

INTRODUCTION

Data published in ALTEX: Edwards et al. (2018); 35(4), 501-512.

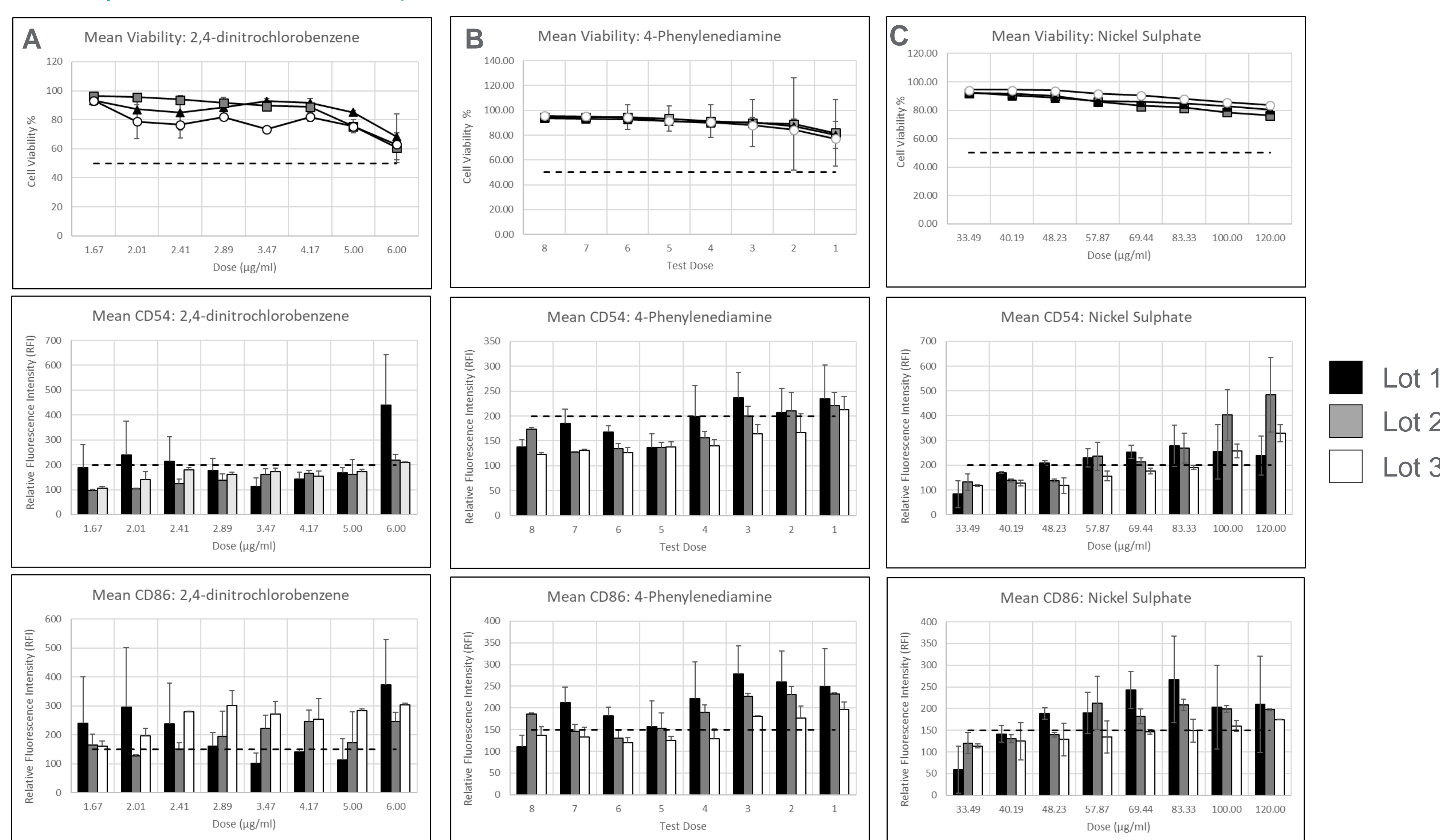
We have previously presented data validating a completely animal-product-free adaptation of the human Cell Line Activation Test (h-CLAT) for skin sensitisation testing using a single lot of human serum. The adapted method provides benefits from both a scientific and ethical standpoint. From a scientific perspective, the elimination of animal-derived components from *in vitro* test systems and replacement with human equivalents directly increases the relevance of the test system as a model of human biology. From an ethical point of view, the adaptations also provide cosmetic companies with an *in vitro* system that can respond to consumer demands to replace the use of animals and animal-derived components altogether in cosmetic testing.

METHOD AND RESULTS

Through consultation with the OECD expert working group on Skin Sensitisation we undertook an inter-lot comparison of 3 lots of commercially available human serum using the animal-product-free adaptation of the h-CLAT method in order to gain additional data to support the inclusion of the animal-product-free method into OECD Test Guideline (TG) 442E. As each human serum lot is comprised of 50-70 pooled donors per batch, this represents a large number of donors overall. A panel of 6 proficiency chemicals, 3 positive; 2,4-dinitrochlorobenzene, 4-Phenylenediamine, Nickel Sulphate and 3 negative; Isopropanol, Glycerol, Lactic Acid, from OECD TG442E were selected and assessed using the dose finding CV75 assay and the CD54/CD86 expression measurement that comprise the h-CLAT method.

Viability and CD54/CD86 expression data for known Skin Sensitisers across 3 lots of Human Serum

XCellR8 animal-free modifications:



In

- ✓ Custom 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)

Relative Fluorescence Intensity (RFI) data for CD54 and CD86 for 2,4-dinitrochlorobenzene (A), 4-Phenylenediamine (B) and Nickel Sulphate (C) generated using 3 separate lots of human serum (n=6 over an 8 dose range) with concurrent cytotoxicity measurement (mean viability). The data presented are Mean and Standard Error of the Mean (SEM) values from two individual experiments for each lot of human serum (n=6 in total).

CV75 Data across 3 lots of Human Serum

EC200 (CD54) and EC150 (CD86) data: 3 lots of Human Serum

Effective concentrations which give RFI values of 200 for CD54 and 150 for CD86 – as determined using the dose response data above (n=6, 2 replicates per lot of human serum).

Proficiency Substance	OECD Range	Human Serum Lot 1 CV75 Average (µg/ml)	Human Serum Lot 2 CV75 Average (µg/ml)	Human Serum Lot 3 CV75 Average (µg/ml)
2,4-dinitrochlorobenzene	2-12	3.20	3.2	2.40
4-Phenylenediamine	5-95	32.60	23.6	14.70
Nickel Sulphate	30-500	44.40	92.5	90.30
Isopropanol	>5000	5000	5000	5000
Glycerol	>5000	5000	5000	5000
Lactic Acid	1500-5000	1546.40	2852.6	2053.40

Proficiency Substance	OECD EC200 range (µg/ml)	Lot 1 Human Serum EC200 (µg/ml)		Lot 2 Human Serum EC200 (µg/ml)		Lot 3 Human Serum EC200 (µg/ml)	
		Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
2,4-dinitrochlorobenzene	0.5-15	1.4	5.3	4.6	5.7	5.8	5.7
4-Phenylenediamine	Neg or >1.5	12.6	Neg	Neg	18.4	Neg	26.3
Nickel sulphate	10-100	48.4	44.8	62.6	52.3	89.1	83.5
Isopropanol	Neg (>5000)	Neg (>5000)	Neg (>5000)	Neg (>5000)	Neg (>5000)	Neg (5812.4)	Neg (>5000)
Glycerol	Neg (>5000)	Neg (>5000)	Neg (>5000)	Neg (>5000)	Neg (>5000)	Neg (>5000)	Neg (>5000)
Lactic acid	Neg (>5000)	Neg (>1856)	Neg (>1856)	Neg (>3423)	Neg (>3423)	Neg (>3423)	Neg (>3423)

Proficiency Substance	OECD EC150 range (µg/ml)	Lot 1 Human Serum EC150 (µg/ml)		Lot 2 Human Serum EC150 (µg/ml)		Lot 3 Human Serum EC150 (µg/ml)	
		Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
2,4-dinitrochlorobenzene	0.5-10	1.1	4.2	2.2	3.3	1.5	1.7
4-Phenylenediamine	<40	11.2	12.5	11.5	15.3	16.2	18.3
Nickel sulphate	<100	39.8	44.3	56.3	49.3	69	37.7
Isopropanol	Neg (>5000)	Neg (>5000)	Neg (>5000)	Neg (>5000)	Neg (>5000)	Neg (>5000)	Neg (>5000)
Glycerol	Neg (>5000)	Neg (>5000)	Neg (>5000)	Neg (>5000)	Neg (>5000)	Neg (>5000)	Neg (>5000)
Lactic acid	Neg (>5000)	Neg (>1856)	Neg (>1856)	Neg (>3423)	Neg (>3423)	Neg (>3423)	Neg (>3423)

DISCUSSION

The CV75 data for each chemical (6/6) across the three lots of human serum met the OECD ranges specified in the test guideline. All 6 proficiency chemicals were correctly classified across the 3 lots of human serum with high levels of consistency in both the cytotoxicity and CD54/CD86 expression measurements. With respect to the OECD ranges, 5/6 proficiency chemicals in all 3 lots of human serum met the ranges specified in the guideline for CD54 and CD86 expression. In conclusion, consistent data that met the OECD proficiency acceptance criteria were generated across three lots of human serum using the animal-product-free h-CLAT method.