Performance of the GARD assay in a blind Cosmetics Europe study

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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.

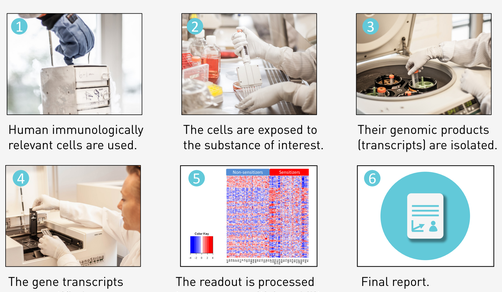

Figure 1. A brief overview of the GARD process.

RESULT

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

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.

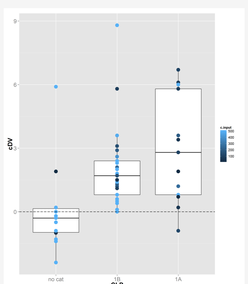

Figure 2. Box plot of mean GARD calibrated decision values, cDVs, grouped by sensitizing potency as defined by the GHS/CLP classification system. The color of each data point is mapped to the GARD input concentration (µM) used for that test substance.

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 predicitive 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:
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