The Validation of GARD®skin

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Introduction

The prevalence of allergic contact dermatitis (ACD) is estimated to >20% in the western world. Not only the individual is affected, but downstream socioeconomic effects are high. To minimize exposure, chemicals must be safety tested. Traditional testing strategies like the murine local lymph node assay (LLNA) comprise animals, but the regulatory authorities, public opinion and economic interests require animal-free models. The Genomic Allergen Rapid Detection skin (GARD®skin) is an *in vitro* assay addressing this need. Here, we present the results of the GARD®skin ring trial (OECD TGP 4.106) for validity of the assay. In addition, we show data for GARD®potency - a complementary assay developed to categorize identified senitizers as CLP 1A or 1B.

Objective

The objective of the study was to assess the transferability and reproducibility of the GARD® assay and to demonstrate that GARD®skin is accurate in identifying skin sensitizers.

The GARD® platform

In brief, the GARD® assay mimics the human immune response during ACD. The method is based on a dendritic cell line, SenzaCell, that is exposed to a test substance at a concentration that generates 90% relative viability. Following the exposure, RNA is harvested and a gene expression panel consisting of 200 genes is analyzed using the NanoString technology (Figure 1).

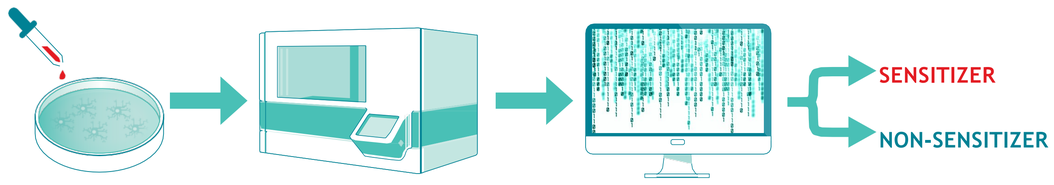

Figure 1. Schematic figure of the GARD® assay. Cells are exposed to a test substance and their gene expression signature is analysed to assess if the test substance is a sensitizer.

Study design

Three laboratories were involved in the validation ring trial – the lead and development laboratory, SenzaGen, and two external naïve contract research laboratories (CROs), Burleson Research Technologies (BRT) and Eurofins. Initially, the two naïve laboratories were trained by SenzaGen personnel to execute the GARD® assay. Next, a study to ensure the transferability was performed by the CROs and finally coded chemicals were tested by all three laboratories in the validation study (Figure 2).

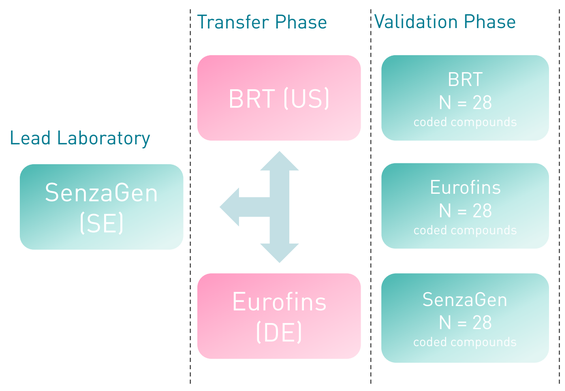

Figure 2. Study design of the GARDskin ring trial.

References Johansson *et al.* ALTEX, 2017, Johansson *et al.* BMC Genomics, 2011, Forreryd *et al.* BMC Genomics, 2014, Johansson *et al.* Toxicol Sci, 2014.

Conclusions

Transfer study
- Transferability: 100%

Validation study
- Reproducibility
  - WLR: 82 - 89%
  - BLR: 92% (92 – 100%)
- Test performance
  - Accuracy: 94%
  - Sensitivity: 93%
  - Specificity: 96%

A blinded ring trial was performed to assess the functionality of the GARD®skin assay. The data demonstrates that GARD®skin is a powerful tool for assessment of chemical skin sensitizers, with a predictive accuracy of 94% and excellent reproducibility between laboratories.

In addition, we show that GARD®potency accurately assesses potency of identified sensitizers.

Transferability

Eleven chemicals (Figure 3) known to be sensitizers or non-sensitizers were analysed according to the GARD®skin SOP. The assay was repeated three times at two contract research laboratories independent from the developing laboratory. All chemicals (11/11), including controls were predicted to their correct class (sensitizer/non-sensitizer). This demonstrates 100% transferability in both laboratories in all three experiments (Figure 3) .

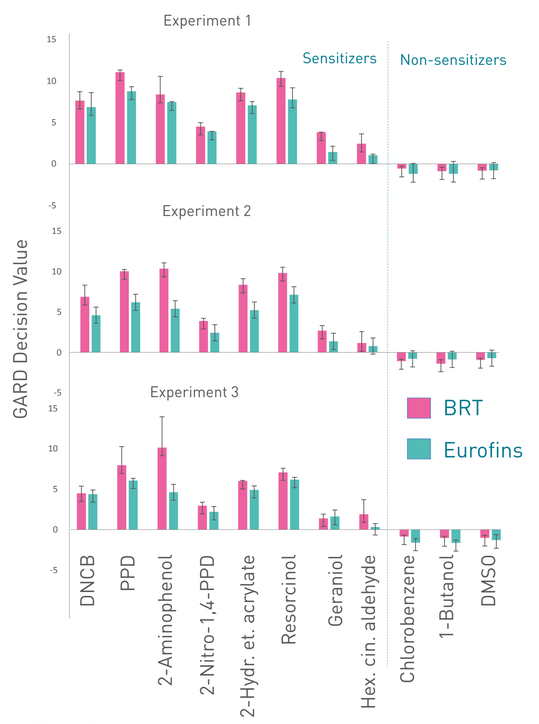

Figure 3. Mean decision values (DVs) of the 11 chemicals. DV ≥ 0 = sensitizer, DV < 0 = non-sensitizer. The error bars represent one standard deviation of three replicates.

Reproducibility

All three laboratories tested the 28 coded chemicals three times using GARD®skin (Table 2). The within laboratory reproducibility (WLR) was calculated to 82% (lead laboratory), 83% and 89% (Table 3). The between laboratory reproducibility (BLR) was assessed to 92% (range 92 – 100%) (Table 4).

Table 3.
Within laboratory reproducibility

Test laboratory	WLR S/NS
SenzaGen	82.1% (23/28)
BRT	83.3% (20/24)
Eurofins	88.9% (24/27)

Table 4. Between laboratory reproducibility

BLR	Agree	Senza/ Euro	Senza/ BRT	Euro/ BRT
Concordance Overall	82% (23/28)	89% (25/28)	82% (23/28)	93% (26/28)
Concordance S/NS	92% (23/25)	96% (25/27)	92% (23/25)	100% (25/25)

Table 2. The 28 chemicals and the GARD®skin test predictions by each laboratory and the concordance between the laboratories. S = sensitizer, NS = non-sensitizer, IC = inconclusive

#	Chemical	True class S/NS	Prediction		
			Senza	Euro	BRT
1	4-Nitrobenzy- bromide	S	S (3/3)	S (3/3)	S (3/3)
2	2-Bromo-2-glutaronitrile	S	S (3/3)	S (3/3)	IC (1/3)
3	Cinnamal	S	S (3/3)	S (3/3)	S (3/3)
4	Formaldehyde	S	S (3/3)	S (3/3)	S (3/3)
5	Lauryl gallate	S	S (3/3)	S (3/3)	S (3/3)
6	4-(Methylamino)phenolsulphate	S	S (3/3)	S (3/3)	S (2/3)
7	Methylisothiazolinone	S	S (3/3)	S (3/3)	S (3/3)
8	Propyl gallate	S	S (3/3)	S (3/3)	S (3/3)
9	Toluene diamine sulphate	S	S (3/3)	S (3/3)	S (3/3)
10	Diethyl maleate	S	S (3/3)	S (3/3)	S (3/3)
11	3-Dimethylamino-propylamine	S	S (3/3)	S (3/3)	S (3/3)
12	Ethylene diamine	S	NS (0/3)	NS (0/3)	NS (1/3)
13	Isoeugenol	S	S (3/3)	S (3/3)	S (3/3)
14	2-Mercapto-benzothiazole	S	S (2/3)	S (3/3)	S (3/3)
15	Benzyl benzoate	S	NS (1/3)	S (2/3)	S (2/3)
16	Cinnamyl alcohol	S	S (3/3)	S (3/3)	S (3/3)
17	Citral	S	S (3/3)	S (3/3)	IC (-)
18	Ethylene glycol dimethacrylate	S	S (3/3)	S (3/3)	S (3/3)
19	Eugenol	S	S (3/3)	S (3/3)	S (3/3)
20	Dextran	NS	NS (3/3)	-	-
21	Glycerol	NS	NS (3/3)	NS (3/3)	NS (3/3)
22	Hexane	NS	NS (3/3)	NS (3/3)	NS (3/3)
23	Isopropanol	NS	NS (3/3)	NS (2/3)	NS (3/3)
24	Kanamycin	NS	NS (3/3)	NS (3/3)	NS (3/3)
25	Lactic acid	NS	NS (3/3)	NS (3/3)	NS (2/3)
26	Propylene glycol	NS	NS (3/3)	NS (2/3)	NS (2/3)
27	Salicylic acid	NS	NS (2/3)	NS (3/3)	NS (3/3)
28	Vanillin	NS	S (1/3)	NS (3/3)	NS (3/3)

Test performance

The performance of GARD®skin in each laboratory is presented in Table 5. Also, the cumulative performance including the results from all laboratories was calculated, illustrating an overall accuracy of 94% (Table 6). Compounds classified as sensitizers were further analyzed with GARD®potency for potency classification with a cumulative accuracy of 82% (Table 7). These results are submitted for OECD validation of GARD®skin and GARD®potency.

Table 5. Performance of GARD®skin in the test laboratories.

Reference results	SenzaGen (19+9)		Eurofins (19+8)		BRT (17+8)	
	S	NS	S	NS	S	NS
S	17	2	18	1	16	1
NS	1	8	0	8	0	8
Total	18	10	18	9	16	9
Accuracy	89%		96%		96%	
Sensitivity	90%		95%		94%	
Specificity	89%		100%		100%	

Table 6. Cumulative performance in all three laboratories.

Reference results	Culmulative (55+25)	
	S	NS
S	51	4
NS	1	24
Total	52	28
Accuracy	94%	
Sensitivity	93%	
Specificity	96%	

Table 7. Cumulative accuracy of potency classification by GARD®potency in all three laboratories.

Reference results	Culmulative (28+22)	
	S	NS
1A	25	3
1B	6	16
Total	31	19
Accuracy	82%	
Correct 1A	89%	
Correct 1B	73%	

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