Abstract Number/Poster Board number: 3381/ P171

ASSESSMENT OF SKIN SENSITISATION VIA *IN VITRO* HUMAN CELL LINE ACTIVATION TEST METHOD (H-CLAT)

Mishra P.K.[1], Vashi K.D.[1], Barad I.M.[1], Nagane R.M.[1], Patel M.V.[1], Piccirillo V.J.[2] and Date R.A.[1]
[1]Jai Research Foundation, Vapi, Gujarat, India and [2]VPTOX, LLC, Ashburn, VA, USA

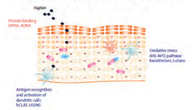
JRF GLOBAL

INTRODUCTION

Allergic contact dermatitis (ACD) is one of the leading causes of occupational diseases affecting approximately 15-20% of the general population in industrialised nations. Skin sensitisation resulting in ACD is an immunologically mediated cutaneous reaction to a substance, characterised by erythema, oedema, etc. in humans. The existing knowledge of the chemical and biological processes associated with skin sensitisation can be summarised in the form of adverse outcome pathway (AOP) [Figure 1]. Dendritic cells (DCs) of the skin (Langerhans cells) play a key role in the development of ACD. After the uptake of a chemical allergen they become activated, migrate to the local lymph node and induce a specific T lymphocyte response. Upregulation of CD86 expression is a hallmark of DC activation and maturation. Non-animal models such as Direct Peptide Reactivity assay (DPRA), human cell line activation test (h-CLAT) are currently looked upon for studying skin sensitisation.

Figure 1(A) Key Events in Skin Sensitisation

Figure 1(B) Schematic representation of key events and associated assays



OBJECTIVE

The study was conducted to validate *in vitro* human cell line activation test by evaluating skin sensitisation potential of proficiency chemicals (i.e., 2,4-Dinitrochlorobenzene, 4-Phenylenediamine, Nickel sulfate, 2-Mercaptobenzothiazole, R(+) Limonene, Imidazolidinyl urea, Isopropanol, Lactic acid, Glycerol and 4-Aminobenzoic acid).

MATERIALS & METHODS

Test System: THP-1, human monocytic leukemia cell line, procured from American Type Culture Collection (ATCC), (TIB-202™).
THP-1 cells are used as a surrogate of dendritic cells.

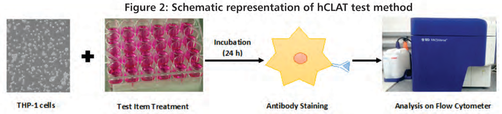
1. Dose Range Finding (DRF) assay

- Determination of CV75 (test item concentration resulting in 75% cell viability compared to the solvent/vehicle control)
- 96-well plates seeded with 1.6 * 10^5 cells/well and exposed to 10 concs. of test item – incubation (24 ± 0.5 h, 37 ± 1 °C, 5 ± 1% CO_2)
- After 24 h, staining with propidium iodide (PI) – analysis on flow cytometer – determination of cell viability
- Results of DRF used for dose selection for CD86/CD54 expression measurement

2. CD86/CD54 Expression Measurement

- To determine upregulation of CD86/CD54 markers upon contact with sensitising chemicals
- Positive control – 2,4-Dinitrochlorobenzene (DNCB) – final test conc. 4 µg/mL
- Negative control – Saline (final conc. 1%) and/or DMSO (final conc. 0.2%)
- 24-well plates seeded with 1 * 10^6 cells/well and exposed to 8 concs. of test item – incubation (24 ± 0.5 h, 37 ± 1 °C, 5 ± 1% CO_2)
- After incubation – staining with antibodies (IgG1, CD86 and CD54) and PI - analysis on flow cytometer
- Determination of Mean Fluorescence Intensity (MFI) and Relative Fluorescence Intensity (RFI)

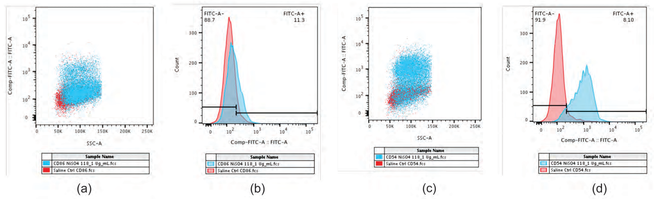
Figure 2: Schematic representation of hCLAT test method



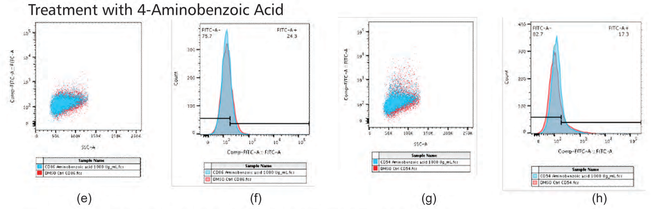
RESULTS

Figure 3: Results of flow cytometer analysis (CD86/CD54 expression measurement) of representative test items

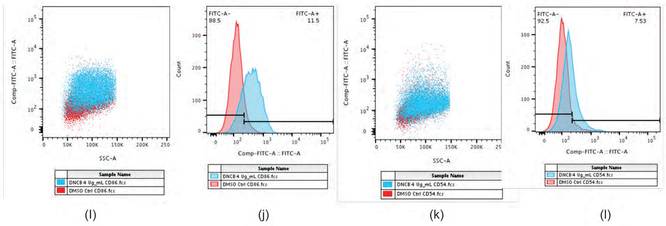
Treatment with Nickel Sulfate



Treatment with 4-Aminobenzoic Acid



Treatment with 2, 4-Dinitrochlorobenzene (Positive Control)



Solvent Control | Treatment

Figure 3: **a-d:** Results of CD86/CD54 Expression analysis of Nickel sulfate, **e-h:** Results of expression analysis of 4-Aminobenzoic acid, **i-l:** Results of expression analysis of positive control 2,4-Dinitrochlorobenzene (DNCB)
The Red dot plots and histograms in figure are of solvent controls [i.e., DMSO (solvent control for 4-Aminobenzoic acid and DNCB) and Saline (for Nickel Sulfate)], while blue dot plots and histograms are of test item. Figure a-d clearly shows increase in expression of CD86 (a-b) and Cd54 (c-d) markers upon treatment with nickel sulfate in comparison to solvent (saline) control. Figure e-h clearly shows no increase in expression of CD86 (e-f) or CD54 (g-h) markers upon treatment with 4-Aminobenzoic acid in comparison to solvent (DMSO) control. Figure i-l shows expression of CD86 and CD54 markers upon treatment with positive control. An increase in expression of both CD86 (i-j) and CD54 (k-l) is observed upon treatment with DNCB.

Table 1: hCLAT Prediction of Test Items

S. N°	Test Item	CV75 (in µg/mL)		EC150(for CD86)[a]		EC200(for CD54)[b]		*In Vivo* Prediction	Observed hCLAT Prediction*
		Observed results (µg/mL)	OECD Reference range (µg/mL)	Observed results (µg/mL)	OECD Reference range (µg/mL)	Observed results (µg/mL)	OECD Reference range (µg/mL)		
1	2,4-Dinitrochlorobenzene	4.9	2-12	2	Positive (0.5-10)	3	Positive (0.5-15)	Sensitiser (extreme)	Positive
2	4-Phenylenediamine	39.8	5-95	27	Positive (<40)	31	Negative (>1.5)	Sensitiser (strong)	Positive
3	2-Mercaptobenzothiazole	139.2	30-400	-	Negative (>10)	48	Positive (10-140)	Sensitiser (moderate)	Positive
4	Nickel Sulfate	204.0	30-500	66	Positive (<100)	65	Positive (10-100)	Sensitiser (moderate)	Positive
5	R(+) Limonene	302.8	>20	-	Negative (>5)	83	Positive (<250)	Sensitiser (weak)	Positive
6	Imidazolidinyl urea	37.2	25-100	39	Positive (20-90)	38	Positive (20-75)	Sensitiser (weak)	Positive
7	Isopropanol	>5000	>5000	>5000	Negative (>5000)	>5000	Negative (>5000)	Non Sensitiser	Negative
8	Glycerol	>5000	>5000	>5000	Negative (>5000)	>5000	Negative (>5000)	Non Sensitiser	Negative
9	Lactic acid	2635.2	1500-5000	>3162.2	Negative (>5000)	>3162.2	Negative (>5000)	Non Sensitiser	Negative
10	4-Aminobenzoic acid	>1000	>1000	>1000	Negative (>1000)	>1000	Negative (>1000)	Non Sensitiser	Negative

EC150 (for CD86)[a]: the concentrations showing the RFI values of 150 in CD86 expression
EC200 (for CD54)[b]: the concentrations showing the RFI values of 200 in CD54 expression
*= A test item is predicted as "positive" if;
RFI CD86 ≥ 150% in at least one tested concentration (cell viability ≥ 50%); and/or
RFI CD54 ≥ 200% in at least one tested concentration (cell viability ≥ 50%)

CONCLUSION

- The sensitisation potential of all 10 proficiency chemicals were correctly predicted with this assay
- In present study, hCLAT assay showed high rate of accuracy for the prediction of skin sensitisation compared with LLNA assay. Thus, this *in vitro* model can be considered as a valid alternative to study sensitisation.

REFERENCES

1. OECD (2018): The Organisation For Economic Co-operation and Development (OECD) Guideline For The Testing of Chemicals, OECD 442E, In Vitro Skin Sensitisation: In Vitro Skin Sensitisation assays addressing the Key Event on activation of dendritic cells on the Adverse Outcome Pathway for Skin Sensitisation, Section 4, OECD Publishing, Paris, adopted by council on 25th June, 2018.
2. DB-ALM (INVITTOX) (2018), Protocol 158: human Cell Line Activation Test (h-CLAT), 23pp. (Last updated on 23rd July, 2018).
3. EC EURL-ECVAM (2013), Recommendation on the human Cell Line Activation Test (h-CLAT) for skin sensitisation testing.

SOT 2019, Baltimore, USA