

## INTRODUCTION

Chemical hypersensitivity is an immunological response to foreign substances. Primarily, these give rise to the clinical symptoms known as allergic contact dermatitis. To mitigate risks associated with consumer products, chemicals are screened for sensitizing effects. Historically, such predictive screenings have been performed using animal models, but industrial and regulatory authorities now demand animal-free methods for the assessment of sensitization. This is a global development spreading across industries and markets. To meet this demand, the Genomic Allergen Rapid Detection (GARD) assay has been developed. Here, we present novel data reconfirming the performance and accuracy of GARD.

## METHODS

The GARD assay was used for the assessment of skin sensitization. GARD is based of a human myeloid cell similar to DCs, which are immunologically active during sensitization. The cell line was separately stimulated with 72 chemicals (table 1) selected by Cosmetics Europe. The RNA was collected 24 h later and the gene expression panel, consisting of 200 biomarkers, was measured by NanoString technology.

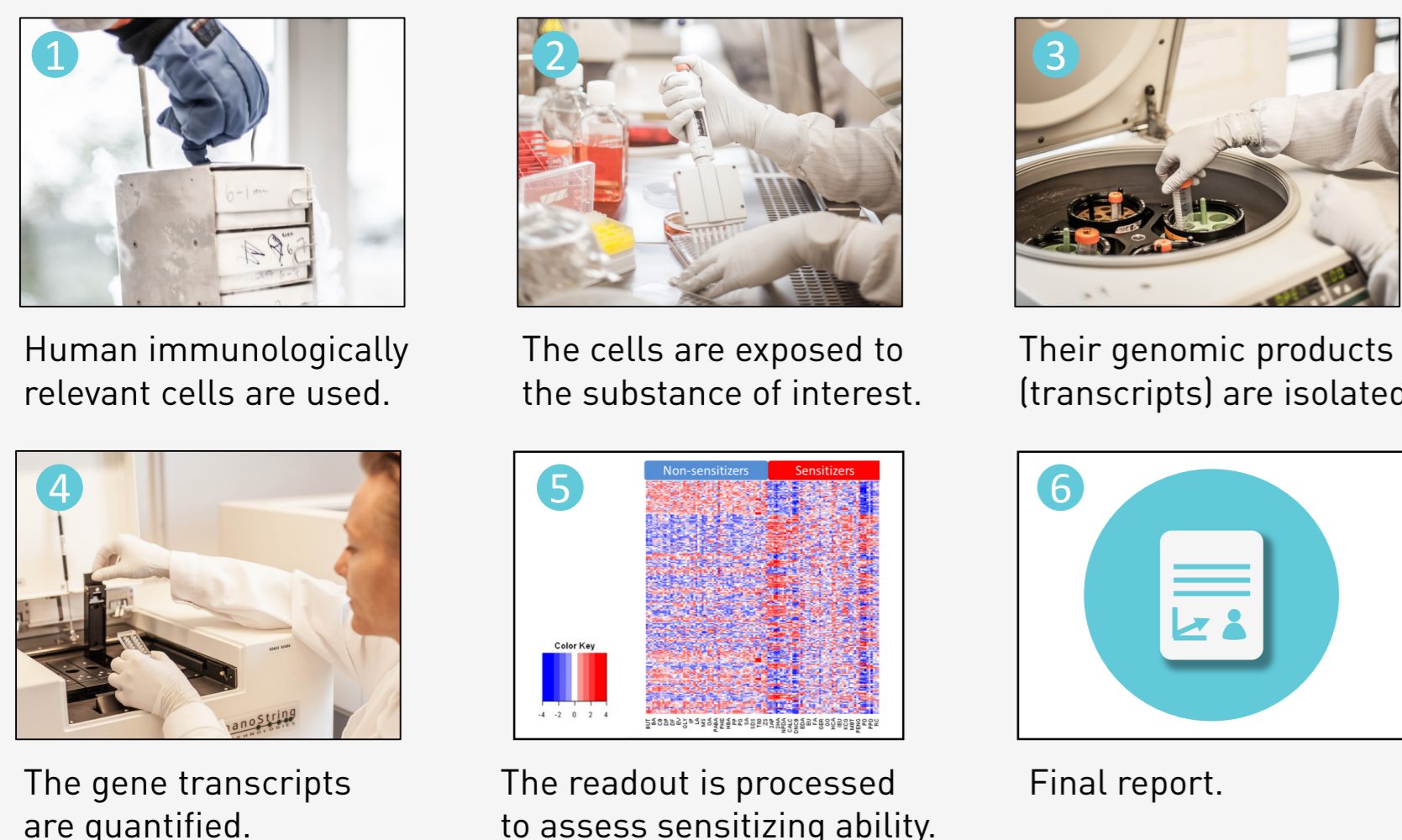


Figure 1. A brief overview of the GARD process.

The GARD predictions of the 72 chemicals are shown in table 1 along with LLNA, Human Potency, and Global Harmonization System (GHS) / Classification for Labelling and Packaging (CLP) classifications.

Table 1. The 72 chemicals selected by Cosmetics Europe and their classification according to LLNA, HP, GHS/CLP and GARD (NS=non-sensitizer).

Substance ID	LLNA	HP	GHS/CLP	GARD	Substance ID	LLNA	HP	GHS/CLP	GARD
<b>Sensitizers</b>					<b>Sensitizers</b>				
1,4-phenylenediamine	strong	1	1A	sensitizer	Benzocaine	NS	4	1B	sensitizer
Tetrachlorosalicylanilide	extreme	1	-	sensitizer	Geraniol	weak	4	1B	sensitizer
Dimethyl fumarate	strong	1	-	sensitizer	Lillial	weak	4	1B	sensitizer
2-aminophenol	strong	2	1A	sensitizer	Linalool	weak	4	1B	sensitizer
2-Nitro-1,4-phenylenediamine	moderate	2	1A	sensitizer	Amyl cinnamic aldehyde	weak	4	-	sensitizer
Formaldehyde (act. 37%)	strong	2	1A	sensitizer	Carvone	weak	4	-	sensitizer
Glutaraldehyde (act. 50%)	extreme	2	1A	sensitizer	Kanamycin	NS	4	-	sensitizer
Methyl heptine carbonate	strong	2	1A	sensitizer	Anethole	moderate	5	1B	sensitizer
Propyl gallate	strong	2	1A	sensitizer	Anisyl alcohol	moderate	5	1B	sensitizer
Toluene diamine sulphate	strong	2	-	sensitizer	Benzyl salicylate	moderate	5	-	sensitizer
Glyoxal (act. 40%)	strong	2	1A	sensitizer	Limonene	weak	5	1B	sensitizer
Isoeugenol	moderate	2	1A	sensitizer	Hexyl cinnamic aldehyde	weak	5	1B	sensitizer
1,2-Benzisothiazolin-3-one	moderate	2	-	sensitizer	Benzyl benzoate	weak	5	1B	sensitizer
3-dimethylaminopropylamine	moderate	2	-	sensitizer	Citronellol	weak	5	1B	sensitizer
Thioglycerol	moderate	2	-	NS	Diethanolamine	weak	5	1B	sensitizer
Lyral	weak	2	1B	sensitizer	Pentachlorophenol	weak	5	1B	sensitizer
Chlorpromazine	moderate	3	1A	sensitizer	Pyridine	weak	5	1B	sensitizer
Benzoyl peroxide	extreme	3	-	NS	<b>Non-sensitizers</b>				
Bisphenol A-diglycidyl ether	moderate	3	1A	sensitizer	Hydrocortisone	NS	5	no cat	sensitizer
Ethylene diamine	moderate	3	1B	sensitizer	Isopropanol	NS	5	no cat	NS
Glyceryl monothioglycolate	moderate	3	-	sensitizer	Methyl salicylate	NS	5	no cat	sensitizer
Farnesol	moderate	3	-	sensitizer	Methyl salicylate	NS	5	no cat	NS
Abietic acid	weak	3	1B	sensitizer	Phenoxyethanol	NS	5	no cat	NS
Butyl glycidyl ether	weak	3	1B	sensitizer	Propylene glycol	NS	5	no cat	NS
Cinnamic alcohol	weak	3	1B	sensitizer	Triethanolamine	NS	5	-	sensitizer
Citral	moderate	3	1B	sensitizer	4-aminobenzoic acid	NS	5	no cat	NS
Eugenol	weak	3	1B	sensitizer	Benzaldehyde	NS	5	no cat	NS
Imidazolidinyl urea	weak	3	1B	sensitizer	Propyl paraben	NS	5	-	sensitizer
Penicillin G	weak	3	-	NS	Vanillinphthalate	NS	5	no cat	NS
5-methyl-2,3-hexanedione	weak	3	-	sensitizer	Dextran	NS	6	no cat	NS
Coumarin	NS	3	-	sensitizer	Glycerol/Glycerin	NS	6	no cat	NS
Hexyl salicylate	strong	4	1A	NS	Octanoic acid	NS	6	no cat	NS
Iodopropnyl butylcarbamate	strong	4	1A	sensitizer	Phenol	NS	6	no cat	NS
Neomycin sulphate	NS	4	-	sensitizer	Tocopherol	moderate	6	-	sensitizer
Resorcinol	moderate	4	1B	sensitizer	Diethyl	NS	6	no cat	sensitizer
Amylcinnamyl alcohol	NS	4	1B	sensitizer	Diethyl toluamide	NS	6	-	sensitizer
Aniline	weak	4	1B	sensitizer	Tween 80	NS	6	no cat	sensitizer

Chemicals were defined as sensitizers if compounds are categorized as Human Potency (HP) (Basketter *et al.*, 2014) 1-4, or HP 5, if it is also predicted as a sensitizer by the LLNA (Table 2). This classification system correlates with GHS/CLP classifications.

Table 2. Definition of the classification system corresponding to GHS/CLP classes.

Sensitizers	Non-sensitizers
HP 1-4	HP 5 and LLNA negative
HP 5 and LLNA positive	HP 6

## RESULT

By this definition, based on the current data the accuracy and sensitivity of GARD, is 83% and 93%, respectively (table 3). Comparing the predictions strictly with either HP or LLNA, the accuracy was estimated to be 81% and 78%, respectively.

Table 3. Cooper statistics of current data.

Characteristics	LLNA	Human potency	Composite
Accuracy (%)	78	81	83
Sensitivity (%)	90	84	93

The data is grouped according to CLP classifications in figure 2. The observed differences in mean cDVs indicate that the GARD predictions correlate with potency classifications.

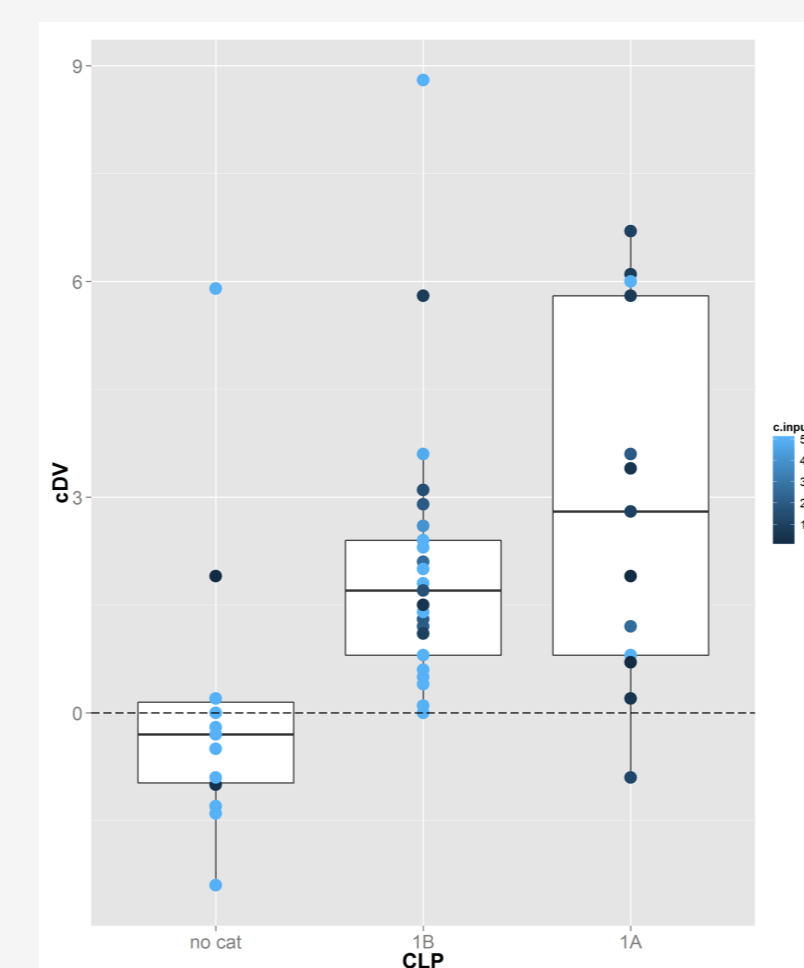


Figure 2. Box plot of mean GARD calibrated decision values, cDVs, grouped by sensitizing potency as defined by the GHS/CLP classification system. The color of each data point is mapped to the GARD input concentration (µM) used for that test substance.

In order to relate the current results to previously published figures of predictive performance, an update of accumulated Cooper statistics for independent GARD assessments across various datasets are presented in Table 4. Combined, the accuracy of GARD was calculated to 86%, including datasets that taken together comprises 127 chemicals.

Table 4. Accumulated predictive performance

Dataset	Sensitivity	Accuracy	Source
GARD in-house validation	89% (17/19)	88% (23/26)	Johansson 2014
Technology transfer and method optimization	94% (16/17)	90% (26/29)	Forreryd 2016
Current study	93% (50/54)	83% (60/72)	Johansson 2017
Accumulated predictive performance	92% (83/90)	86% (109/127)	Johansson 2017

## RESUME

- ✓ The performance of GARD is highly accurate
- ✓ 83% (72 chemicals)
- ✓ 86% (127 chemicals)
- ✓ Correlated GARD predictions and potency classifications

References:

Johansson *et al.* BMC Genomics, 2011  
 Forreryd *et al.* BMC Genomics, 2014  
 Forreryd *et al.* PloSOne, 2015  
 Johansson *et al.* Toxicol Sci, 2014  
 Johansson *et al.* Altex, 2017

