In Chemico Skin Sensitisation Methods and Photosensitisation Assays

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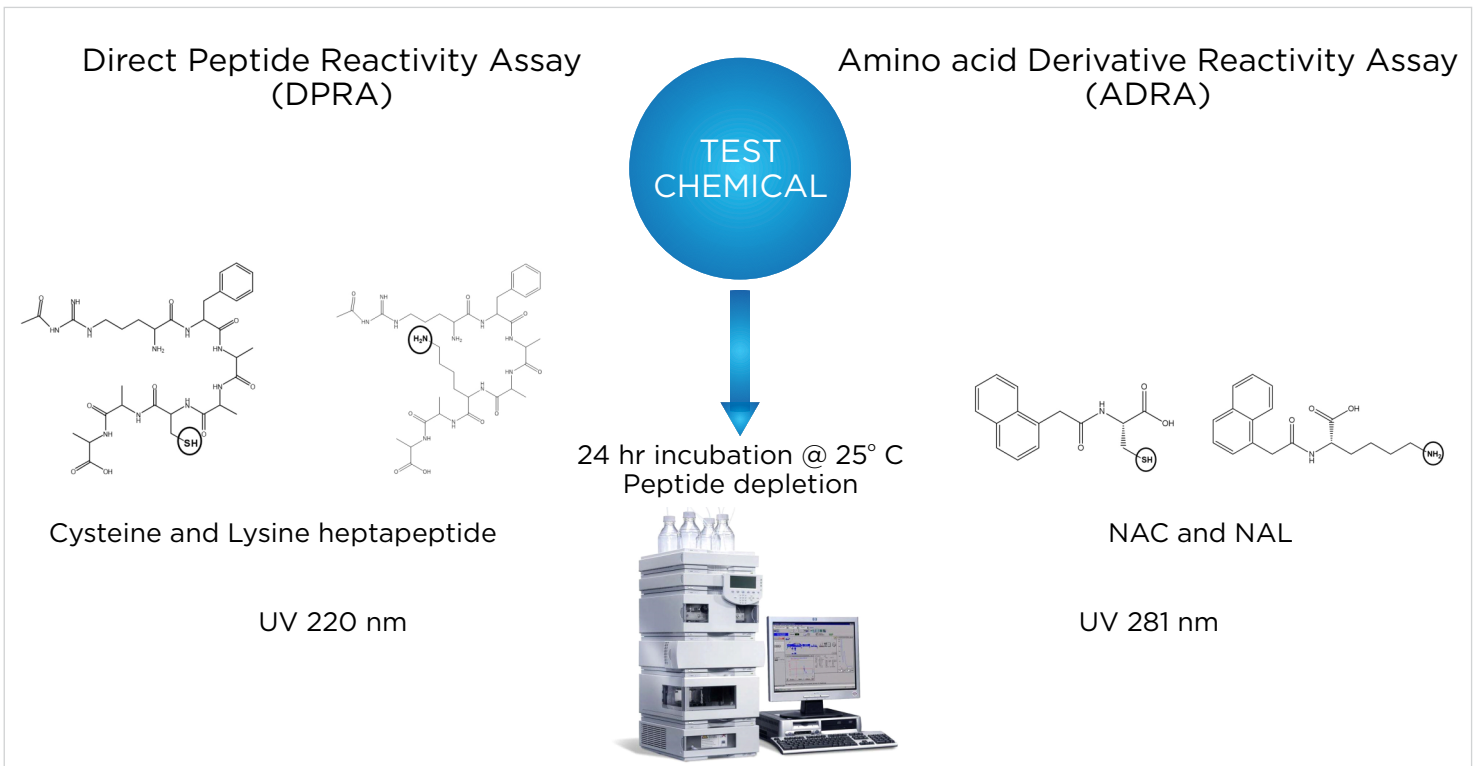
Introduction

Chemical substances that induce an allergic response in the skin upon contact are called skin allergens, while chemical substances that elicit an allergic response in the presence of light are called photo allergens.

Based on the adverse outcome pathway (AOP) of skin sensitisation, this complex process is subdivided into various sequential key events corresponding to two main phases, sensitisation, and elicitation. In the first sensitisation (or induction) phase, chemical is exposed to the skin, where skin proteins get modified upon contact with the allergen. In the second elicitation phase, upon the repeated exposure to the allergen to the skin, dendritic cells, keratinocytes are activated. Allergen modified peptides are then presented to the T-lymphocytes in the nearest lymph nodes. The more robust antigen-specific immune response may be elucidated, causing the adverse cutaneous hypersensitivity reaction.



Schematic representation of DPRA and ADRA assay setup

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These events can be studied *in vitro* by targeting well accepted specific key events with biological significance. Covalent binding to proteins, i.e., binding of haptens to endogenous proteins in the skin can be studied *in chemico* Direct peptide reactivity assay (DPRA) or amino acid reactivity assay (ADRA)(Gerberick, 2016; *Test No. 442C: In Chemico Skin Sensitisation,* 2020)[1,3], Keratinocyte activation by KeratinoSens/LuSens assay (*Test No. 442D: In Vitro Skin Sensitisation,* 2018)[4], and Dendritic cell activation assay by hCLAT or USens assay (*Test No. 442E: In Vitro Skin Sensitisation*, 2018)[5].

DPRA and ADRA are in chemico assays used to discriminate between allergens and non-allergens as described in OECD TG 442C. These assays respectively monitor the depletion of model heptapeptide and modified amino acids induced by crosslinking with test chemicals using high-performance liquid chromatography. This process of haptenisation is one of the initial and crucial events in sensitisation.

We at Jai Research Foundation (JRF) compared these two assays and analysed their suitability to predict the skin sensitisation potential of several chemical substances (Patel et al., 2019)[2]. These substances were selected to represent a range of responses for skin sensitisation hazard. The results suggested that both methods predict and discriminate between sensitisers and non-sensitisers equally well.

Test Chemical	Avg % depletion DPRA				DPRA ΔUV	Avg % depletion ADRA				ADRA ΔUV
	+UV	SD	-UV	SD		+UV	SD	-UV	SD	
Cinnamaldehyde	66.36	0.65	64.35	1.44	2.01	70.00	6.16	60.22	3.74	9.78
Chlorpromazine	54.34	0.92	0.25	0.43	54.09	59.92	0.98	7.48	3.91	52.44
SDS	48.21	0.61	47.54	0.14	0.67	0.21	0.36	0.00	0.00	0.21
L-Histidine	0.14	0.24	0.17	0.29	0.00	0.09	0.16	0.05	0.09	0.04
Amiodarone HCl	49.89	0.05	0.42	0.66	49.47	51.05	1.69	1.43	2.46	49.62
Protoporhyrin IX	55.97	4.17	29.04	2.86	26.93	57.98	1.79	26.14	5.78	31.84
Anthracene	50.78	1.36	6.04	5.79	44.74	49.58	0.46	1.71	0.86	47.87
Hexachlorophene	62.12	0.95	52.54	2.82	9.58	63.08	3.25	56.14	1.49	6.94
Norfloxacin	61.66	0.62	15.00	4.52	46.66	50.97	0.95	2.24	1.67	48.73

This table represents the % mean, the depletion of cysteine, and lysine heptapeptide with and without 5 J/cm^2 UVA treatment. Samples were incubated with the test chemical, in a dark ambience, for 24 ± 2 hours, at 25 ± 2.5 °C, before the HPLC analysis (Patel et al., 2019)[2].

Given the inherent complexity of the processes underlying skin sensitisation, it is unlikely that any single non-animal test can replace animal use for hazard identification. While these methods have proven particularly promising for the prediction of skin sensitisation potential, each method has its limitations when used in isolation. In this assay, effect of UV irritation on the compound was lacking. Thus, we at JRF modified these assays by introducing a photo-irradiation parameter and termed these assays as photo-DPRA or photo ADRA.

We have exposed the chemicals to 5 Joules/cm^2 of UVA irradiation and compared the depletion of heptapetides or the amino acid derivates.

Using this photo-DPRA and photo-ADRA, we were correctly able to distinguish known photoallergens from non-photoallergens. As shown in the table above, upon irradiation, photoallergens selectively showed higher depletion of model peptides or modified amino acids indicated by higher delta (Δ UV) value.

Thus, using these recently published, photo-DPRA and/or photo-ADRA assays can provide as non-animal *in vitro* methods for the identification and assessment of photo allergens/photosensitisers (Patel et al., 2019)[2].

References

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3. *Test No. 442C: In Chemico Skin Sensitisation.* (2020). OECD. https://doi.org/10.1787/9789264229709-en
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Dr. Rahul Date is a Biochemist with more than 20 years of experience, leading R&D team at JRF. His team is focused on developing various in vitro assays specifically in the field of skin sensitisation, endocrine disruptor and ADME assays. His work on Skin sensitisation has been published in Science and ALTEX journals.

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