XCellR8
Redefining testing

A new definition for animal-free testing

REPLACING ANIMAL-DERIVED COMPONENTS IN REGULATORY *IN VITRO* METHODS

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PISC webinar: Replacing Foetal Bovine Serum in Cell Culture Media
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What is truly animal-free testing?

- Most *in vitro* methods utilise animal components
  - Fetal bovine serum
  - Tissue extracts
  - Antibodies
- Reasons: largely historical
- Scientific and ethical considerations
- Truly animal-free testing needs to be animal-product-free
- Driven by consumer and industry demand for sustainable, ethical products (*and* ethical testing)
- Vegan products require vegan testing

Everything we do at XCellR8 is animal-product-free (“vegan testing”) - GLP

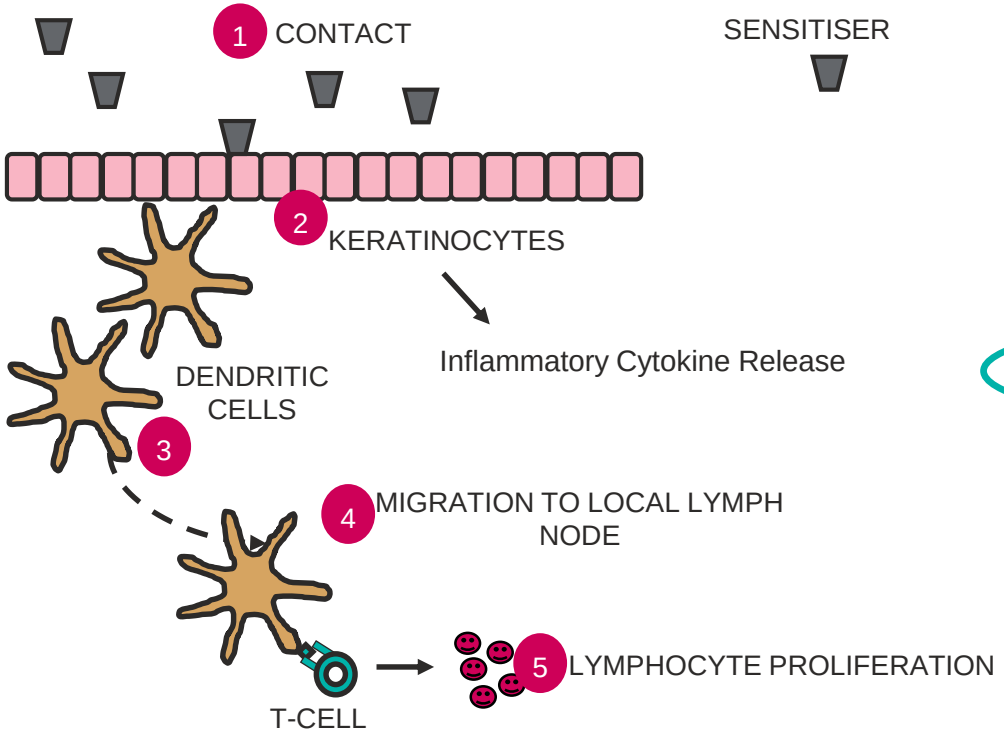
Current regulatory guidance favours “2 out of 3” approach

Animal-product-free skin sensitisation testing

THE CHALLENGE

- DPRA (OECD TG 442c): **OK**
- KeratinoSens™ (OECD TG 442d): **animal components**
- h-CLAT (OECD TG 442e): **animal components**

Skin sensitisation adverse outcome pathway (AOP)



KEY EVENTS IN SKIN SENSITISATION AND RELATED TESTS

1. Contact
   (Direct Peptide Reactivity Assay – **DPRA**)
2. Release of Pro-Inflammatory Cytokines by Keratinocytes (**KeratinoSens™**)
3. Dendritic Cell Activation/Maturation
   (human Cell Line Activation Test – **h-CLAT**)
4. Migration
5. T-cell Proliferation
   (Local Lymph Node Assay - LLNA)

Regulatory guidance: “2 out of 3” approach

Adaptation of the KeratinoSens™ Skin Sensitisation Test (OECD TG 442d) to Xeno-Free Conditions

Published in ALTEX:

Belot, N., Sim, B., Longmore, CL., Roscoe, L. and Treasure, C. (2017)

Adaptation of the KeratinoSens™ skin sensitisation test to animal-product-free cell culture >

KeratinoSens™ - Method outline

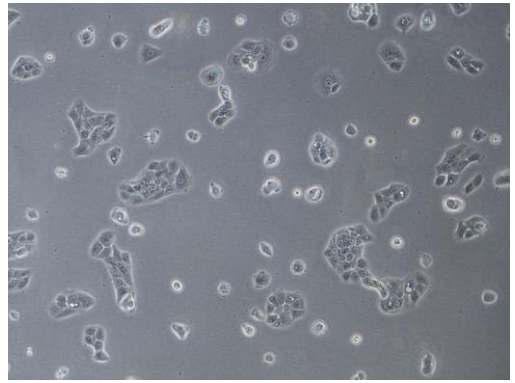
- Human keratinocyte cell line (HaCaT) transfected with a luciferase reporter linked to Nrf2-mediated activation of Antioxidant Response Element (ARE)-linked genes
- 12 concentrations of test chemical incubated for 48 hours (in triplicate; 3 independent runs)
- Luciferase response measured by luminescence and cytotoxicity measured by MTT

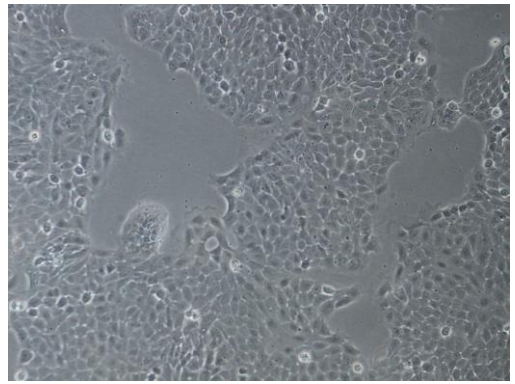
Xeno-Free adaptation of KeratinoSens™

- Animal-derived components were replaced with human-derived & recombinant equivalents:
  - FBS replaced with pooled human serum (60-70 donors) obtained from FDA-approved source / Sigma Aldrich – cells adapted to new culture conditions
  - Porcine trypsin replaced with recombinant Trypzean™
- In-house validation using the panel of proficiency chemicals and performance standards for OECD TG 442d

KeratinoSens™ cell line adaptation to culture in human serum

- Basal medium: Dulbecco's Modified Eagle's Medium (DMEM)
- Gradual adaptation (weaning) from 10% FBS to 10% pooled human serum
- Comparable morphology and growth rates (population doubling times)
- Sample cell counts after 72 hours in culture (seeding density 1.2 x 10^5 cells/ml):
  - FBS: 3.6 x 10^5 cells/ml
  - Human serum: 4.9 x 10^5 cells/ml
- Sub-cultured at 80-90% confluence using TrypZean® (recombinant trypsin product)
- Growth rates sustained up to passage 22 (compared with p25 reported in FBS)



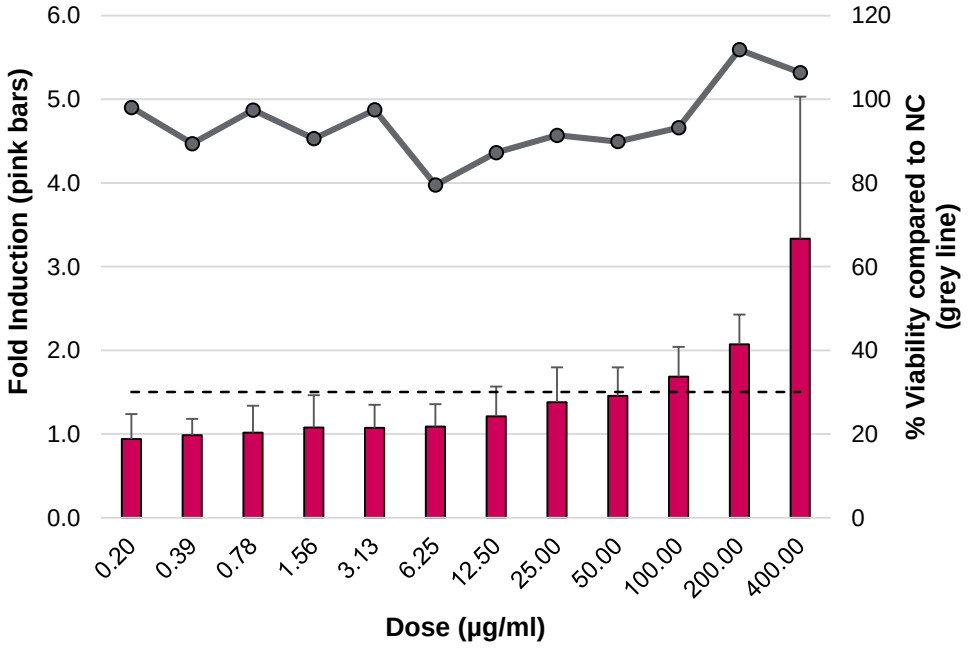


KeratinoSens™ xeno-free adaptation: In-house validation

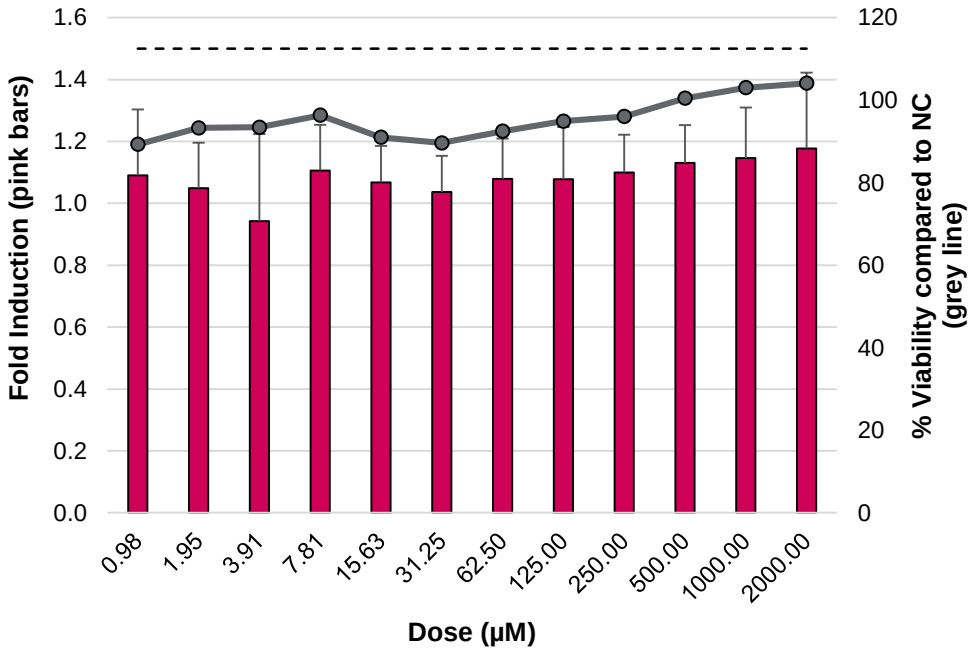
- 10 chemicals listed in the proficiency testing requirements of OECD Test Guideline 442D
- Additional 11 chemicals as per the KeratinoSens™ Performance Standards
- Total dataset 21 chemicals – known sensitisers and non-sensitisers
- 3 independent runs per chemical
- Triplicate samples per run
- Total 9 datapoints

KeratinoSens™ sample results

Sensitiser



Non-Sensitiser



Key parameters measured: EC1.5 (concentration to achieve 1.5-fold induction); Imax (maximum fold induction)

Results: Non-Sensitisers (*as per LLNA*)

Chemical Name	Validated Reference Method (VRM)			XCellR8 Animal-Product-Free Adaptation		
	I_{Max}	EC1.5 (µM)	Prediction	I_{Max}	EC1.5 (µM)	Prediction
Isopropanol	1.2	n.i.	Non-Sensitiser	1.2	n.i.	Non-Sensitiser
Salicylic Acid	1.1	n.i.	Non-Sensitiser	1.4	n.i.	Non-Sensitiser
Lactic Acid	1.3	n.i.	Non-Sensitiser	1.3	n.i.	Non-Sensitiser
Glycerol	1.2	n.i.	Non-Sensitiser	1.4	n.i.	Non-Sensitiser
4-methoxy-acetophenone	1.7	449.3	*Sensitiser*	2.1	620	*Sensitiser*
Chlorobenzene	1.2	n.i.	Non-Sensitiser	1.2	n.i.	Non-Sensitiser
Methyl Salicylate	1.2	n.i.	Non-Sensitiser	1.2	n.i.	Non-Sensitiser
Sulfanilamide	1.4	n.i.	Non-Sensitiser	1.1	n.i.	Non-Sensitiser

n.i. = not induced

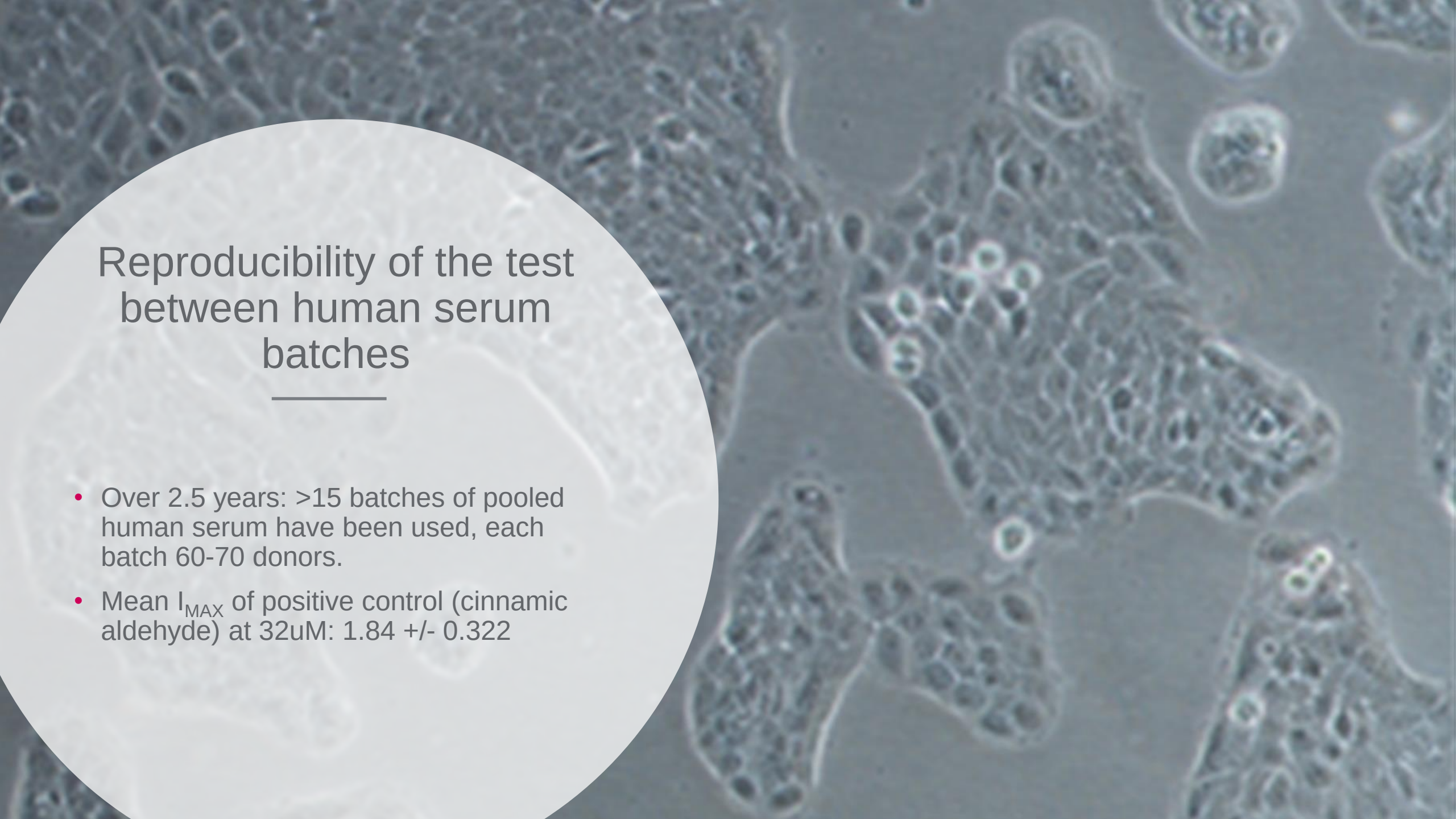
Results: Sensitisers (*as per LLNA*)

Chemical Name	Validated Reference Method (VRM)			XCellR8 Animal-Product-Free Adaptation		
	I_{Max}	EC1.5 (µM)	Prediction	I_{Max}	EC1.5 (µM)	Prediction
Cinnamyl alcohol	1.7	123.6	Sensitiser	4.2	20	Sensitiser
Ethylene Glycol Dimethacrylate	188	57.4	Sensitiser	4.8	29	Sensitiser
Phenyl Benzoate	1.3	n.i.	*Non-Sensitiser*	1.1	n.i.	*Non-Sensitiser*
Eugenol	1.3	n.i.	*Non-Sensitiser*	2.2	286	*Non-Sensitiser (borderline)*
2-Mercaptobenzothiazole	8.8	48.1	Sensitiser	6.9	57	Sensitiser
Citral	96.4	23.2	Sensitiser	3.8	18	Sensitiser
Isoeugenol	6.4	16.1	Sensitiser	3.4	20	Sensitiser
Methyldibromo Glutaronitrile	4	7.8	Sensitiser	2.7	8	Sensitiser
4-Methylaminophenol Sulfate	5.9	9.4	Sensitiser	36.1	4	Sensitiser
Para-phenylene Diamine	26.8	5	Sensitiser	28.2	6	Sensitiser
2,4-Dinitrochlorobenzene	14.8	2.5	Sensitiser	8.5	1	Sensitiser
4-Nitrobenzyl Bromide	6.9	1.3	Sensitiser	10.5	<0.98	Sensitiser
Oxazolone	2.4	175.5	Sensitiser	5.4	129	Sensitiser

n.i. = not induced

Reproducibility of the test between human serum batches

- Over 2.5 years: >15 batches of pooled human serum have been used, each batch 60-70 donors.
- Mean I_{MAX} of positive control (cinnamic aldehyde) at 32uM: 1.84 +/- 0.322



Animal-product-free (APF) adaptation of KeratinoSens™ Conclusions

- All 20 reference chemicals correctly classified in line with Validated Reference Method (VRM)
- Data accepted by the OECD Expert Working Group on Skin Sensitisation and WNT National Co-Ordinators' Committee
- Adapted method published as an Annex to the VRM in the new version of OECD TG 442d 2018
- Therefore full acceptance as a regulatory method

Current and future work

- Participation in thought-starter paper and OECD workshop on the ethical use of human reagents:
  - Addressing potential ethical issues regarding the supply of human-derived products or reagents in *in vitro* OECD Test Guidelines. Published in ALTEX 2019
- Xeno-free adaptation of h-CLAT including human serum and animal-free antibodies:
  - Edwards *et al*, ALTEX, 2018
- Adaptation of KeratinoSens™ and h-CLAT to fully defined conditions
- Auditing supply chains for all products used in the XCellR8 lab

Thanks to the XCellR8 team!

Thank you!

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Further reading

- Getting under the skin of *in vitro* skin sensitisation testing ebook
- Topics include potency assessment and testing finished products

Download your copy >

Available at x-cellr8.com/in-vitro-skin-sensitisation-testing/