Potency ranking of skin sensitizers using the Reconstituted human Epidermis (RhE) IL-18 test and the Genomic Allergen Rapid Detection (GARD) test.

Erwin L Roggen[1], Els Adriaens[2], Eric Andres[3], Fanny Boisleve[4], Sun-A Cho[5], Emanuela Corsini[6], George DeGeorge[7], Horst W Fuchs[8], Pierre-Jacques Ferret[9], Sue Gibbs[10], Patrick Hayden[11], Henrik Johansson[12], Dagmar Lehmeier[13], Silvia Letasiova[14], Malin Lindstedt[15] , Jean-Philippe Therrien[16]

[1] 3Rs Management and Consulting, [2] Adriaens Consulting, [3] Oroxcell, [4] Yves Rocher, [5] Amorepacific, [6] University of Milan, [7] MB Research Laboratories, [8] CellSystems, [9] Pierre-Fabre, [10] Vrije Universiteit Medical Center, [11] MatTek Cooperation, [12] SenzaGen, [13] Eurofins, [14] MatTek IVSL, [15] Lund University, [16] GlaxoSmithKline

Background

IL-18 production by keratinocytes is a potentially useful endpoint for determination of contact sensitization potential of low molecular weight chemicals (Corsini et al., 2009). Potency classification of skin sensitizers relates to the irritant potential of the chemical in a RhE model (dos Santos et al., 2011). Gibbs et al. (2013) successfully integrated the IL-18 endpoint in various established RhE models, currently used to assess chemical substances for their potential to trigger irritation and corrosion. This test addresses Key Event (KE) 2 of the Adverse Outcome Pathway (AOP) for skin sensitization.

The GARD assay is based on a predictive biomarker signature of 200 transcripts differentially regulated in a myeloid cell line when stimulated with sensitizing compounds, as compared to non-sensitizing compounds. The GARD Prediction Signature (GPS) participates in signaling pathways involved in cyto-protective mechanisms and dendritic cell maturation. Johansson et al. (2014) provided a proof of concept for the functionality of the GARD test using 37 blinded test substances, including 11 'difficult' substances (Sensitivity: 89%; specificity: 88%; accuracy: 89%). This test addresses KE 3 of the AOP for skin sensitization.

Both test methods more accurately discriminate contact sensitizers from non-sensitizers as compared to current Test Guidelines (TG) (hazard identification) (Table 1).

Table 1:

	DPRA[(1)] (TG442C)	KeratinoSense[(1)] (TG442D)	RhE IL-18[(2)]	h-CLAT[(1)] (TG-draft)	GARD[(3)]
Number of chemicals tested[(1)]	145	145	30	141	90
Sensitivity	82	79	91	71	96
Specificity	74	72	90	70	95
Accuracy	80	77	95	71	96

[(1)]: Natsch et al. (2013) J. Appl. Toxicol DOI 10.1002/jat.2868; [(2)]: Gibbs et al (2013) Toxicol Appl. Pharmacol. 272: 529-541; [(3)]: Johansson et al (2013) Toxicol. In Vitro 27: 1163-1169

Results and Discussion

RhE IL-18 potency test:

A generic protocol was developed using different 3D-epidermal models including the *in house* VUMC model, epiCS®, MatTek EpiDerm™ and SkinEthic™ RHE.
Critical issues were identified that need to be controlled in order to obtain comparable quantitative data (e.g. for relative potency determination) when using different skin models.

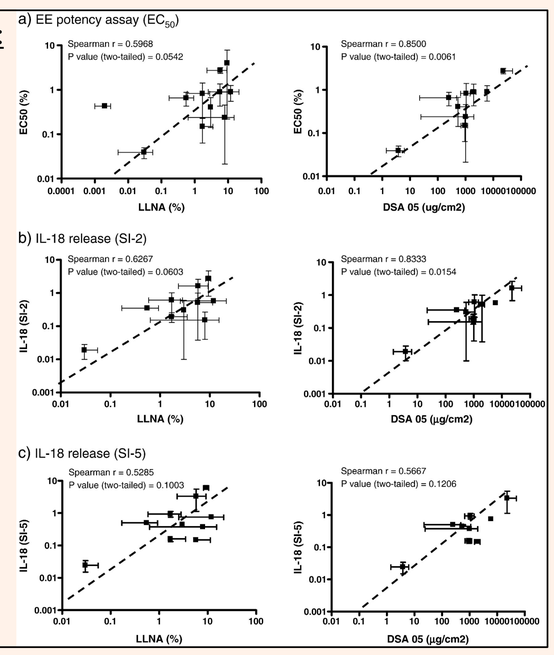
The available data (17 contact allergens, 13 non-sensitizers) indicate that potency is assessable on the basis of the amount of substance required to result in (i) 50% cytotoxicity (EC50) and (ii) an IL-18 response exceeding the cut-off stimulation index (Fig. 1).

(i) Correlation of the EC50 with human and animal data showed a superior correlation with human DSA05[(4)] (µg/cm2) data (Spearman r = 0.8500; P value (two-tailed) = 0.0061) compared to Local Lymph Node Assay (LLNA) data (Spearman r = 0.5968; P value (two-tailed) = 0.0542).

[(4)] *DSA05: the induction dose per skin area that produces a positive response in 5% of the tested population.*

(ii) A equally good correlation with human DSA05 data was observed for release of IL-18 (Spearman r = 0.8333; P value (two-tailed) = 0.0154).

Figure 1:



GARDskin potency assessment:

Myeloid cells were exposed to chemicals selected to complement the existing data set (Background) and the chemical stimulations, phenotypic and viability assessments were performed as described in the standardized protocol.

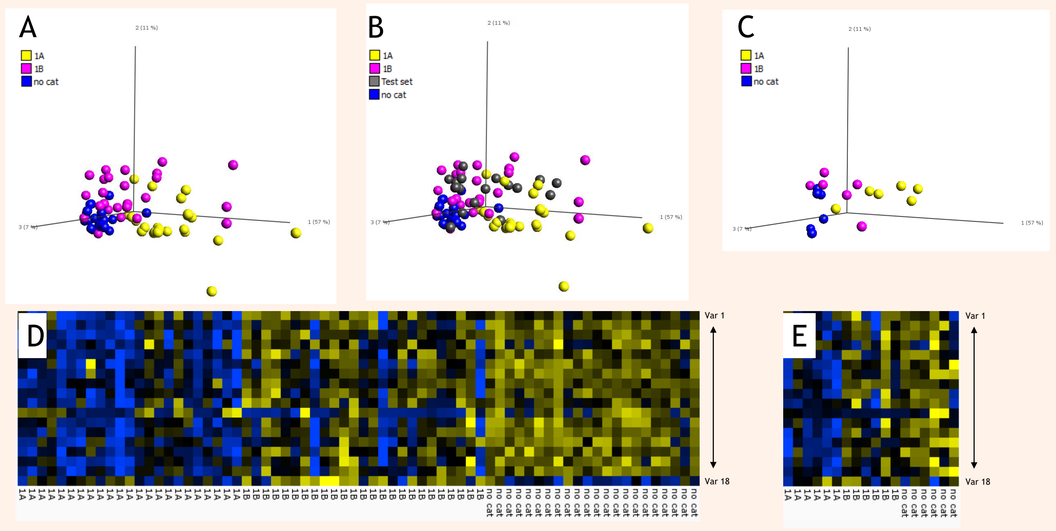
A training set of 68 unique chemicals and an additional test set of 18 chemicals were selected to balance CLP (Classification, Labelling and Packaging) classification and chemical reactivity mechanisms. The prediction model was built on the arithmetic mean of the transcript intensities from replicates in the training data using Random Forest (Fig. 2 A-E).

Using complete genome transcriptomics, a Random Forest model was developed. This model is based on 18 biomarkers, and is able to predict CLP sensitizer potency classifications of 18 chemicals previously unseen to the model (Table 2).

Table 2:

	CLP 1A	CLP 1B	CLP no category
Sensitivity	0.86	0.63	1.00
Specificity	1.00	0.90	0.80
Accuracy	0.93	0.76	0.90

Figure 2:



PCA plots and heat map. PCA of training set **(A)**, training and test set **(B)** and test set only **(C)** based on an input of 18 variables identified by Random Forest modeling**.** Each sphere represents a chemical, colored according to CLP classifications (yellow = 1A, pink = 1B, blue = no category). **(D)** Heat map of training set, **(E)** heat map of test set.

Lindberg et al (2016) , Abstract 2205, SOT2016.

Conclusion

The available data suggest a potential use of the RhE IL-18 (KE 2) and the GARDskin (KE 3) test methods in the identification of skin sensitizers. While the GARDskin can be considered as a stand-alone test for hazard identification of hydrophilic substances, the RhE IL-18 potency test may have added value when hydrophobic or water-unstable substances have to be tested.

For chemicals with human data available, the potency information acquired with the RhE IL-18 and the GARD test methods is more reliable than the data acquired with the LLNA. This conclusion is based on RhE IL-18 data (Fig. 1) and a study by the US interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) concluding that 22% of human sensitizers were not detected by the LLNA, while, overall, 48% were placed in the wrong GHS sub-category[(5)]. The GARDskin is currently being improved for CLP 1B classification.

[(5)] http://ntp.niehs.nih.gov/pubhealth/evalatm/test-method-evaluations/immunotoxicity/llna-potency/tmer/index.html

Integrated in a testing and assessment strategy, the test methods have the potential to meet the regulatory needs and data requirements related to skin sensitization assessment in the context of the REACH legislation (Regulation 1907/2006/EC), the EU Cosmetics Regulation (1223/2009), the EU Regulation on classification, labelling and packaging of substances and mixtures (1272/2008), the EU Legislation on Plant Protection Products (1107/2009) and the EU Regulation on Biocidal Products (528/2012).

The RhE IL-18 and the GARDskin test methods are in the process of being formally validated.