Skin sensitization: VITOSENS™

- **Objective:** *in vitro* assay for the identification of chemical compounds with sensitizing potency

- **Principle:** cord blood $CD34^+$-derived dentritic cells are exposed to the IC20 concentration of a chemical compound. Using a classification model based on gene expression, the compound is classified as sensitizer or non-sensitizer.

- **Development Stage:** R&D / validation stage / routine

- **Results / data:** classification of the compound as sensitising or non-sensitising

- **Applicability Domain:** hazard identification, cosmetic and pharmaceutical industry

Skin sensitization: VITOSENS™

Patent pending: WO2008037806

A cell-based *in vitro* alternative
to identify skin sensitizers
by gene expression

Jef Hooyberghs, PhD
CARDAM-VITO (BE)

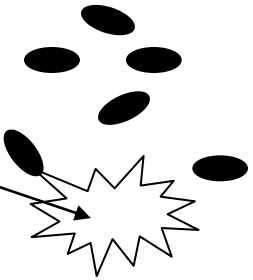
Skin sensitization: VITOSENS™

1 Mechanism and strategy

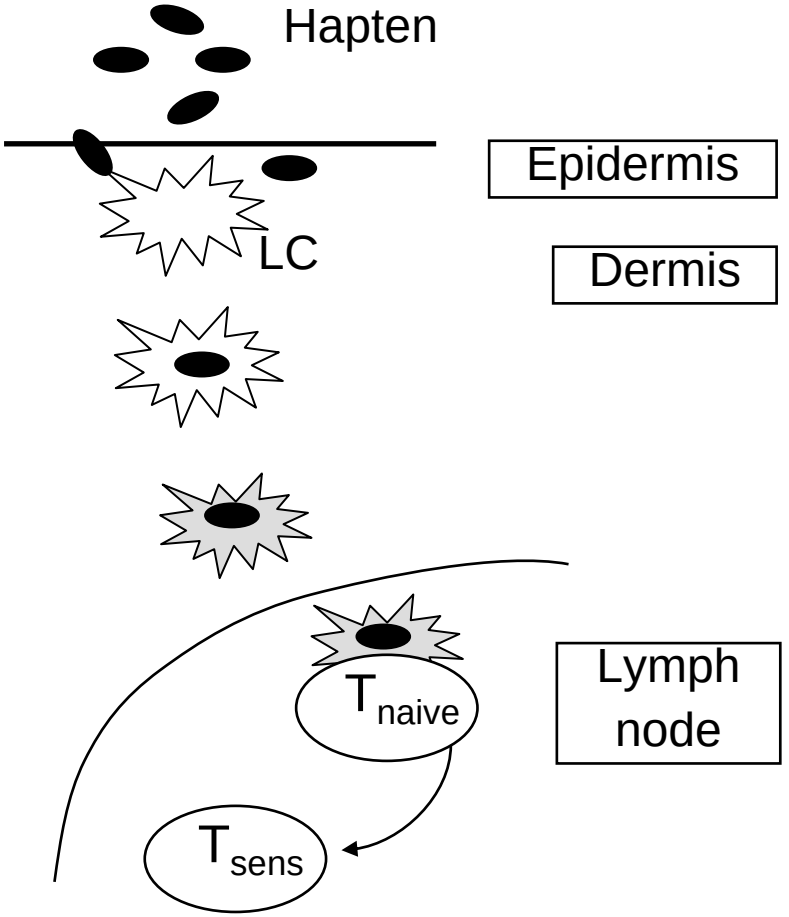
IN VITRO

strategy

Human CD34⁺ progenitor-derived dendritic cells



IN VIVO



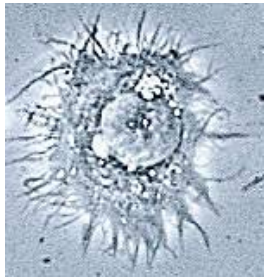
Skin sensitization: VITOSENS™

1 Mechanism and strategy

1998 -2002

Cell-culture optimization

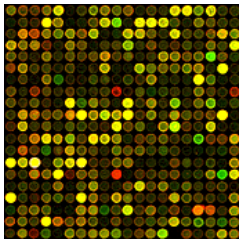
Surface markers and cytokines



2002 – 2006

Transcriptomics (microarrays)

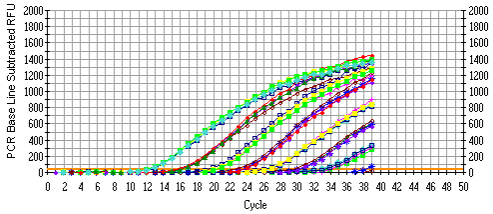
Selection of discriminative genes



2006 – current

Real-time RT-PCR

Prediction model & pathway analysis



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2 Prediction model

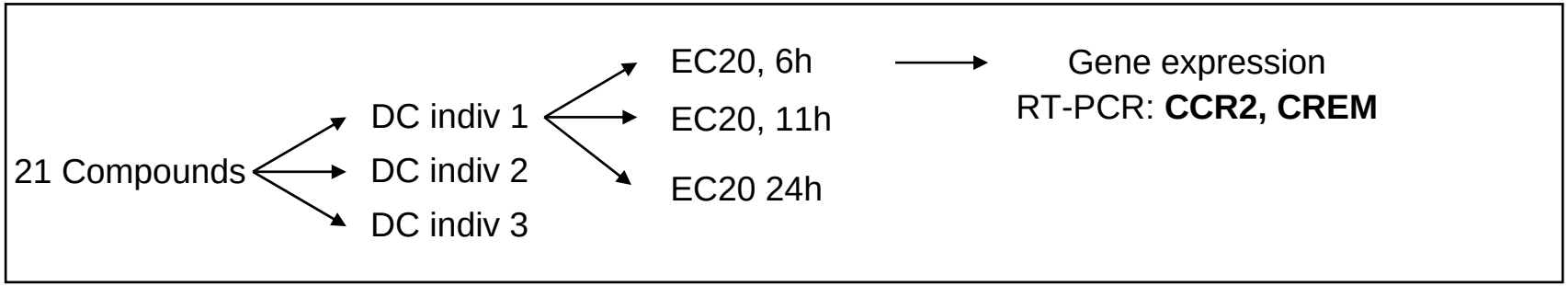
21 chemical compounds

Sensitizers		non-sensitizers	
Dinitrobenzenesulfonic acid	DNBS	Benzalkonium Chloride	BC
Dinitrofluorobenzene	DNFB	Dimethylsulfoxide	DMSO
Dinitrochlorobenzene	DNCB	L-Ascorbic Acid	L-AA
p-phenylenediamine	PPD	L-Glutamic Acid	L-GA
2-mercaptobenzothiazole	2MBT	Methyl salicylate	MeSA
Cinnamaldehyde	CA	p-Aminobenzoic Acid	PABA
Tetramethylthiuram disulfide	TMTD	Phenol	phenol
Ammonium hexachloroplatinate IV	HCPt	Sodium Lauryl/Dodecyl Sulphate	SDS
Eugenol	eugenol	Tributyltin Chloride	TBT
Nickel Sulfate	$NiSO_4$	Triton X-100	triton
		Zinc sulphate	$ZnSO_4$

Skin sensitization: VITOSENS™

2 Prediction model

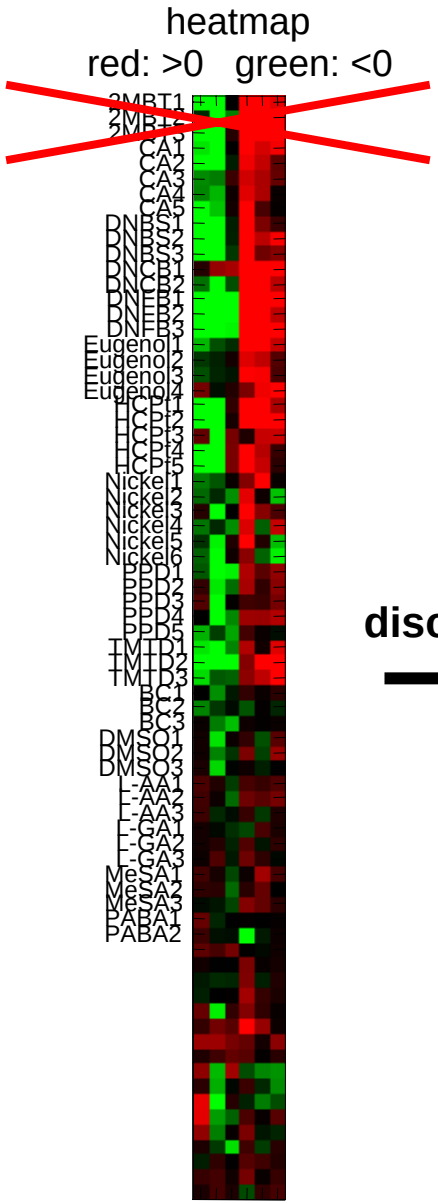
Experimental setup



Skin sensitization: VITOSENS™

2 Prediction model

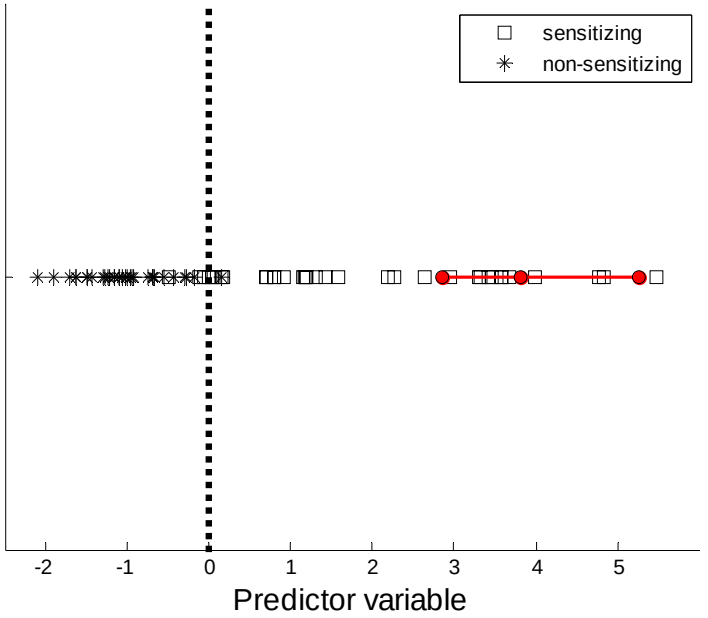
Cross-validation



CCR2 (6h, 11h, 24h)

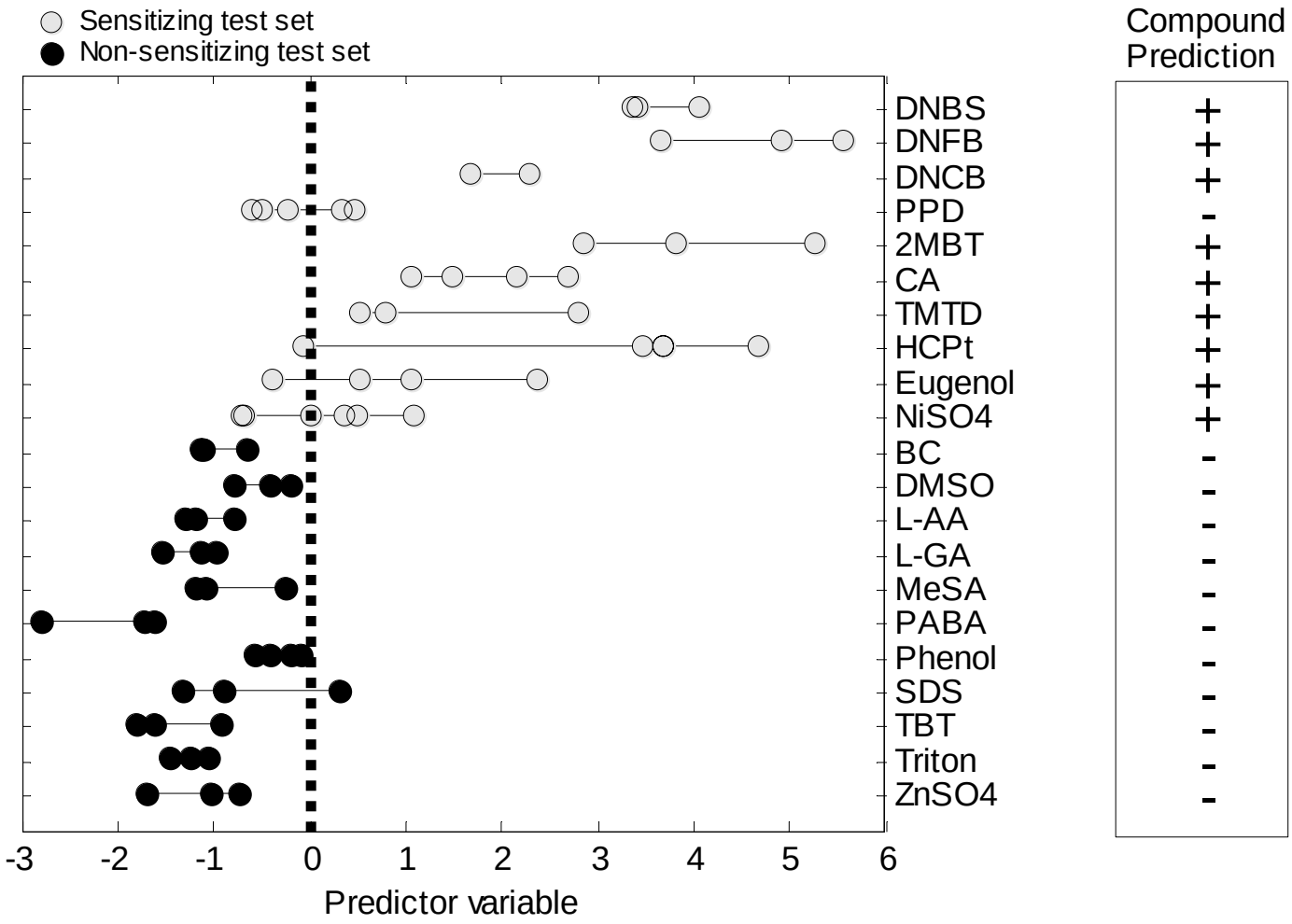
CREM (6h, 11h, 24h)

discriminant analysis



Skin sensitization: VITOSENS™

2 Prediction model



Skin sensitization: VITOSENS™

2 Prediction model

Contingency table of the VITOSENS® model

On the level of donor samples

	Predicted sensitizing	Predicted non-sensitizing	Total
Sensitizing	**32**	**7**	39
Non-sensitizing	**1**	**33**	34
Total	33	40	73

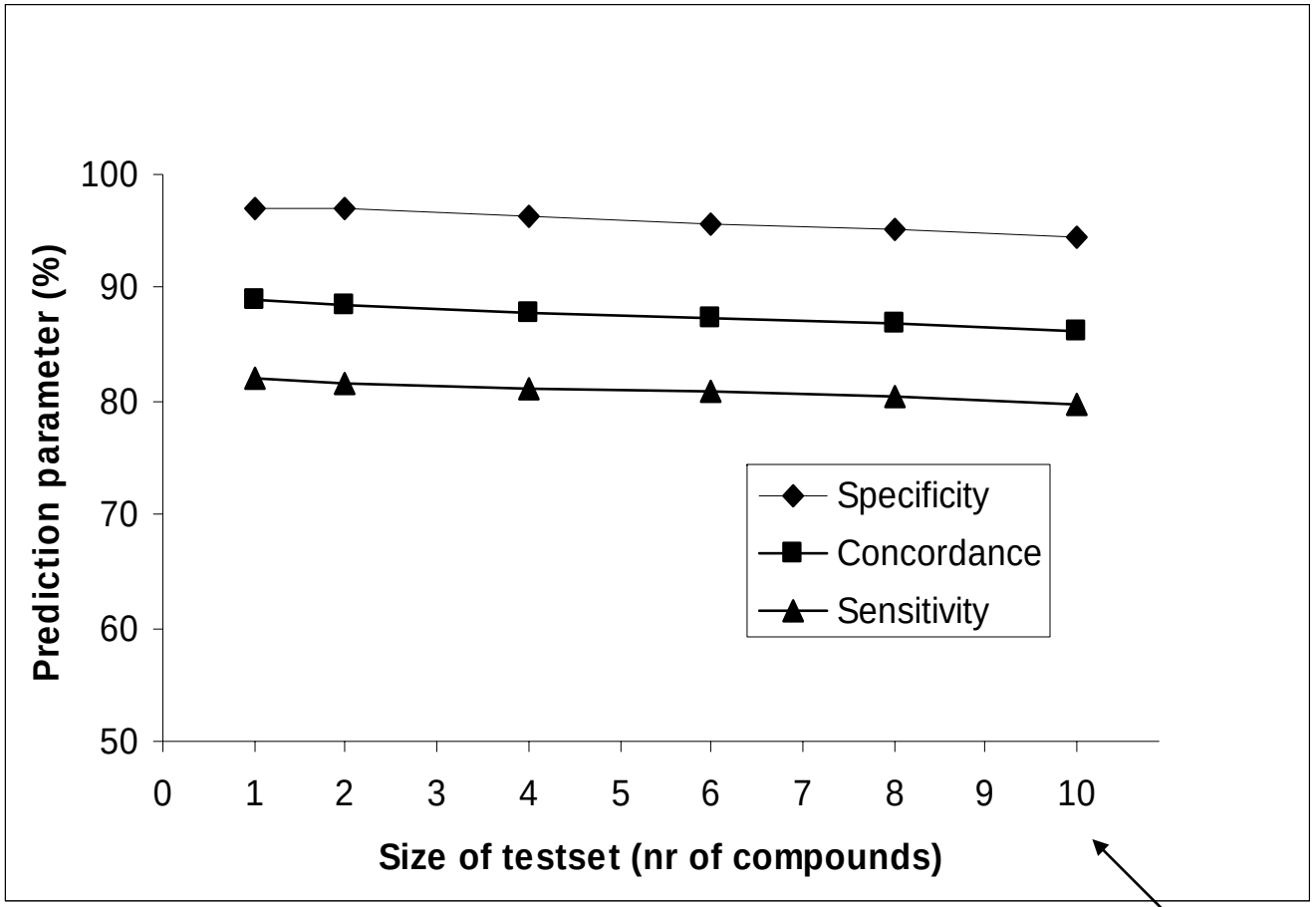
Sensitivity = 32/39	**82%**
Specificity = 33/34	**97%**
Concordance = (32+33)/73	**89%**

On the level of compounds

Sensitivity 9/10	**90%**
Specificity 11/11	**100%**
Concordance 20/21	**95%**

2 Prediction model

Effect of larger test set



training set, test set about 50% 50%

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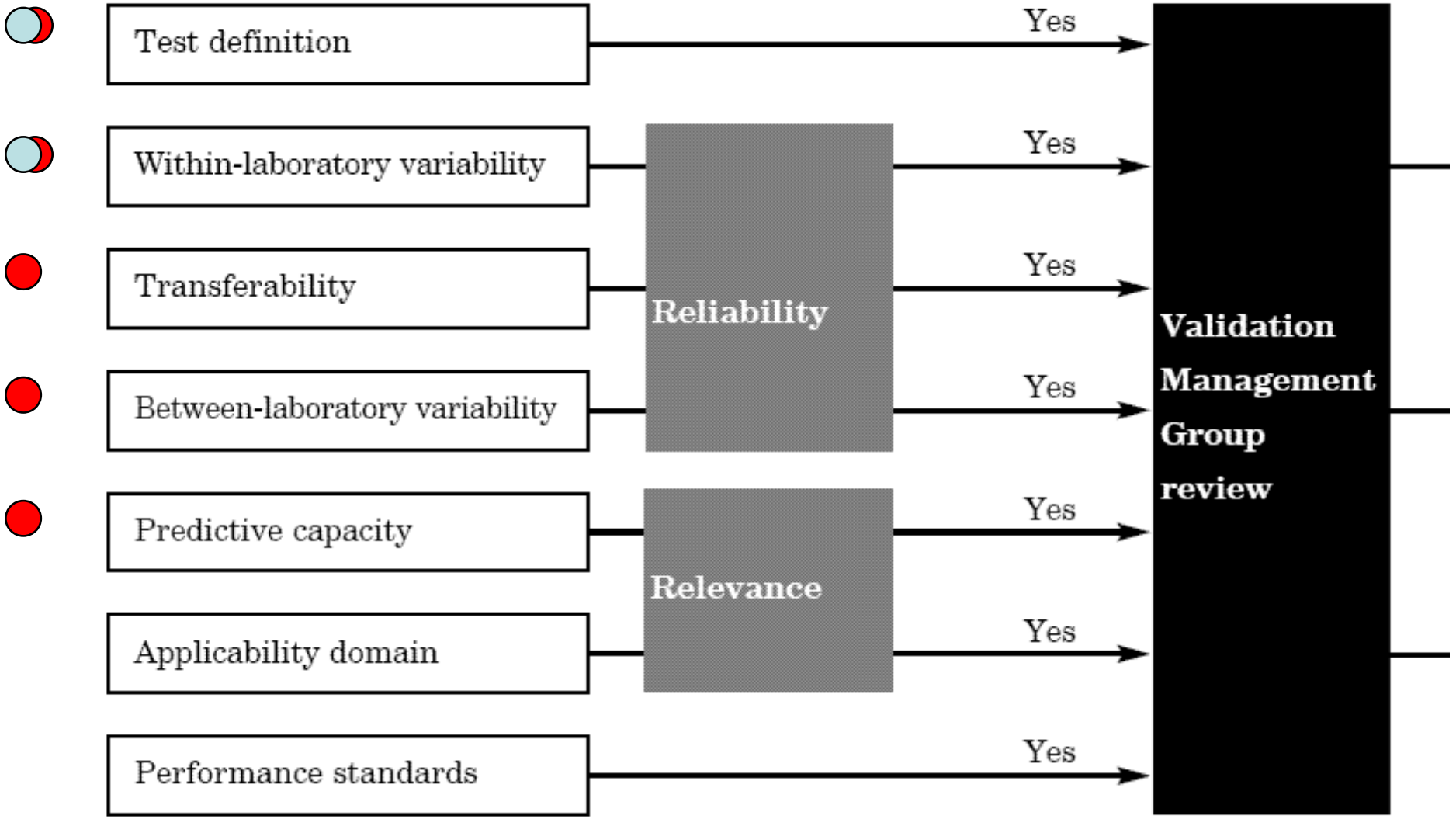
3 Future

The modular approach for applying the ECVAM principles on test validity



Skin sensitization: VITOSENS™

VITOSENS is operational for screening

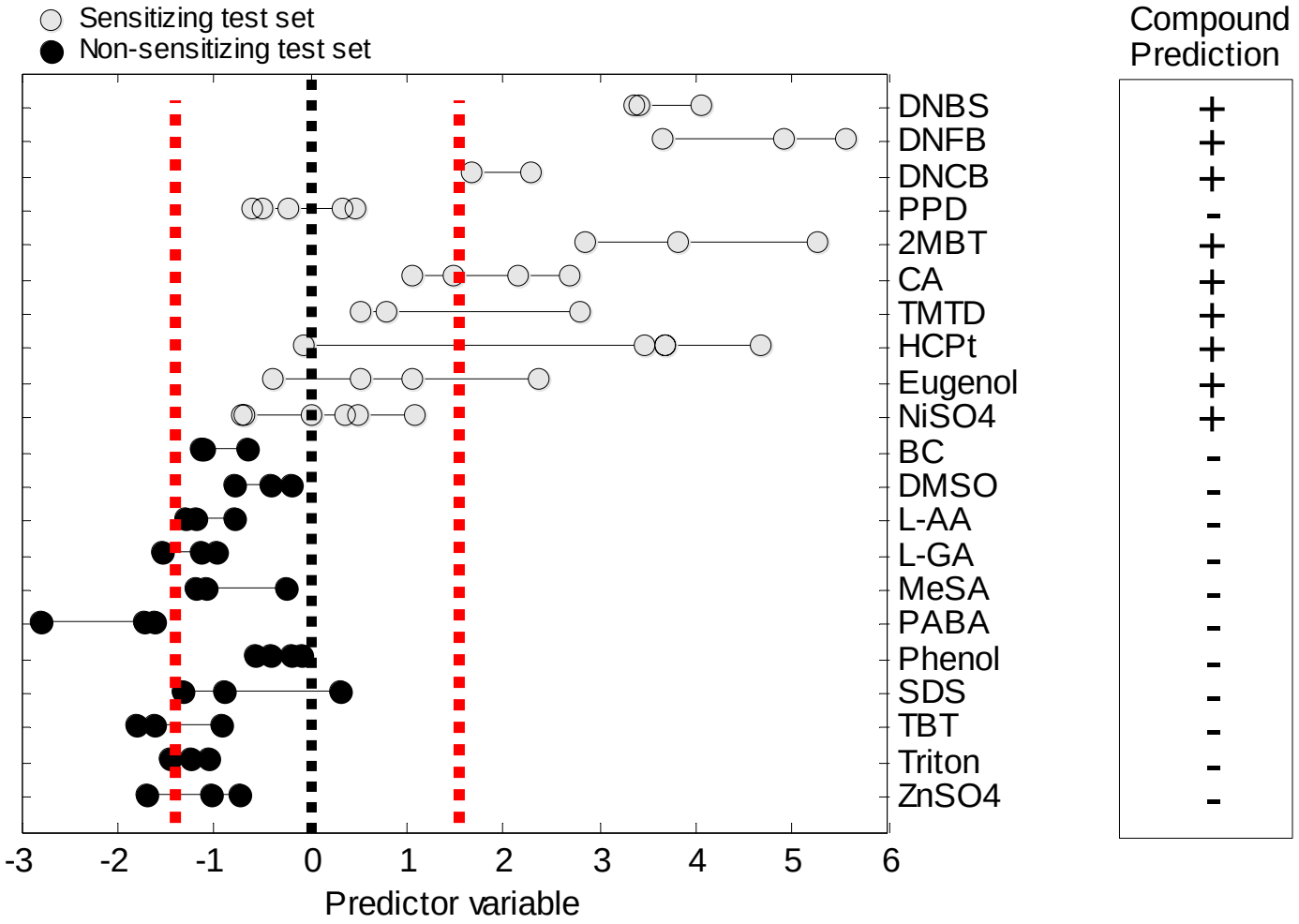
Test can currently be performed at CARDAM

Strategies for cost reduction:

1) Evaluate results per cell culture
2) Reduction of exposure conditions

Skin sensitization: VITOSENS™

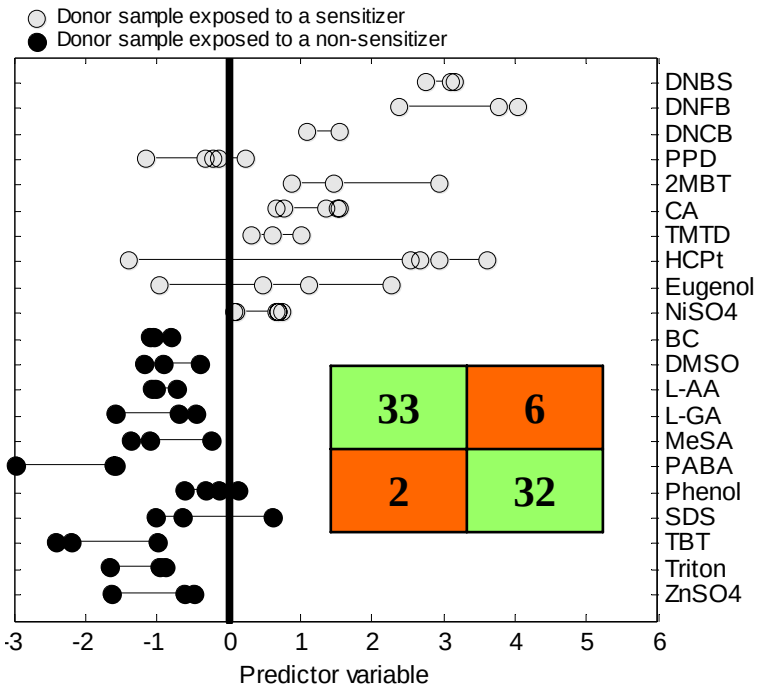
Evaluate results per cell culture

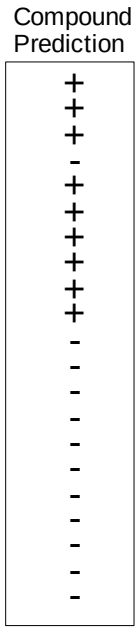


Skin sensitization: VITOSENS™

Reduction of exposure conditions

only 6h





6h & 11h & 24h