

Conclusions

- GARD[®]skin is well adapted to risk assess the skin sensitizing potential of medical devices in accordance with ISO 10993.
- GARD[®]skin Medical Device classified the tested commercially available face masks as non-sensitizers and the nitrile glove as sensitizer.
- New *in vitro* technologies, like GARD[®], is well suited as a routine tool to increase the speed of decision making in extraordinary situations as a pandemic.

Introduction

Since the start of the SARS-CoV-2 pandemic there has been a growing demand for protective gear, such as face masks and nitrile gloves, both in hospital setting and for the general public. As a consequence, instances of adverse skin reactions have increased.

The assessment of skin sensitization is a part of the biological evaluation of both face masks and nitrile gloves as they are classified as medical devices, and this is done in accordance with the ISO 10993 standards [1]. Historically, the Guinea Pig Maximization Test has been used for this purpose, but recent development of *in vitro* testing strategies has spurred the investigation into the use of new approach methodologies to replace animal tests.

Here, a commercially available face mask and nitrile gloves are investigated using the GARDskin Medical Device assay, which is an *in vitro* assay for hazard identification of skin sensitizers. The assay uses the transcriptional readout from a dendritic-like cell line together with a genomic biomarker signature to perform hazard assessment of unknown substances. The assay has been adapted to be able to use polar- and non-polar extraction vehicles to assess solid materials from e.g. medical device products, which is in accordance with ISO 10993-12 [2].

Method

The GARDskin assay [3] is a next-generation *in vitro* assay for hazard assessment of skin sensitizers. The method evaluates the transcriptional patterns of a genomic biomarker signature in a human dendritic-like cell line following exposure, in order to provide hazard assessments of substances. The method has been adapted to testing of medical device products, by application of extraction protocols using polar- and non-polar extraction vehicles, in accordance with ISO 10993-12 [2].

In short, a human dendritic cell-like cell line, SenzaCell, was exposed to the test item extracts followed by RNA purification and gene expression measurements. The transcriptional levels of the genes in the GARDskin prediction signature were measured and used as input in the GARDskin classification algorithm, which is based on a Support Vector Machine prediction model. Final classifications were given depending on the algorithm's predicted value, the decision value (DV), where a positive DV (DV ≥ 0) classifies the test item as a sensitizer and a negative classification (DV < 0) classified the test item as a non-sensitizer. The stepwise procedure for conducting the assay is illustrated in Figure 1.

The assessment of skin sensitization potential of chemicals was done using the GARDskin Medical Device assay using a polar extraction vehicle for the extraction of the face mask and nitril glove. For this study, the face masks were investigated coming from four different production batches and the nitrile gloves from one production batch.

Extracts from the face masks and the nitrile glove were made using a polar extraction vehicle, PBS supplemented with 1% PEST, with an extraction ratio of 0.2 g per ml for the facial mask and 6 cm² per ml for the nitrile glove. The extraction was performed at 37°C for 72 h. Input concentrations for the GARDskin Medical Device assay were determined based on cytotoxic properties of the extracts, with a maximum concentration of 10% if non-cytotoxic and a targeted concentration inducing 90% relative viability of the cells if cytotoxic.

Results

In total, four different production batches of face masks and one production batch of nitrile gloves were investigated for their skin sensitizing potential using the GARDskin Medical Device assay. The extracts from the face masks showed no cytotoxic activity and were used at an in-well final concentration of 10%. The PBS extract of the nitrile glove was strongly cytotoxic and was therefore titrated to achieve a 90% relative viability of the cells in the assay (Figure 2A). An in-well concentration of 5% nitrile glove extract yielded a relative viability of 90% and was chosen as input concentration in the assay.

For final classifications, as sensitizers or non-sensitizers, the saline extracts were analyzed using the GARDskin Medical Device assay, assigning GARD DVs to each test item based on their intrinsic sensitizing capacity (Figure 2B). The mean GARD DVs for all four production batches of face masks were negative, resulting in a final classification as non-sensitizers. The nitrile glove on the other hand showed a positive mean DV, resulting in a final classification as skin sensitizer.

Discussion

In the current situation of a pandemic, the use of protective gear has increased. This has led to more instances of reported adverse effects, such as allergic skin reactions. In order to meet the demand for product safety as well as a safe personal and occupational environment, new technologies that have better accuracy and short turn-around time, need to be developed. *In vitro* technologies are currently being investigated to replace the conventional animal tests for this purpose.

The work presented in this study uses the next generation *in vitro* assay, GARD, for assessment of skin sensitization potential of two commercially available protective gears, a face mask and nitrile gloves. The results indicate that no skin sensitizing chemicals are extracted during simulated use conditions, as the extracts from four batches of face masks were classified as non-sensitizing. The nitrile glove extract was however classified as skin sensitizing while also showing cytotoxic effects on the cells.

The results presented here may implicate that the increase in skin reactions seen under the pandemic when using face masks can be driven by the combination of nitrile gloves and face masks. However, other reasons behind the increase in adverse reactions can also be irritant-related responses caused by friction through continuous wear of the mask or exposure to chemicals with skin irritating properties, endpoints not investigated in the GARD assay.

In conclusion, the increase in adverse reactions during the pandemic, caused by a rapidly increased market for protective equipment, has shown the need for accurate safety testing for risk assessment of products. The GARDskin Medical Device assay is a non-animal approach that can be utilized as a reliable and fast method to risk assess new materials and product candidates, but also existing products already established on the market, in order to prevent instances of adverse reactions caused by the use of protective gear.

#P23-34, Abstract ID 402

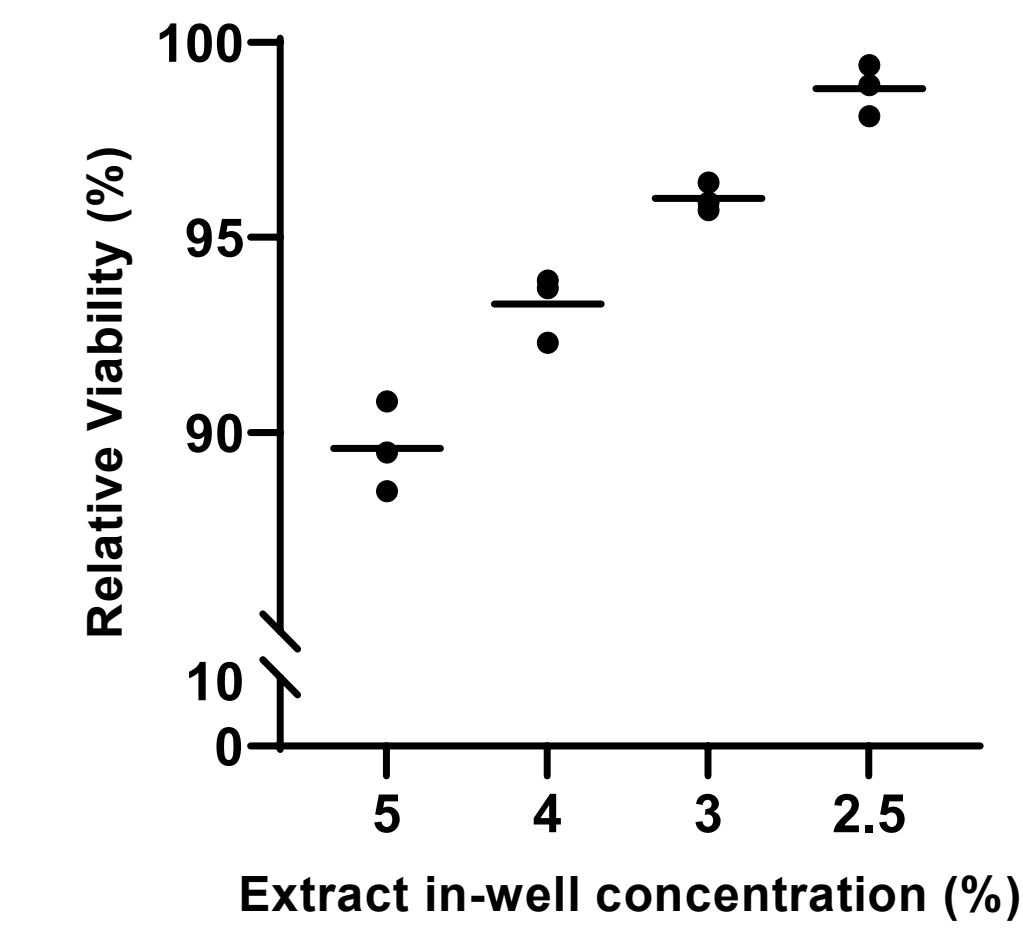
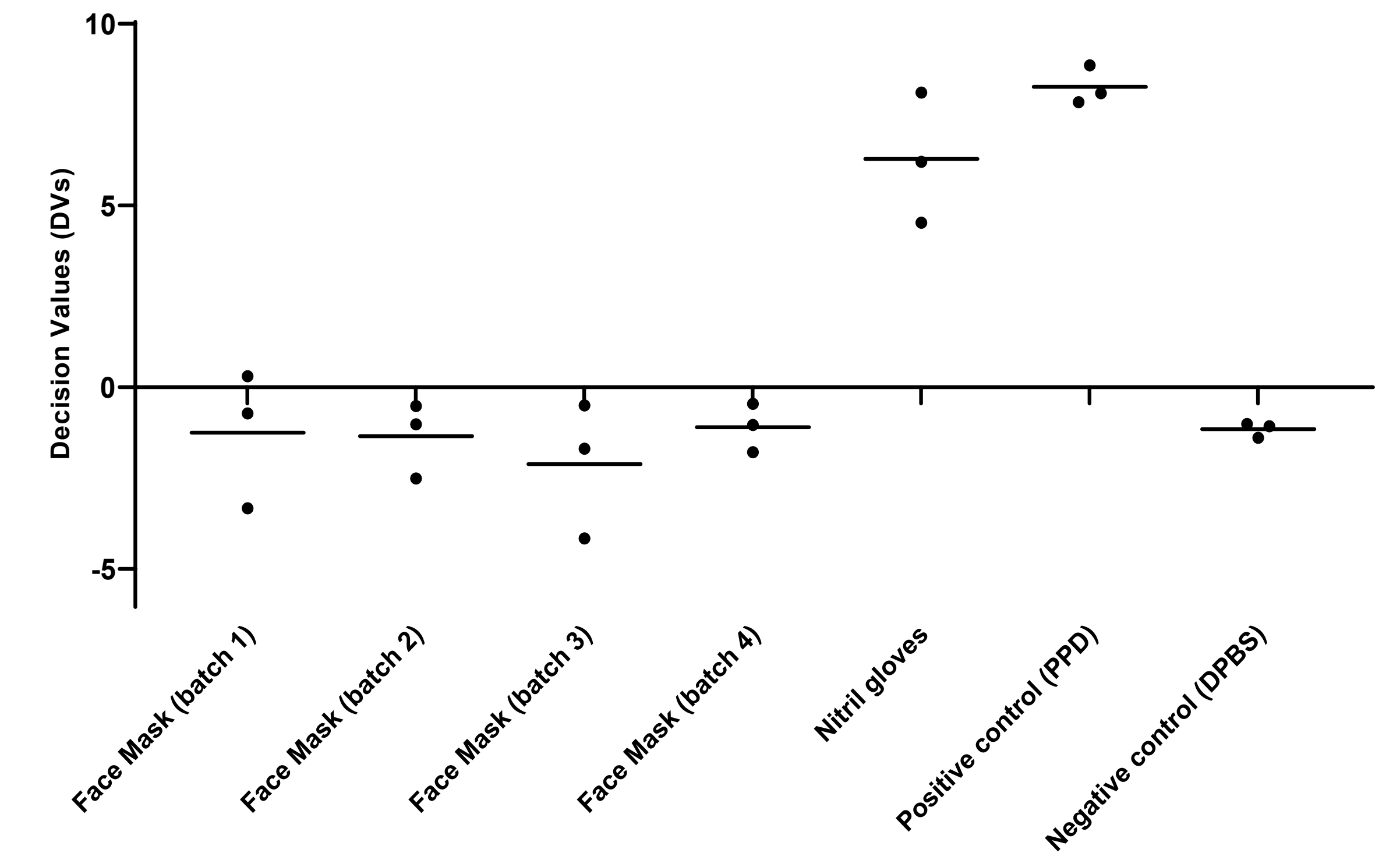


Figure 2A. Titrated range of in-well concentration for nitrile glove extract.

At the maximum concentration of 10% the nitrile glove extract was cytotoxic, so a titrated range of in-well concentrations was investigated to achieve a relative viability of 90%.

Figure 2B. GARD[®]skin Medical Device hazard classifications

DVs for polar extracted face mask and nitrile gloves. Each test item was run in triplicates with mean DV (straight line) being used for final classification.



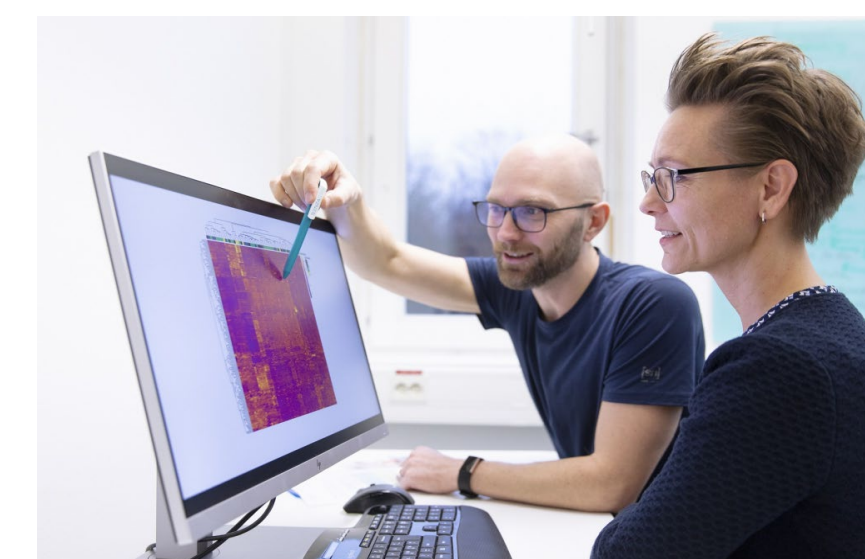
Step 1. Prepare extracts from test items according to ISO 10993-12:2021 [2].



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.

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

Figure 1: The GARD[®]skin Medical Device assay in four steps, including extraction procedure.

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