Use of 3 Animal Product-Free Methods as Part of a Novel Integrated Approach to Skin Sensitisation Testing

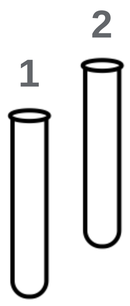
Alexander Edwards[1], Nathalie Belot[1], Lottie Roscoe[1], Christopher Longmore[1], Bushra Sim[1], Carol Treasure[1].
[1]XCellR8 Ltd, The Innovation Centre, Sci-Tech Daresbury, Cheshire WA4 4FS, UK
Email: info@x-cellr8.com

INTRODUCTION

An integrated approach to *in chemico* and *in vitro* skin sensitisation using three methods; Direct Peptide Reactivity Assay (DPRA), KeratinoSens™ and human Cell Line Activation Test (h-CLAT), has recently been approved by the OECD. Here, we present novel data from 2 representative case studies using animal product free adaptations of the 2 *in vitro* skin sensitisation methods alongside data from the *in chemico* DPRA method. Animal product free adaptations to h-CLAT and KeratinoSens™ include the use of Human Serum, Human Serum Albumin, custom anti-CD54 and anti-CD86 antibodies produced using phage display, and Trypzean from plant sources to replace the use of animal derived components. All 3 methods have been validated in-house and approved for use in REACH submissions. We are seeking inclusion of the *in vitro* adaptations into OECD TGs 442D and 442E. The 2 test items from the case study are the first to be used in this completely animal free workflow and have been successfully classified using the 3 methods, with a 2/3 approach being taken to decide upon the outcome of the testing.

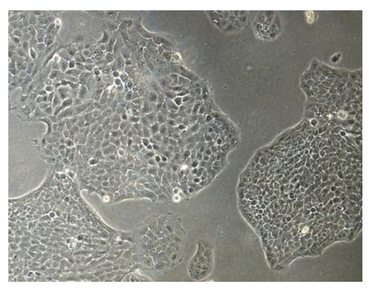
METHODS AND RESULTS

DPRA (*in chemico*)



Test Items

KeratinoSens™ (*in vitro*)



XCellR8 animal-free modifications:

In
- ✓ Human Serum
- ✓ Trypzean (plant based enzyme)

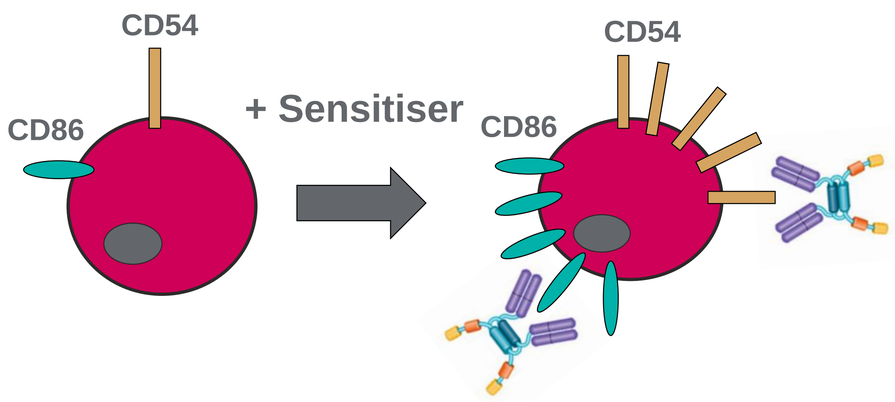
Out
- × Foetal Bovine/Calf Serum (FBS/FCS)
- × Porcine Trypsin

Test Item	Cysteine % Depletion	Lysine % Depletion	Mean % Depletion	Classification
1	5.108	0.000	2.554	**Non-Sensitiser (No or Minimal Reactivity)**
2	1.449	16.529	8.989	**Sensitiser (Low Reactivity)**

Test Item	EC1.5 (mg/ml)	I_{MAX} (fold induction)	Classification
1	0.475	4.217	**Sensitiser**
2	N/A	1.127	**Non-Sensitiser**

3rd confirmatory test required

h-CLAT (*in vitro*)



Test Item	EC200 (CD54)	EC150 (CD86)	Classification
1	529 µg/ml	1120 µg/ml	**Sensitiser**
2	N/A	N/A	**Non-Sensitiser**

XCellR8 animal-free modifications:

In
- ✓ Custom project to produce HuCAL anti-CD54 and anti-CD86 antibodies (Human Combinatorial Antibody Libraries; BioRad) from non-animal source using phage display. Bivalent Fab-dHLX format i.e. no Fc regions.
- ✓ Human Serum
- ✓ Human Serum Albumin (HSA)

Out
- × Antibodies originally derived from animal source
- × Foetal Bovine/Calf Serum (FBS/FCS)
- × Bovine Serum Albumin (BSA)

DISCUSSION

The results show that methods adapted to animal product free conditions can be successfully used as part of an integrated approach to testing and assessment (IATA) for skin sensitisation. The overall outcome using the 2/3 testing approach for these methods was as follows; Test Item 1 = Sensitiser and Test Item 2 = Non-Sensitiser. It is interesting to note that both *in vitro* methods, KeratinoSens™ and h-CLAT correlated in terms of classification and gave similar EC values for the respective assay sensitisation thresholds. Differences between the results generated using *in vitro* and *in chemico* methods further support the use of an IATA for skin sensitisation testing.