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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 (GARDskin) is an *in vitro* assay addressing this need. Here, we present the results of the GARDskin ring trial (OECD TGP 4.106) for validity of the assay.

Objective

The objective of the study was to assess the transferability and reproducibility of the GARDskin assay and to demonstrate that the assay is an accurate assay for assessing 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 are 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 by the NanoString technology (Figure 1).

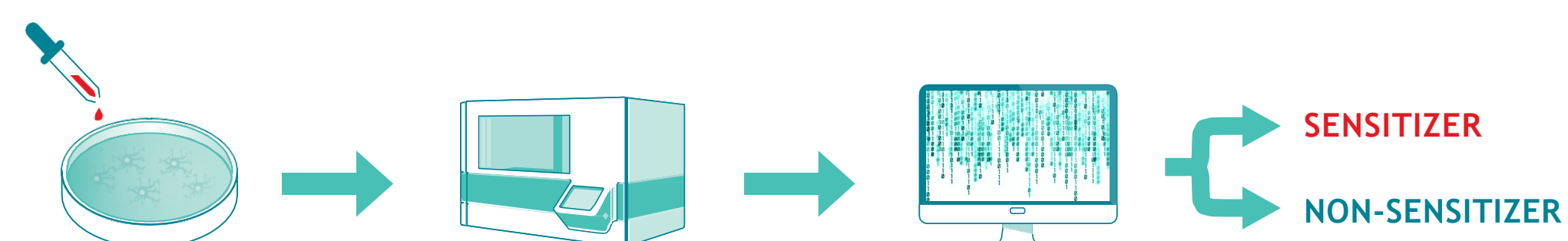


Figure 1. Schematic figure of the GARD assay. Cells are exposed to a test substance and their gene expression signature is analysed to assess if the test substance is a sensitizer.

Study design

Three laboratories were involved in the ring trial – the lead and development laboratory, SenzaGen, and two external naïve laboratories, Burlison Research Technologies (BRT) and Eurofins. Initially, the two naïve laboratories were trained to execute the GARD assay by SenzaGen personnel. 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).

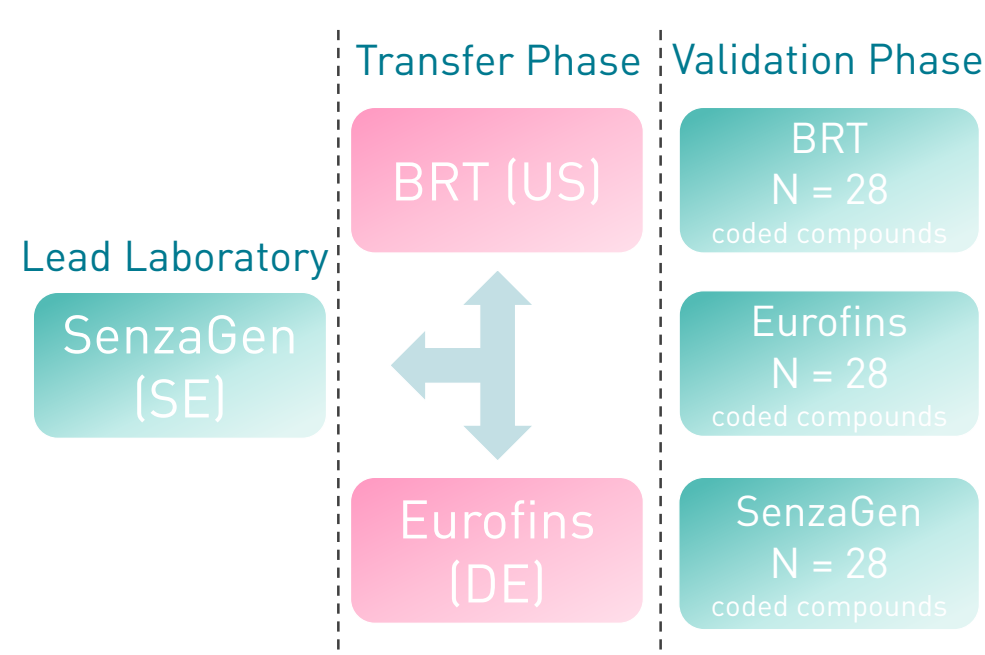


Figure 2. Study design of the GARDskin ring trial.

Transferability

Eleven chemicals (Figure 3) known to be sensitizers or non-sensitizers were analysed according to the GARDskin 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).

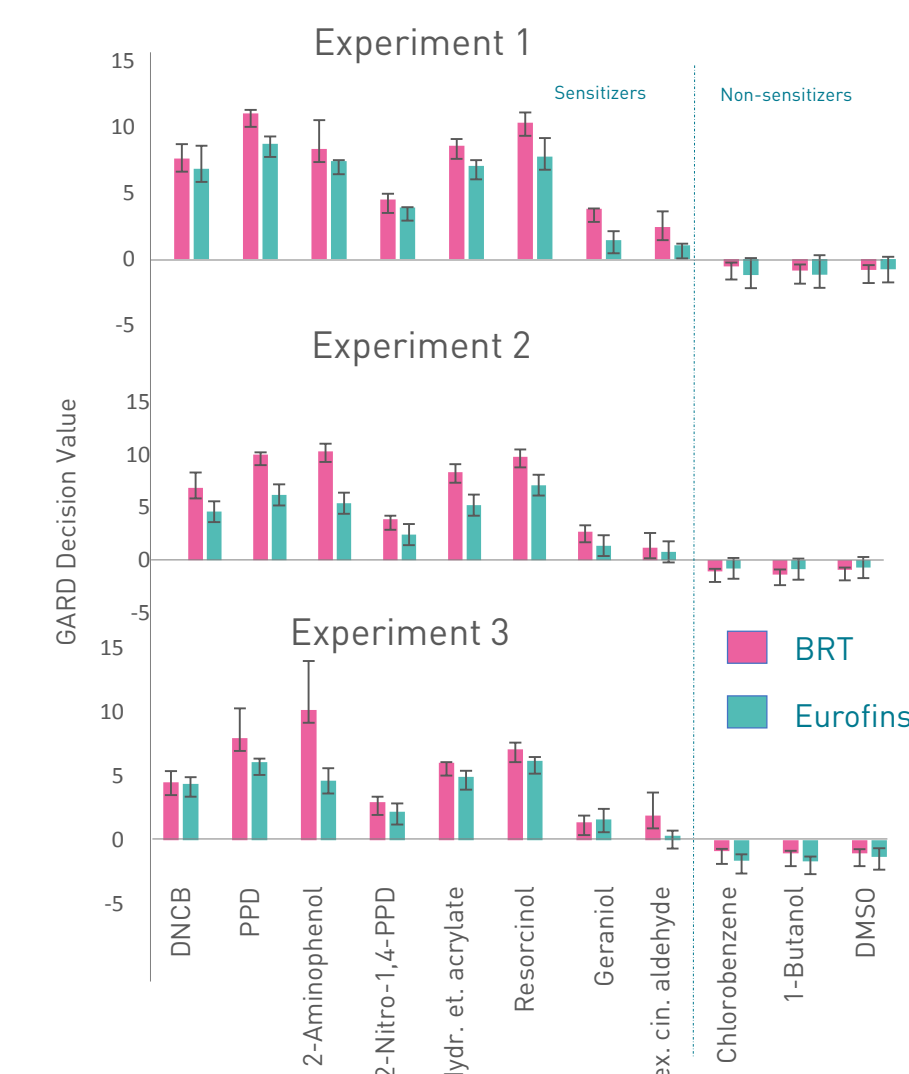


Figure 3. Mean decision values (DVs) of the 11 chemicals. DV ≥ 0 = sensitizer, DV < 0 = non-sensitizer. The error bars represent one standard deviation of three replicates.

Reproducibility

All three laboratories tested the 28 coded chemicals three times using GARDskin (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	82%	89%	82%	93%	
Overall	(23/28)	(25/28)	(23/28)	(26/28)	
Concordance	92%	96%	92%	100%	
S/NS	(23/25)	(25/27)	(23/25)	(25/25)	

Table 2. The 28 chemicals and the GARDskin 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	Cinnamat	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-(Methylaminolphenolsulphate	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 (3/3)
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)	NS (3/3)	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 GARDskin 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).

Table 5. Performance of GARDskin 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%	

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 GARDskin assay. The data demonstrates that GARDskin is a powerful tool for assessment of chemical skin sensitizers, with a predictive accuracy of 94% and excellent reproducibility between laboratories.

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