able to predict the skin sensitization potential of compounds released from medical device materials with a high degree of sensitivity and specificity, including:
  - metals
  - lipophilic compounds
  - pre/pro haptens

## Introduction

The potential for a medical device to induce a skin sensitization response in patients must be evaluated as part of the biological evaluation of the device, as specified in the ISO 10993-1:2018 standard[5]. Currently, animal-based tests (e.g., GPMT, LLNA) are typically used to assess skin sensitization potential. However, several *in vitro* skin sensitization test methods have shown promise as alternatives to the animal tests. To date, the predictive ability of these *in vitro* methods has not been specifically evaluated using compounds typically found in materials used to manufacture medical devices. Therefore, the goal of this study is to assess the applicability domain of the *in vitro* assay, GARDskin Medical Device, and determine the predictive ability of the test method for chemical compounds that may be released from medical device materials.

## Methods

Two approaches were used to evaluate the predictive applicability domain of the GARDskin Medical Device assay. First, the functional applicability domain of GARDskin was evaluated by examining the ability of the assay to assess the skin sensitization potential of a wide range of compounds released from medical devices including difficult to test compounds that are pre/pro haptens and lipophilic compounds as well as several metal salts.

In addition, the applicability domain of the assay was evaluated by visually comparing the chemical space of compounds tested in GARDskin to the chemical space of compounds known to be released from polymeric materials using the Chemical Characterization module provided in the National Toxicology Program's Integrated Chemical Environment (ICE) software: https://ice.ntp.niehs.nih.gov/.

Evaluation of the applicability domain was done using results from the GARDskin assay. The GARDskin Medical Device is an adaptation of GARDskin using the same genomic biomarker signature, and validated for use with saline, sesame oil, and olive oil. The step-wise procedure for conducting the assay is illustrated in **Figure 1**.

Step 1. Prepare extracts from test items according to ISO 10993-12:2021[6].

Step 2. Expose cells to the extracts at determined concentration.

Step 3. Measure the gene expression levels of 200 biomarkers.

Step 4. Binary prediction based on gene expression analysis.

**Figure 1.** The GARDskin Medial Device assay in four steps.

## Results and Discussion

### Metals and inorganic compounds

Predicting the sensitization potential of metals released from medical devices can be challenging using conventional animal-based tests since the results of these tests do not always reflect the human response. Here we show that the GARDskin assay was able to correctly predict the human skin sensitization potential of four metal ions; zinc, cobalt, nickel, and chromium (+6), as well as the inorganic compound, potassium permanganate (**Table 1**). The ability of the GARDskin assay to correctly predict the skin sensitization potential of these compounds is notable given the clinical significance of metals such as nickel and cobalt as sensitizing agents which can be released from a variety of products, including medical devices.

Importantly, the GARDskin assay was able to correctly identify zinc sulphate and potassium permanganate as non-sensitizers, demonstrating the ability of the assay to accurately distinguish between metallic/inorganic sensitizers and non-sensitizers.

**Table 1.** GARDskin prediction of the skin sensitization potential of metal ions and inorganic compounds. HP: Human potency[1].

| | GARD | HP |
|---|---|---|
| Cobalt Chloride | | |
| Nickel sulfate hexahydrate | | |
| Potassium dichromate | | |
| Potassium permangenate | | |
| Zinc sulphate | | |

skin sensitizer
non-sensitizer

### Lipophilic compounds and Pre/Pro haptens

Due to the characteristics of lipophilic compounds and pre/pro haptens, it can be challenging to assess the skin sensitization potential of these compounds using *in vitro* assays.

Nevertheless, our results show that the GARDskin assay is able to correctly predict the skin sensitization potential of compounds with these characteristics. As shown in **Table 2** the GARDskin assay correctly predicted the *in vivo* skin sensitization potential of 13 out of 14 lipophilic compounds and 12 out of 13 of the pre/pro haptens that are known to be released from materials used for medical devices, for a total accuracy of 90.5% with one false negative and one false positive prediction. The high predictive performance of the test method for compounds with high LogP indicates that GARDskin has a high sensitivity and hence has the potential to pick up low concentrations of skin sensitizers in extracts from medical devices. The results also show that the assay has the metabolic capacity to convert pre or pro haptens to their active haptenic form.

**Table 2.** GARDskin prediction of the skin sensitization potential of organic compounds[1,2,3,4]. *In vivo*, results from LLNA and Human potency[1].

| | LogP | Pre/pro hapten | GARD | *In vivo* |
|---|---|---|---|---|
| Tocopherol | 12 | - | | |
| Lauryl gallate | 6.75 | YES | | |
| Abietic acid | 6.28 | YES | | |
| Sodium dodecyl sulphate | 4.99 | - | | |
| 2-Ethylhexyl acrylate | 4.37 | - | | |
| Benzyl salicylate | 4.16 | YES | | |
| Bisphenol A-diglycidyl ether | 3.98 | - | | |
| Benzyl benzoate | 3.94 | - | | |
| Hexane | 3.87 | - | | |
| Octanoic acid | 2.98 | - | | |
| 2-Mercaptobenzothiazole | 2.96 | YES | | |
| Benzoyl peroxide | 2.78 | YES | | |
| Diethyl phthalate | 2.62 | - | | |
| Salicylic acid | 2.19 | YES | | |
| 4-Aminobenzoic acid | - | YES | | |
| Ethylenediamine | - | YES | | |
| Hydroquinone | - | YES | | |
| p-Phenylendiamine | - | YES | | |
| Propyl gallate | - | YES | | |
| Resorcinol | - | YES | | |
| Toluene diamine sulphate | - | YES | | |

### Chemical Space Analysis

A substantial overlap of the chemical space was seen for compounds tested in the GARDskin assay and compounds released from polymeric materials when compared using Principal Component Analysis (PCA) on the basis of molecular descriptors (**Figure 2**) and chemical properties (**Figure 3**).

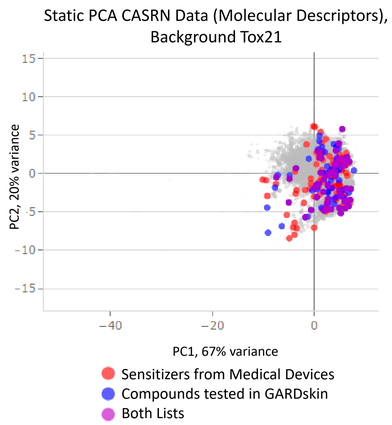


**Figure 2.** Chemical space analysis by structure comparing the compounds tested in the GARDskin assay (blue) and compounds released from polymeric materials (red).

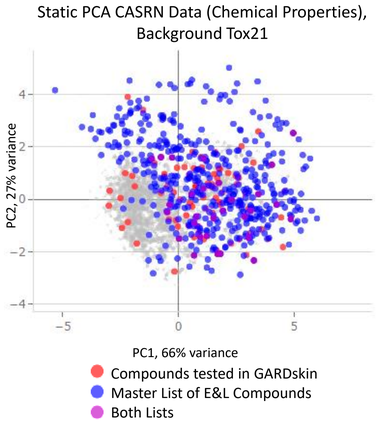


**Figure 3.** Chemical space analysis by chemical properties comparing the compounds tested in the GARDskin assay (red]) and compounds released from polymeric materials (blue).

Overall, the evaluation of the applicability domain for the GARDskin Medical Device assay shows that the test method has the ability to predict the skin sensitization potential of compounds that may be released from materials used to manufacture medical devices with a high degree of accuracy, specificity, and sensitivity.

### References:

[1] Basketter et al., 2014 Dermatitis 25(1), p 11-21.
[2] Johansson et al. 2019, Toxicological Sciences, 170 (2), p 374–38
[3] Johansson et al., 2017 ALTEX, 34(4), p 515-523
[4] Forreryd et al., 2016, Toxicol In Vitro. 37, p 178-188
[5] ISO 10993-1:2018 , Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process
[6] ISO 10993-12:2021 Biological evaluation of medical devices — Part 12: Sample preparation and reference materials

Contact: Rose-Marie Jenvert, PhD, rose-marie.jenvert@senzagen.com