In vitro skin sensitization testing of Medical Devices

All you need to know about the GARD™ technology for *in vitro* sensitization testing of Medical Devices

Rose-Marie Jenvert, PhD

About SenzaGen

- Founded in 2010
- Spin-out from Lund University after over 10 years research
- Highly multidisciplinary team: 20+ employees today

- Business model: global industrial and CRO partnerships
- Own laboratory, continuous development of the technology

Our lead product, GARD™ stands for Genomic Allergen Rapid Detection and is a state-of-the art test platform for assessment of chemical sensitizers

Launched assays:
- GARD™skin/GARD™potency for skin sensitization
- GARD™air for respiratory sensitization
- GARD™skin Medical Device for skin sensitization assessment of medical devices

Items tested include:
- Active pharmaceutical ingredients
- Cosmetic ingredients
- Industrial chemicals
- Agrochemicals
- Medical device materials
- UVCBs, Pre-/pro haptens

- Since Sept. 2017, SenzaGen AB's shares have been traded on Nasdaq First North, Stockholm (SENZA)
- Partners in USA, EU, China, Korea

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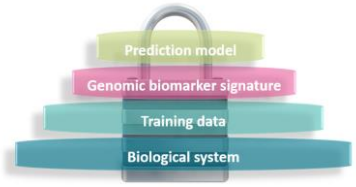
The GARD platform – Currently available assays

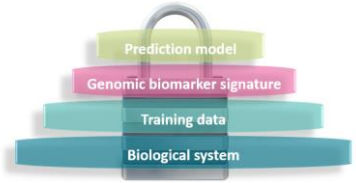


GARD™skin (200 genes)

To identify the skin sensitization hazard of chemicals

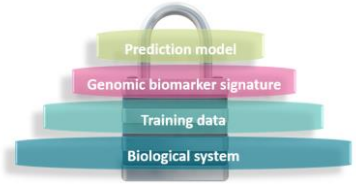
GARD™skin Medical Device (200 genes)

To identify the skin sensitization hazard of medical devices



GARD™potency (51 genes)

Skin sensitization potency classification according to GHS/CLP



GARD™air (28 genes)

To identify the respiratory sensitization hazard of chemicals

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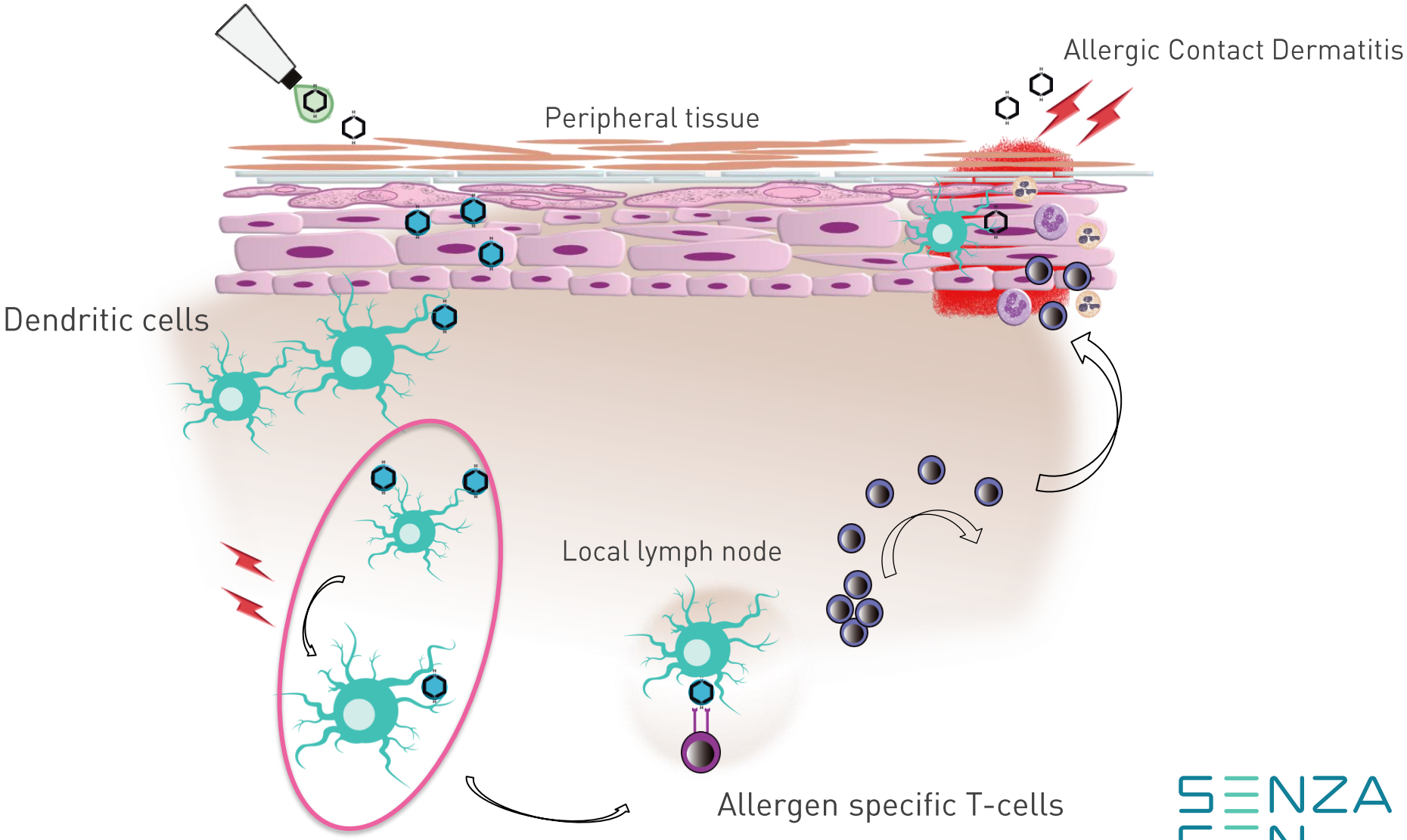
The GARD technology platform

Human relevant cells in combination with Genomics and machine learning

An intricate response to foreign substances



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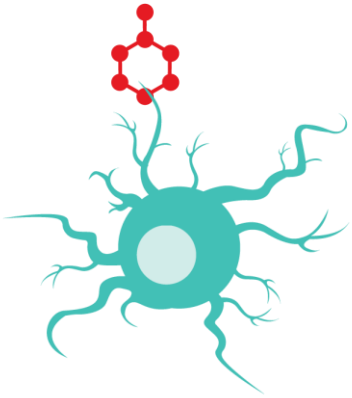
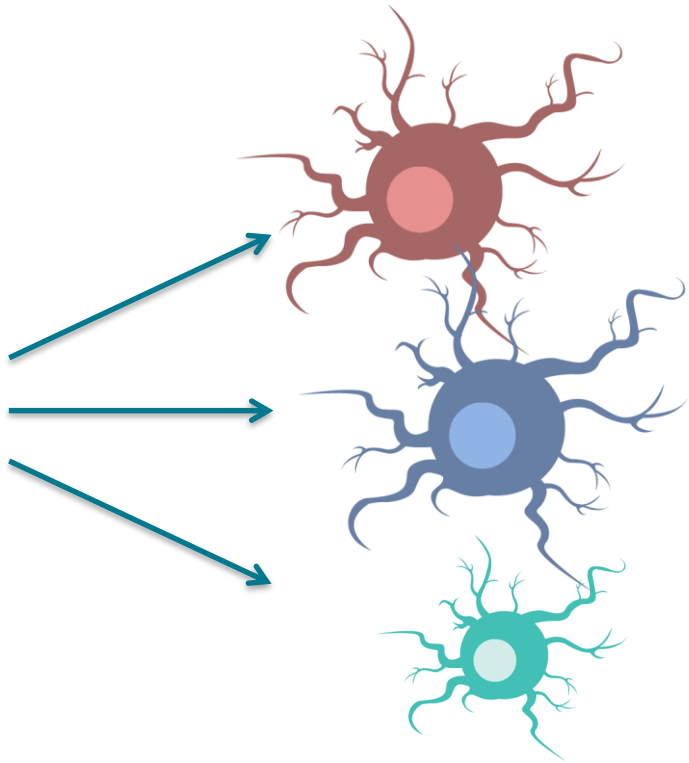
The GARD platform – how it works

SenzaCells: a human dendritic-like cell-line



Cellular responses



Skin sensitizer

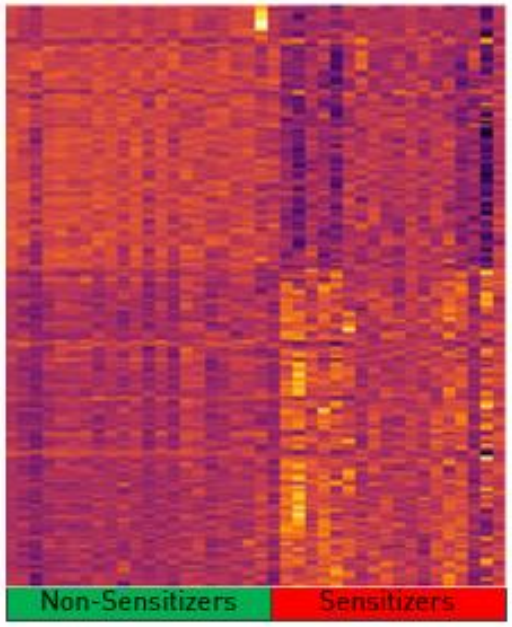
Respiratory Sensitizer

Non-sensitizer

The GARD platform – how it works

Cellular response is monitored using biomarker signatures – **Not only a single biomarker**

GARDskin prediction signature
200 genes



Recognition of foreign substances
e.g. **TLR**s, **RXR**, **AHR**

Immunological self-defence mechanisms
e.g. **CD80**, **CD86**

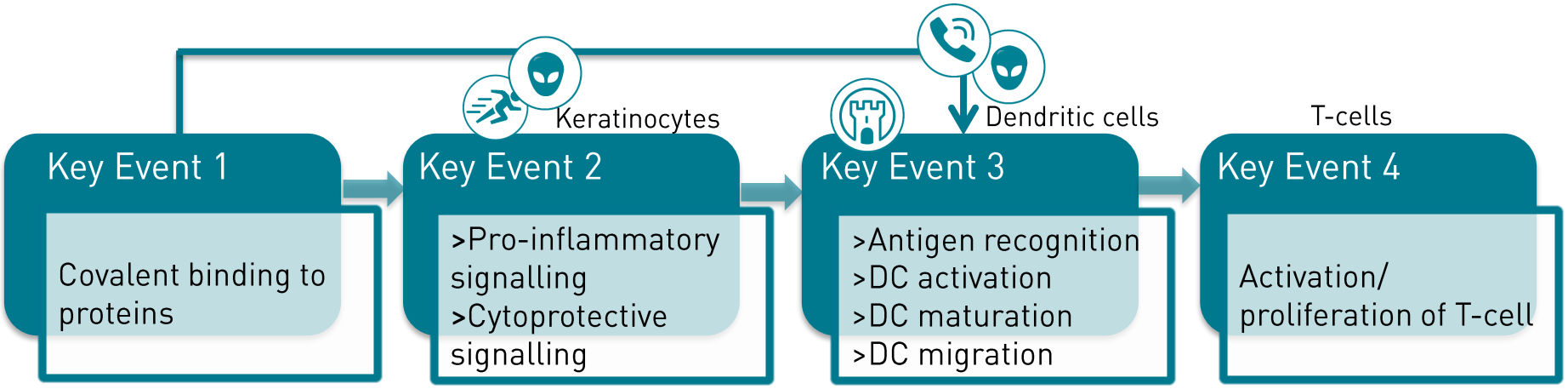
Cellular stress responses
e.g. **NRF2**-pathway

Communication
e.g. chemotaxis receptors

BMC Genomics

Johansson *et al. BMC Genomics* 2011, **12**:399
http://www.biomedcentral.com/1471-2164/12/399

The GARD platform – how it works



Captures events downstream of KE1

Metabolic activity & identifies pre/pro haptens
ALDH
NAT-1
CYP - Cytochrome p-450

> Keap1-Nrf2-ARE pathway & AHR signalling
NQO1
HMOX1
Thioredoxin reductase I

> Pro-Inflammatory cytokines mediating e.g. TNFα, **INFγ, IL-8**
FAS
MAP2KI
COX20

> Inflammasome
NLRP
PSTPIP1

> DC migration & maturation
CD86
MAPK- activation
PKA- and GPCR- mediated signaling

> Antigen recognition & Innate immune activation
TLR-4
TLR-6
RXRA – retinoic X receptor
NLRP
PSTPIP1

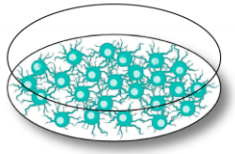
> Self-defence mechanisms
C3a/C5a-activation pathways

Covers the 3 Key steps for T-cell activation:

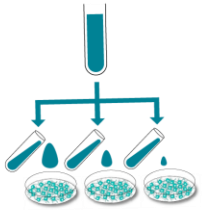
Antigen presentation
Co-stimulation
Cytokine secretion

How to GARD your product - in 6 Steps

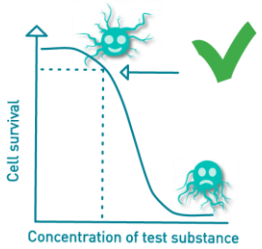
1 GARD Input Finder



Grown SenzaCells

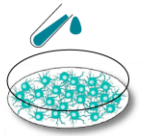


Add different concentrations of the test substance to the cells



Determine the concentration of the test substance where the cells react and 90% survive

2 GARD Main Stimulation



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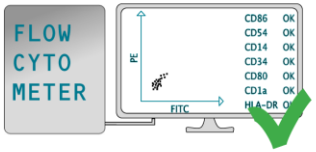


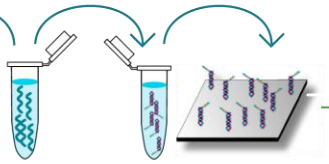
Quality control of the cells

3 RNA extraction

Extract RNA from the cells

4 Gene expression profiling

Check the RNA quality



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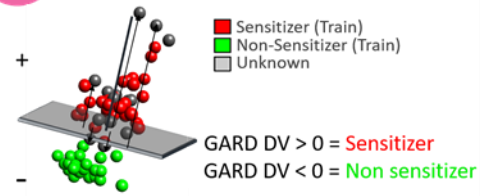
Analyze the probe cassette to quantify the RNA

5 GARD data analysis application



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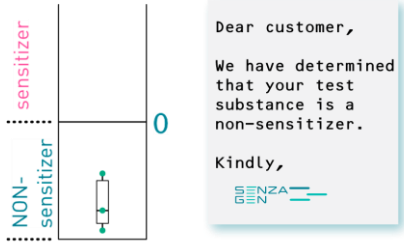
6 Results



The results are yours!

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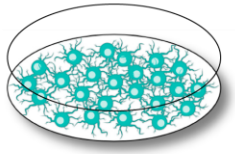

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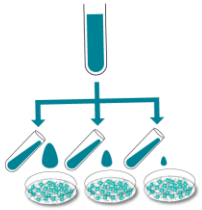

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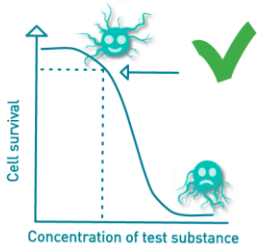

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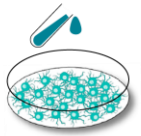

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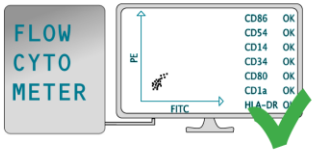

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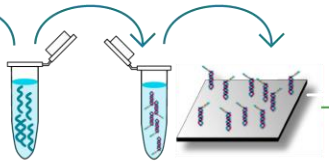

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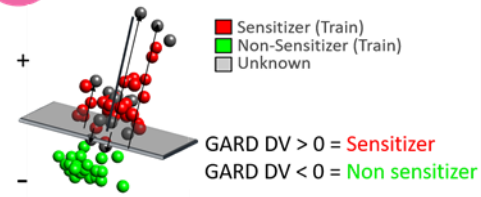

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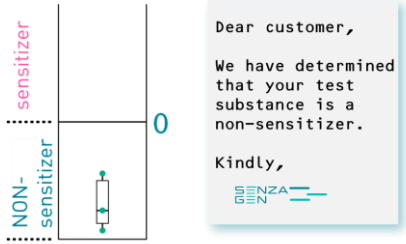

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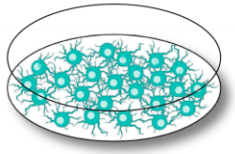

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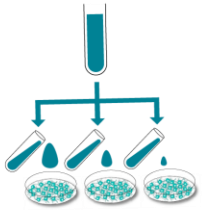

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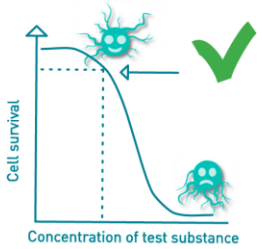

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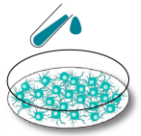

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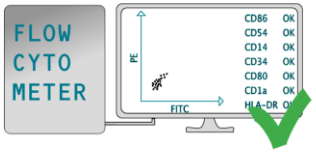

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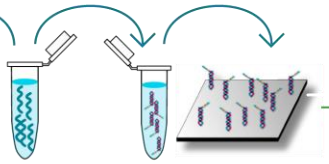

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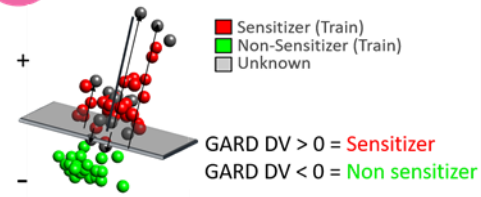

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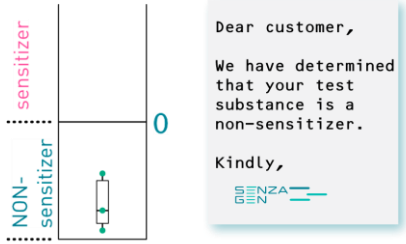

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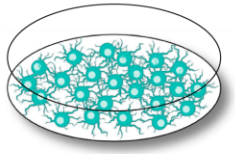

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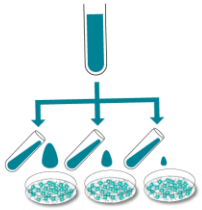

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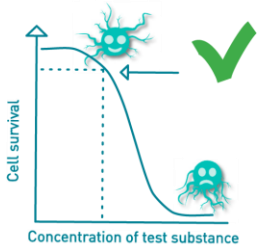

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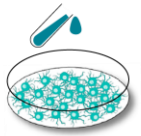

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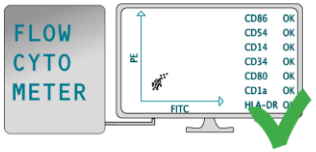

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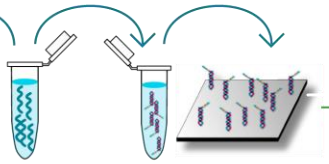

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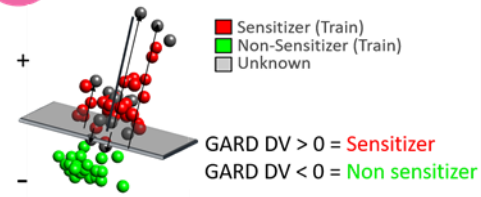

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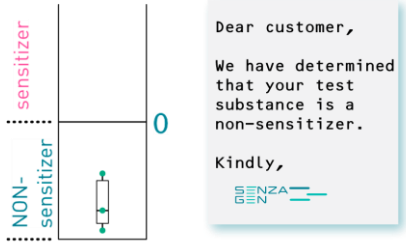

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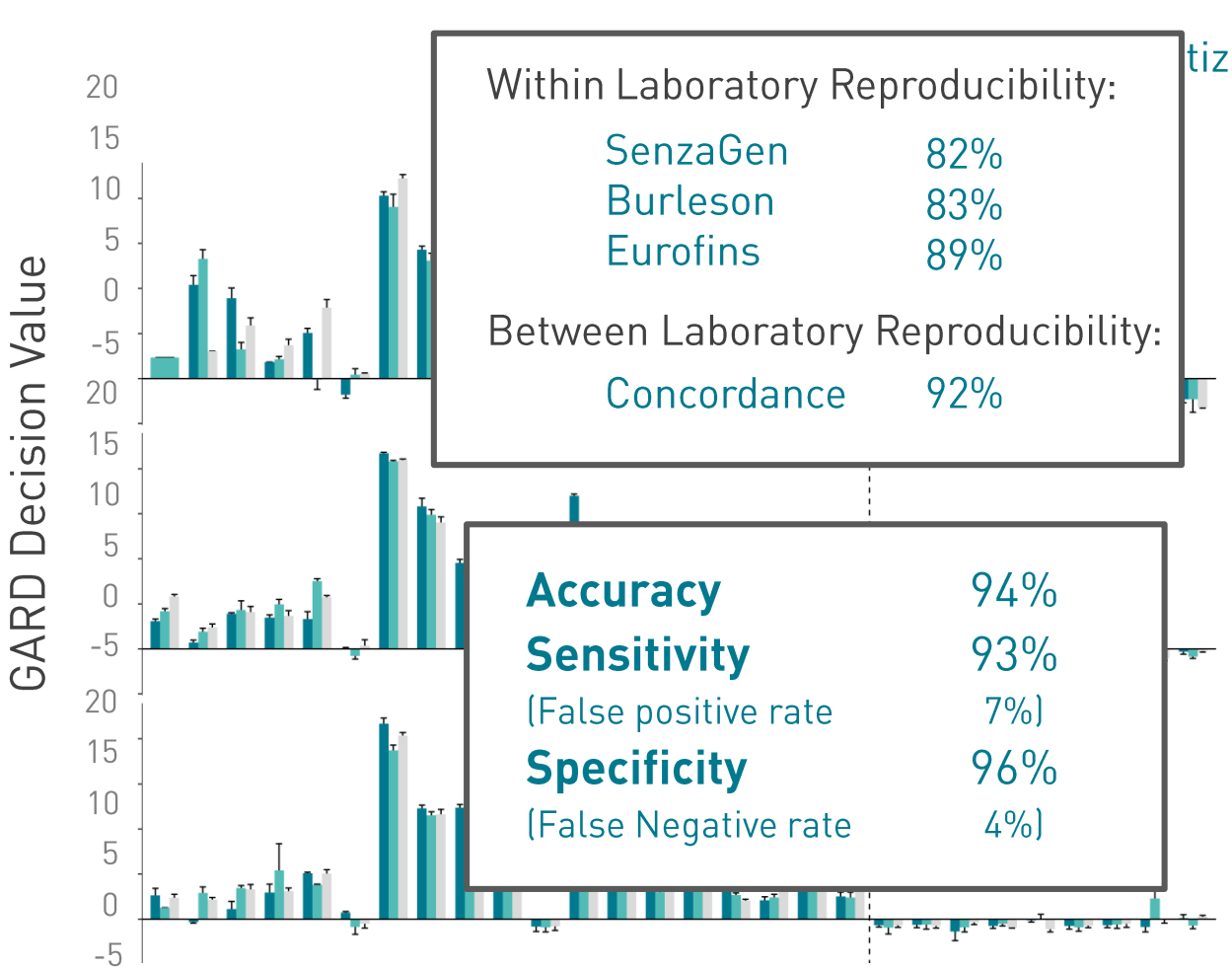
GARD™skin validation



TOXICOLOGICAL SCIENCES, 170(2), 2019, 374–381

doi: 10.1093/toxsci/kfz108
Advance Access Publication Date: May 17, 2019
Research Article

Validation of the GARD™skin Assay for Assessment of Chemical Skin Sensitizers: Ring Trial Results of Predictive Performance and Reproducibility

Henrik Johansson,[1] Robin Gradin,[1] Angelica Johansson,[1] Els Adriaens,[2] Amber Edwards,[3] Veronika Zuckerstätter,[4] Anders Jerre,[1] Florence Burleson,[3] Helge Gehrke,[4] and Erwin L Roggen[5]

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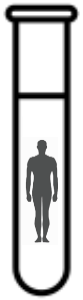
Development of GARD™skin Medical Device

Genomics in combination with machine learning and pattern recognition

In vivo

70-75%
Accuracy

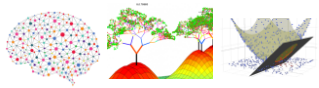
In vitro



New generation toxicology testing

HUMAN GENOME ORGANISATION
HUGO

$$maximize\ f(c_1 \dots c_n) = \sum_{i=1}^{n} c_i - \frac{1}{2}\sum_{i=1}^{n}\sum_{j=1}^{n} y_i c_i \left(\varphi(x_i) * \varphi(x_j)\right) y_j c_j$$
$$= \sum_{i=1}^{n} c_i - \frac{1}{2}\sum_{i=1}^{n}\sum_{j=1}^{n} y_i c_i k(x_i, x_j) y_j c_j$$
$$Subject\ to \sum_{i=1}^{n} c_i y_i = 0, and\ 0 \le c_i \le \frac{1}{2n\lambda}\ for\ all\ i$$



- Artificial Neural Networks
- Random Forests
- Support Vector Machines

90-95%
Accuracy

SENZA GEN

Development of GARD™skin Medical Device

Requirements

- Polar and non-polar extraction vehicles according to ISO 10993-12:2012
- Sensitive enough to detect low-level of skin sensitizers in extracts

Development

- Find oil that works with the assay
- Demonstrate sensitivity of the assay, by perform analysis on materials spiked with known skin sensitizers

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GARD™skin Medical Device – in house validation

Extraction vehicles

- Saline
- Super Refined Olive Oil
- Sesame oil, Ph Eur

Material

- Silicone and TPU spiked with five known skin sensitizers
- Tubes (Silicone, TPU and PVC)

Controls

- Negative control, vehicle control
- Positive control, vehicle spiked with P-phenylenediamine (PPD)

Extraction conditions

- 0.2 g/ml
- 37 ± 1°C for 72 ± 2h

SENZA GEN

GARD™skin Medical Device – in house validation

Extraction conditions: 0.2 g/ml, 37 ± 1°C for 72 ± 2h

Summary of the GARDskin Medical Device results from the materials used in this study compared with LLNA (as listed in the CE STTF database) and Human potency classification (HP) for the chemicals (Basketter et al. 2014)

Test material	Chemical	Sensitizing potential		GARD®skin Medical Device Prediction		
		LLNA	HP	Saline	Olive oil	Sesame oil
Silicone	None	N/A	N/A	Non-sensitizer	Non-sensitizer	Non-sensitizer
	2-aminophenol	Strong	Cat 2	Sensitizer	Sensitizer	Sensitizer
	Cinnamic aldehyde	Moderate	Cat 2	Sensitizer	Sensitizer	Sensitizer
	Propyl gallate	Strong	Cat 2	Sensitizer	Sensitizer	Sensitizer
	Phenyl benzoate	Weak	Cat 3	Sensitizer	Sensitizer	Sensitizer
TPU	None	N/A	N/A	Non-sensitizer	Non-sensitizer	Not tested
	Propyl gallate	Strong	Cat 2	Sensitizer	Sensitizer	Not tested
	Phenyl benzoate	Weak	Cat 3	Sensitizer	Sensitizer	Not tested
Silicone tube	-	N/A	N/A	Non-sensitizer	Non-sensitizer	Non-sensitizer
TPU tube	-	N/A	N/A	Non-sensitizer	Non-sensitizer	Non-sensitizer
PVC tube	-	N/A	N/A	Non-sensitizer	Non-sensitizer	Non-sensitizer
Vehicle control	-	Neg		Non-sensitizer	Non-sensitizer	Non-sensitizer
Positive control	p-Phenylenediamine	Pos		Sensitizer	Sensitizer	Sensitizer

The spiked materials were produced by Research Institute of Sweden (RISE)
The tubes are supplied by Medizintechnik Promedt

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How can you use GARD™ for skin sensitization testing of Medical Devices

- **Product development**

- **Changes during product life cycle**
  - Change of material
  - Change of supplier
  - Sterilization changes
  - Change of production site

- **Final product testing**

SENZA GEN

Case study I
– Change in sterilization procedure

Problem

New batch of the product caused allergic dermatitis during clinical studies.

Method

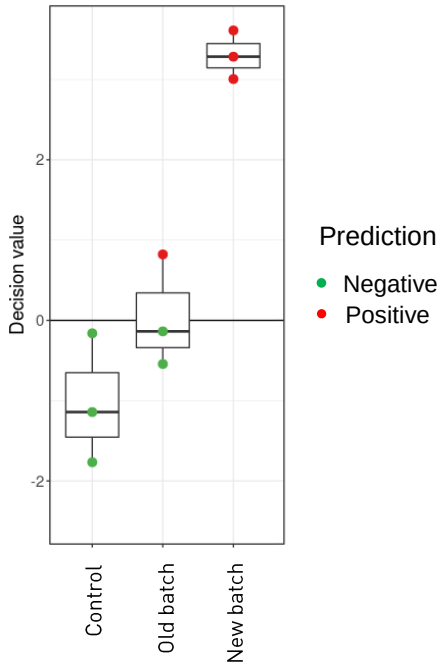
Compare the old batch with the new batch

- Extraction in cell culture media with serum
- 37°C for 72 h
- Safety assessment of skin sensitizers using GARD

Case study I
– Change of sterilization procedure

Results

Impurities in new batch of the product were linked to adverse effects seen in clinical studies. Skin sensitizer probably introduced during gamma-sterilization.



Case study II
– Comparison of different products/materials

Problem

Company wants to compare already marketed products with R&D projects and avoid unnecessary animal testing.

Method

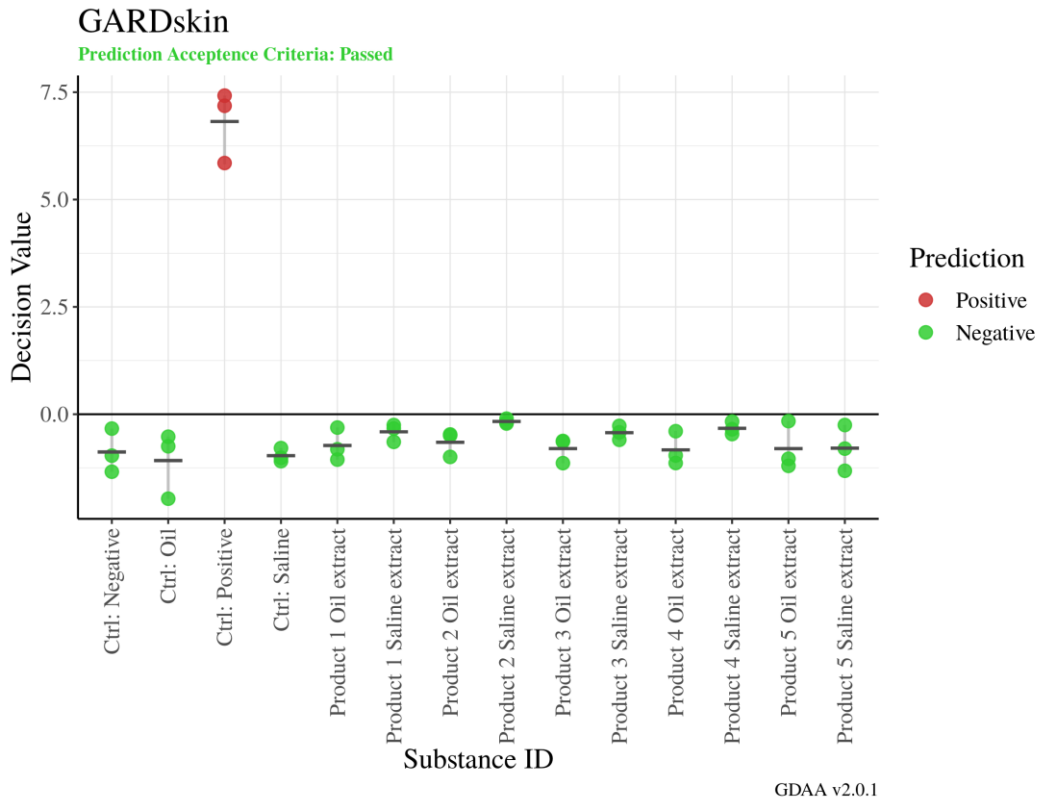
Compare the product on the market with product under development

- Extraction in saline and Super Refined Olive oil
- 37°C for 72 h
- Safety assessment of skin sensitizers using GARD

Case study II
– Comparison of different products/materials

Results

Products on the market compared to product under development.



The GARD platform
– How can I get my product/material tested?

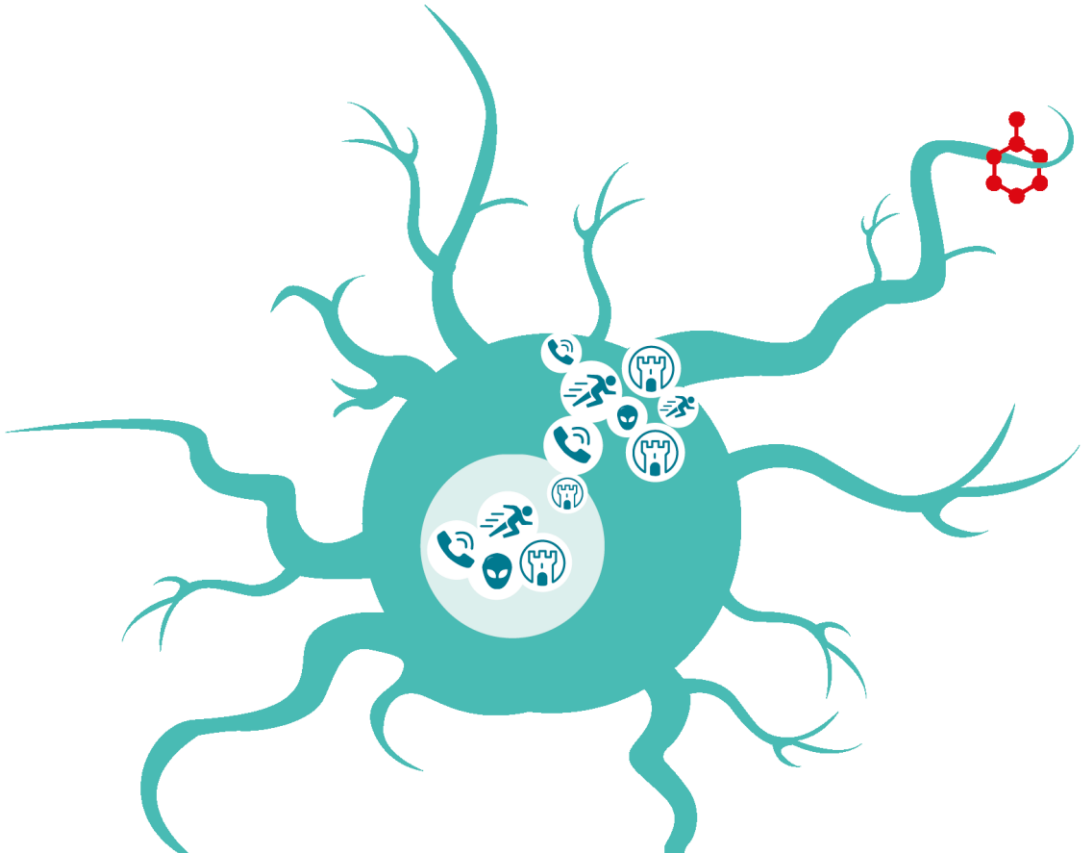
Contact us:
We help to design the testing strategy for your specific substances, products or materials.

Select assay(s):
GARD™skin, GARD™potency, GARD™air, GARD™skin Medical Device

Test Substance Questionnaire:
Do you have information on vehicles? If not, we evaluate it for you.

Turnaround time:
4-6 weeks.

Your preferred testing site:
Select where you like to have your testing done.



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The GARD platform
– Where can I get my testing done?

- **Licence Labs*:**
  Burleson Research Technologies
  Eurofins BPT
  MB Research Laboratories

- **Distributors*:**
  Charles River Laboratories
  Eurosafe
  Guangzhou CHN-ALT Biotech Co., Ltd
  PKDerm
  Woo Jung BSC
  XCellR8

* In alphabetic order

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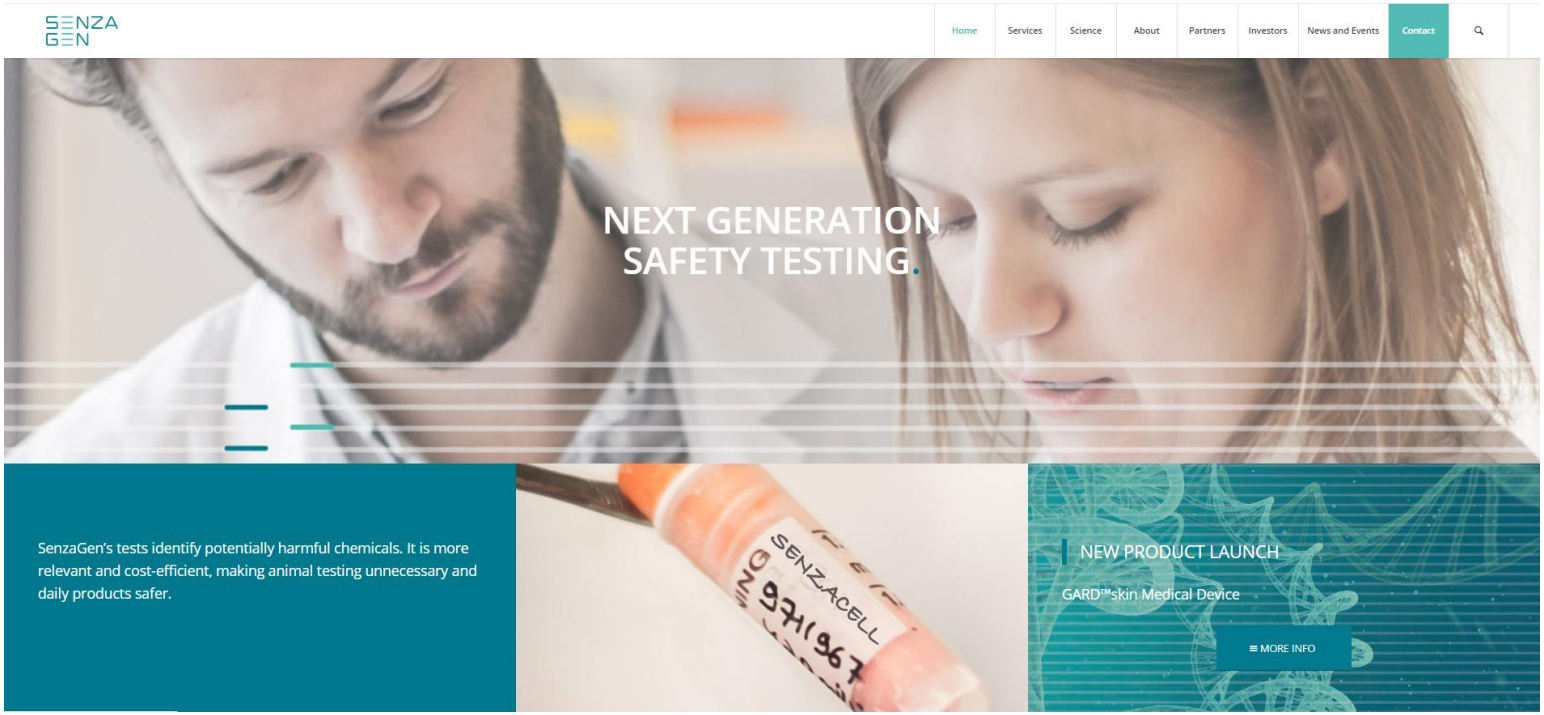
Information and contacts

www.senzagen.com



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