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Kao STS

EPA Skin Sensitization Webinar

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Outline

Background

Key Events of Allergic Contact Dermatitis

Operational Summary and Limitations of DPRA & h-CLAT

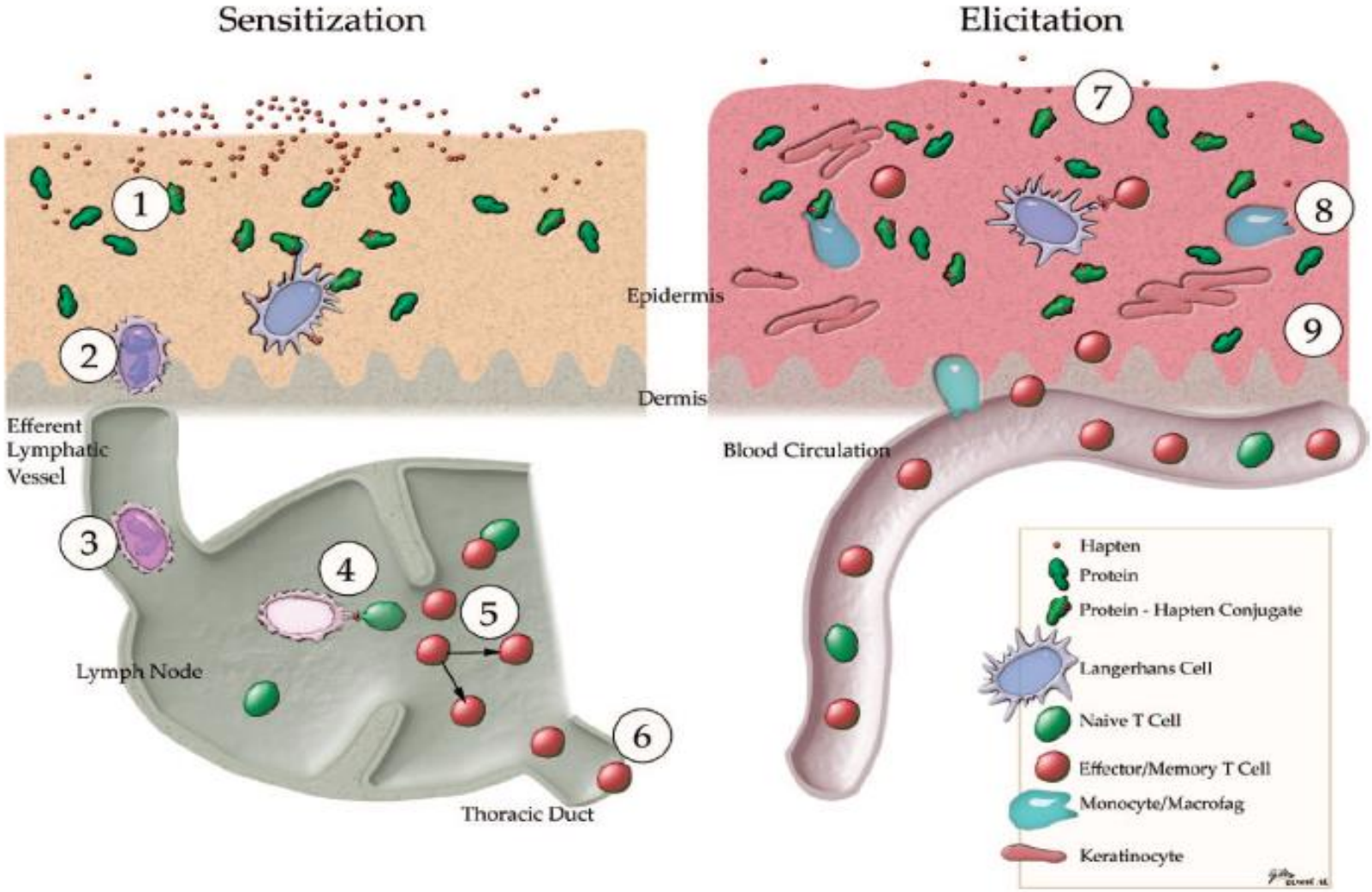
Sequential Testing Strategy with DPRA & h-CLAT

Predictive Performance

Limitation

Uncertainty

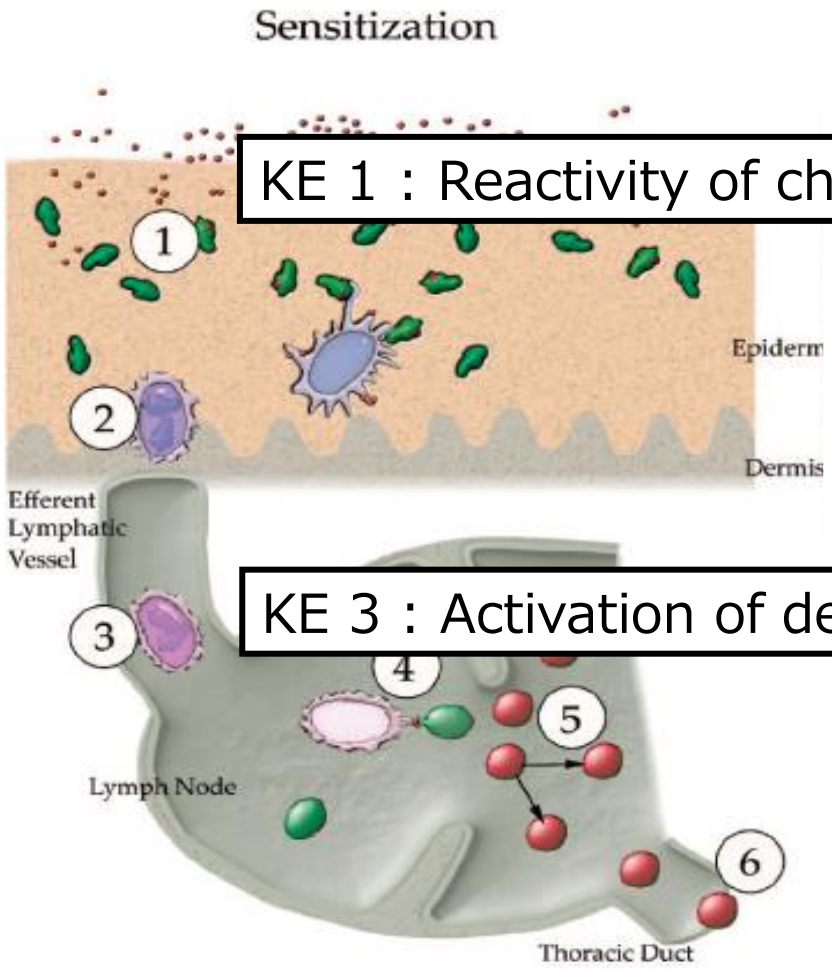
Allergic Contact Dermatitis



Karlberg *et al*., 2008

Several key events are required for acquisition of skin sensitization

In vitro Test Methods based on Key Events



Karlberg *et al.*, Chem Res Toxicol, 2008

KE 1 : Reactivity of chemical with proteins => DPRA*

- Focus on chemical reaction
- Developed by The Proctor & Gamble

*Direct Peptide Reactivity Assay

KE 3 : Activation of dendritic cells by chemical => h-CLAT**

- Focus on biological reaction
- Developed by Kao and Shiseido

**human Cell Line Activation Test

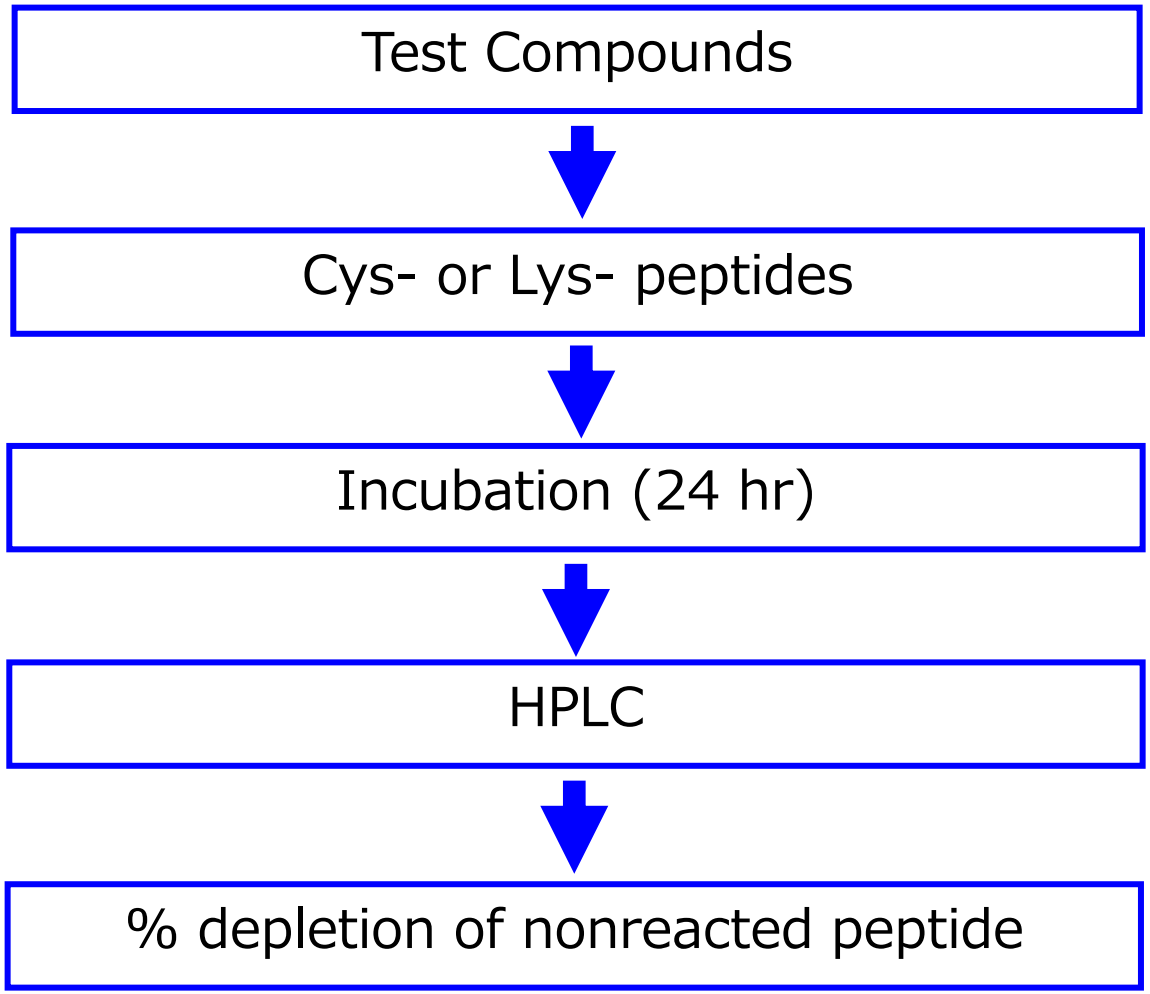
DPRA & h-CLAT have been accepted by OECD TG

Consideration of KE 2 (Keratinocyte Activation)

- In STS, the assay related to KE 2 is not included, but DPRA cysteine depletion (KE 1) and KeratinoSens™ covering KE 2 are mechanistically relevant (Joanna *et al.*, 2013).
- Key molecular pathway (Nrf2-ARE pathway) induced in KeratinoSens™ corresponds to cysteine reactivity with the Keap1 sensor protein.
- In addition, the Nrf2 activation is induced by sensitizers and not by non-sensitizers in THP-1 cells, and could function as one of the danger signals to lead to the phenotypic alterations on THP-1 cells (Migdal *et al.*, 2013; Ade *et al.*, 2009).

Mechanistic rationale that DPRA and h-CLAT could be linked to KE 2

Operational Summary of DPRA

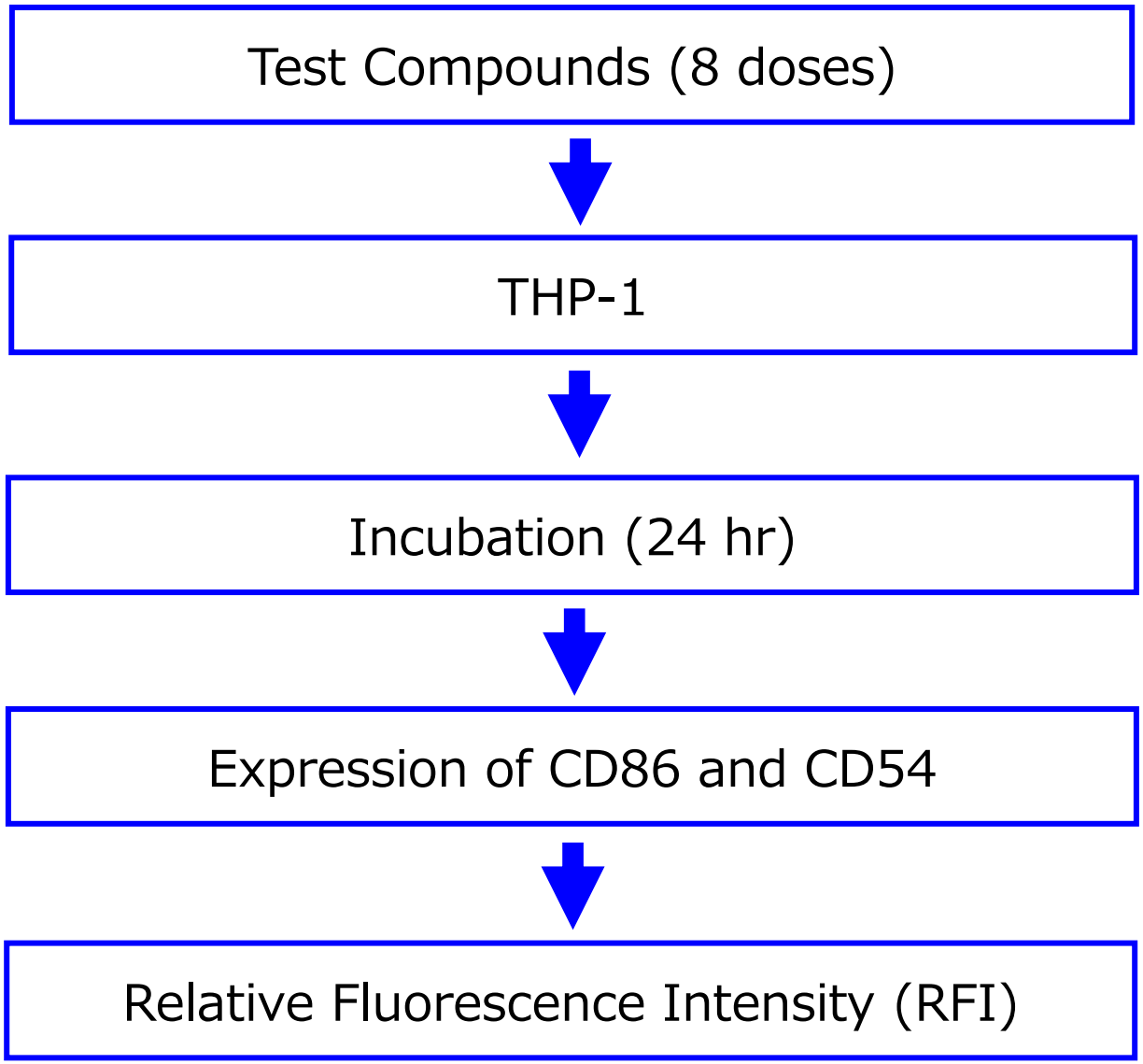


Gerberick *et al*., 2004, *Toxicol. Sci.*

Positive Criteria : Avg. Score* > 6.376%

* Average Score: (Depletion ratio of Cys peptide + Lys peptide) / 2

Operational Summary of h-CLAT



Prediction Model for h-CLAT

Positive Criteria : CD86 RFI ≥ 150% and/or CD54 RFI ≥ 200%

in at least 2 of 3 independent experiments at any dose

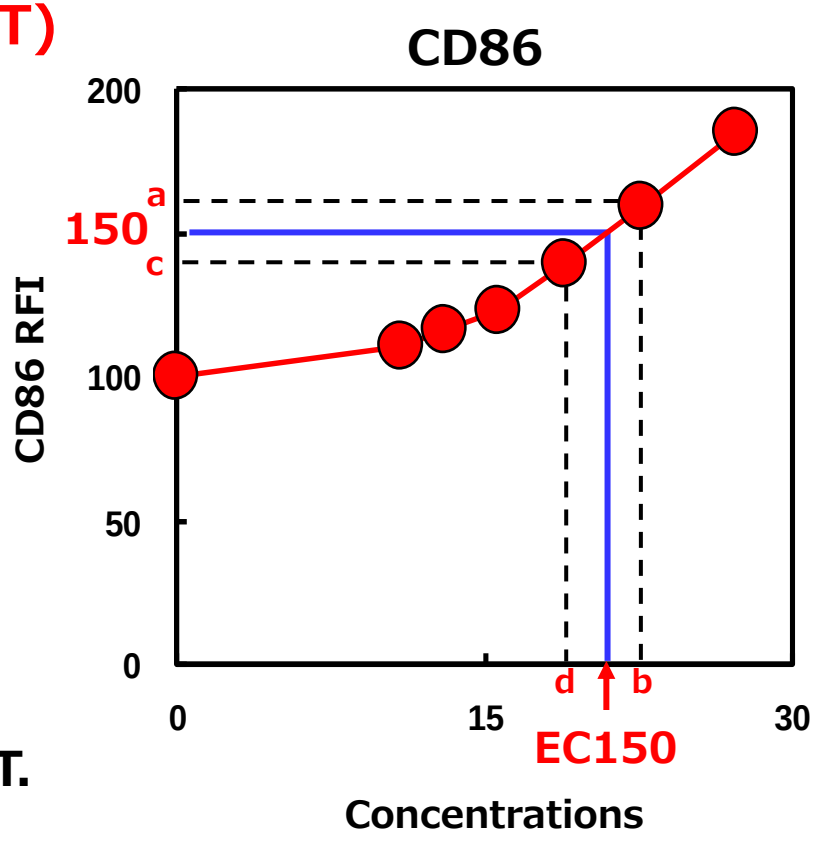
Minimal induction threshold (MIT)

Linear extrapolation

EC150 (CD86)= c + [(150-d)/(b-d)]x(a-c)

EC200 (CD54) is calculated in the same way

The median concentration of three experiments is defined as EC150 or EC200.
The lower EC value is defined as MIT.



Predictive Performance for Each Single Test

139 chemicals		h-CLAT		DPRA	
		+	-	+	-
LLNA	+	82	20	74	28
	-	11	26	9	28
Accuracy (%)		78		73	
Sensitivity (%)		80		75	
Specificity (%)		70		73	

Takenouchi *et al*., 2015

Single test method is insufficient to cover the AOP and have high accuracy

Summary of In vitro Test Methods

	DPRA	h-CLAT
Procedure	• Incubate test chemical with model peptide • Analyze the non-reacted peptide by HPLC	• Incubate test chemical with THP-1 cells for 24h • Measure fluorescence intensity by flow cytometry
Exposure condition	Cys pep.: test chemical = 1:50 M ratio Lys pep.: test chemical = 1:10 M ratio	8 doses based on CV75
Limitation	• **Lipophilic chemicals** • Pre-/pro-haptens • **Unknown molecular weights** • Chemicals having the same retention time as model peptides	• **Lipophilic chemicals (log Kow>3.5)** • Pre-/pro-haptens • Strong fluorescent chemicals

Discussion for Limitation of h-CLAT

OECD TG 442E *(under review)*

- If cytotoxicity (< 90% cell viability) observed with test chemicals with a Log Kow >3.5 is reached at a maximum soluble test concentration, criteria for negativity can be applied.
- If a negative result is observed with test chemicals with a Log >3.5 and no cytotoxicity is reached, the result should be considered as inconclusive.
- Positive results obtained with test chemicals with a Log Kow >3.5 could still be used to support the identification of the test chemical as a skin sensitizer.
- In general, mono constituent substances with a high Log Kow may be insoluble in the exposure medium, however, if solubility or stable dispersion can be obtained and documented, testing may still be conducted.

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Approaches to Test Battery

Integrated Testing Strategy

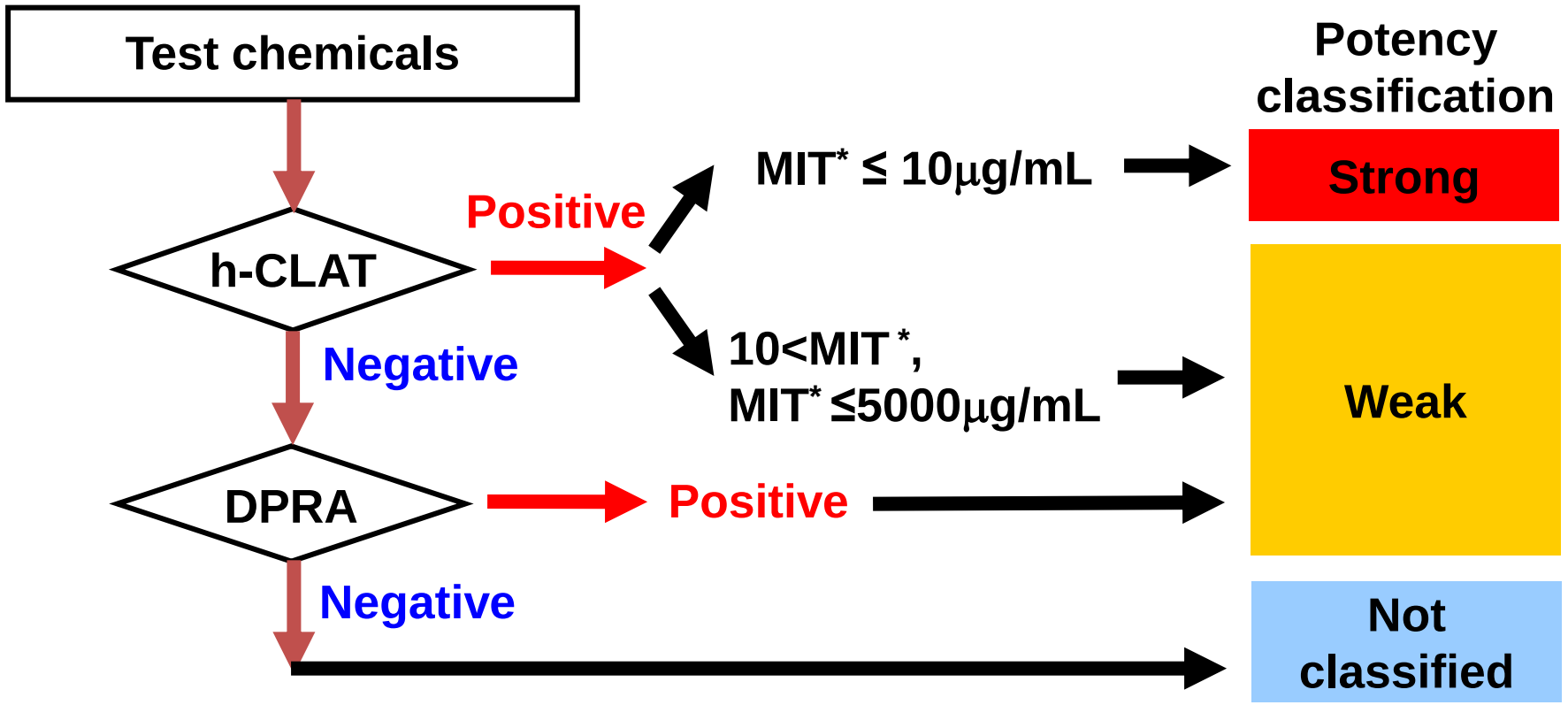
Assign scores to the outcomes in each single test and integrate the scores for the best predictivity

The ITS concept proposed by Jowsey *et al*. (2006)

Sequential Testing Strategy

Develop a stepwise system, weighing the evidence from h-CLAT and DPRA with 139 chemical dataset

Sequential Testing Strategy with DPRA & h-CLAT



*MIT : Lower value of EC150/EC200

Hazard identification by STS

139 chemicals		STS	
		Strong/Weak	Not-classified
LLNA	Sensitizer (102)	*92*	***10***
	Non-sensitizer (37)	*17*	*20*
Hazard identification		***Accuracy (%)***	***81*** *(112/139)*
		Sensitivity (%)	*90 (92/102)*
		Specificity (%)	*54 (20/37)*

Takenouchi *et al*., 2015

- **High sensitivity (90%: 92 of 102 sensitizers) but low specificity (54%: 20 of 37 non-sensitizers)**
- **Compared to LLNA prediction, the accuracy was 81% for hazard identification.**
- **10 chemicals were false negatives in STS**

Potency Classification by STS

139 chemicals		STS		
		Strong	Weak	Not-classified
LLNA	Extreme + Strong (29)	*19*	*10*	*0*
	Moderate + Weak (73)	*6*	*57*	*10*
	Non-sensitizer (37)	*0*	*17*	*20*
Potency classification		***Accuracy (%)***		***69*** *(96/139)*
		Overprediction rate (%)		*17 (23/139)*
		Underprediction rate (%)		*14 (20/139)*

Takenouchi *et al.*, 2015

Compared to LLNA prediction,
the accuracy was 69 % for potency classification.

Performance in Predicting Human Hazard

		STS (n=128)			LLNA (n=128)		
		Strong	Weak	Not-classified	Ext/St	Mod/Weak	NS
Human*	1+2	13	16	0	14	14	2
	3+4	2	51	5	5	42	11
	NS(5+6)	0	23	16	1	19	20
Hazard identification		Accuracy (%)		80 (95/123)	Accuracy (%)		74 (95/128)
		Sensitivity (%)		98 (82/84)	Sensitivity (%)		85 (75/88)
		Specificity (%)		41 (16/39)	Specificity (%)		50 (20/40)
Potency classification		Accuracy (%)		64 (80/123)	Accuracy (%)		59 (66/128)

*Basketter *et al*. 2014

Kleinstreuer *et al*., 2018

Predictive performance is comparable to the LLNA for human hazard identification.

Limitations

Technical limitations

- Low water soluble chemicals.
- For the DPRA, test chemicals should be soluble in an appropriate solvent such as acetonitrile or water.
- For the h-CLAT, test chemicals should be soluble or form a stable dispersion in DMSO or saline.

Substance related limitations

- Pre-/pro-haptens might not be reliably predicted due to lack of metabolic capacities in both the DPRA and h-CLAT.

Uncertainty

- KE 4 (T-cell activation) is not included due to lack of available tests.
- STS covers KE 1 and 3 of AOP and is based on a dataset of 139 chemicals. The confidence in the prediction for hazard identification is high, when similar chemicals are available in this data set and the limitations are taken into account.
- The confidence is lower for chemicals with low water solubility.
- The confidence is lower for pre-/pro-haptens due to limited metabolic capacities of test methods.

Complexity: Data Interpretation Procedure

- In STS, in order to idetify skin sensitizing potential, the conservative decision is conducted by weighing one positive result in the individual assay.

- Conservative decision approach using two assays (DPRA and h-CLAT) vs three assays (DPRA, KeratinoSens™, and h-CLAT) could be compared to identify skin sensitizing potential (Otsubo *et al.,* 2017).

- But it was found that decision approach using three assays only slightly improves sensitivity and markedly decrease specificity.

References

- Kleinstreuer *et al.* (2018). Non-animal methods to predict skin sensitization (II): an assessment of defined approaches. Crit Rev Toxicol 48(5), 359-374

- Takenouchi *et al.* (2015). Test battery with the human cell line activation test, direct peptide reactivity assay and DEREK based on a 139 chemical data set for predicting skin sensitizing potential and potency of chemicals. J Appl Toxicol 35, 1318-1332.

- Nukada *et al.* (2013). Data integration of non-animal tests for the development of a test battery to predict the skin sensitizing potential and potency of chemicals. Toxicol in Vitro, 27, 609-618.

- Nukada *et al.* (2012). Prediction of skin sensitization potency of chemicals by human Cell Line Activation Test (h-CLAT) and an attempt at classifying skin sensitization potency. Toxicol in Vitro, 26:1150-60.

Summary

- In STS, the test methods used (DPRA and h-CLAT) address two KEs 1 (protein binding) and 3 (dendritic cell activation).
- Tested chemical which is positive by either DPRA or h-CLAT is judged as a sensitizer.
- The strong class in the h-CLAT is available to predict EC3<1% in LLNA (Strong). Either the weak class in the h-CLAT or the positive result in the DPRA is available to predict EC3≥1% in LLNA.
- STS has high predictive performance, which is comparable to the LLNA for human hazard identification.
- The confidence is lower for chemicals with low water solubility and pre-/pro-haptens.

Thank you very much!