National Institute of Environmental Health Sciences
Division of Translational Toxicology

Peer Review Report of the EpiSensA Skin Sensitization Assay

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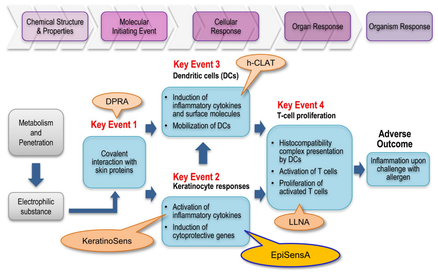
Introduction

- The EpiSensA assay is proposed as an additional test method designed to target the second key event (KE) in the skin sensitization adverse outcome pathway (AOP) (**Figure 1**; OECD 2014).
- Three guidelines have been published by the Organisation for Economic Co-operation and Development (OECD) describing methods that address the first three KEs of the AOP (OECD 2018a, 2018b, 2021a).
- New methods are still needed to address and overcome some of the limitations of the currently accepted methods, which have difficulty testing highly lipophilic compounds and detecting pre-/pro-haptens.
- Reconstructed human epidermis (RhE) models overcome these limitations because:
  - The tissues used have limited metabolic capacity in order to better predict pre-/pro-haptens.
  - The test chemical is directly applied to the epidermal layer allowing for application of lipophilic compounds.
- The EpiSensA is an RhE-based assay developed by the Kao Corporation (Japan) (Saito et al. 2013, 2017; Mizumachi et al. 2018, 2021). It was developed using the LabCyte EPI-MODEL 24 RhE skin model.
- The EpiSensA evaluates gene expression of four markers of the keratinocyte response to the early phase of skin sensitization: induction of cytoprotective gene pathways and inflammatory responses. These four markers are:
  - Encoding activating transcription factor 3 (ATF3)
  - Glutamate-cysteine ligase, modifier subunit (GCLM)
  - DnaJ (Hsp40) homolog, subfamily B, member 4 (DNAJB4)
  - Interleukin-8 (IL-8).
- The Japanese Center for the Validation of Alternative Methods (JaCVAM) convened a peer review panel (PRP) to assess the completed validation study (2018-2022) of the EpiSensA. JaCVAM also supported the Validation Management Team for the validation project.
- The panel met virtually several times and once face-to-face from June to November 2022. This presentation summarizes the findings described in the report (draft available: https://www.oecd.org/chemicalsafety/testing/episensa-validation-report.pdf).

Figure 1: Skin Sensitization AOP



OECD, 2014

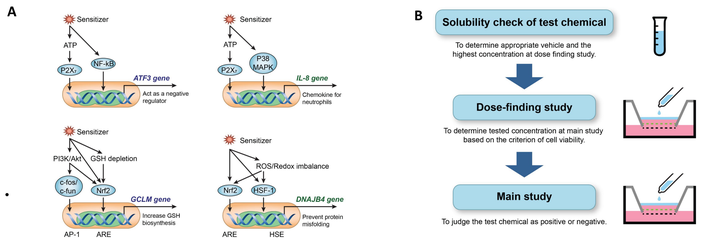
GHS Skin Sensitization Categories	
Category	**Potency**
1A	Strong sensitizer
1B	Other sensitizers
NC	Not Classified

UN, 2019

Objectives

- The PRP evaluated the validation report on 13 criteria, based on metrics determined by the Validation Management Team prior to the onset of the multi-laboratory validation project.
- A rationale for the test method should be available, including a description of the human health effect, a clear statement of scientific need, and regulatory application.
- The toxicological mechanisms and the relationship between the test method endpoint(s) with the biological effect and the toxicity of interest should be addressed, describing the limitations of the test method.
- A detailed test method protocol should be available. Transferability to other labs should be demonstrated.
- The within- and between- laboratory reproducibility of the test method should be demonstrated.
- Demonstration of the test method's performance should be based on testing a diversity of chemicals, preferably coded reference chemicals.
- Predictive capacity should be demonstrated using representative chemicals.
- All data from the validation study supporting the validity of a test method should be obtained in accordance with the principles of Good Laboratory Practice (GLP).
- The applicability domain of the test method should be defined.
- Proficiency chemicals should be specified in the proposed protocol.
- Performance standards should be specified with the proposed protocol.
- Advantages in terms of time, cost, and animal welfare should be described.
- Completeness of all data and documents supporting the assessment of the validity of the test method should be demonstrated.
- The validation study should be managed and conducted adequately.
- Additional recommendations and further considerations were made by the panel for future studies and evaluations.

Basis and Conduct of the Assay



A) Potential mechanism of the stimulus-specific regulation of the four marker genes. B) Flow of EpiSensA testing (both images are from the Validation Study). These images address Evaluation Criteria 1-3.

Testing Scheme for Within- and Between-laboratory Reproducibility

Coded test chemicals were provided to test facilities by JaCVAM by test phases as follows:

- Phase I-A: 4 sensitizers, 1 non-sensitizer
- Phase I-B: 2 sensitizers, 3 non-sensitizers
- Phase I-C: 4 sensitizers, 1 non-sensitizer
- Phase II: 8 sensitizers, 4 non-sensitizers

Within-laboratory Reproducibility

No.	Chemicals	Lab 1			Lab 2			Lab 3		
		Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
1	Glyoxal 40% solution in water	P	P	P	P	P	P	P	P	P
2	Lauryl gallate	N	N	N	N	N	N	N	P	N
3	Benzisothiazolinone	N/P	N/N	N/N	PP	P	N/P	P	P	N/P
4	Diethyl phthalate	P	P	P	P	P	P	P	P	P
5	Sodium lauryl sulfate	P	P	P	P	N/P	P	P	P	P
6	Hexane	N	N	N	N	N	N	N	N	N
7	Dextran	N	N	N	N	N	N	N	N	N
8	Tween80	N	N	N	N	N	N	N	N	N
9	Ethyl acrylate	P	P	P	P	P	P	P	P	P
10	2,4-Dinitrochlorobenzene (DNCB)	P	P	P	P	P	P	P	P	P
11	Lactic acid	N	N	N	N	N	P	N	N	P
12	P-Phenylenediamine	P	P	P	P	P	P	P	P	P
13	Methyl heptane carbonate	P	P	P	P	P	P	P	P	P
14	Abietic Acid	P	P	P	P	P	P	P	P	P
15	Farnesol	P	P	P	P	P	P	P	P	P

This table lists chemicals used to demonstrate within-laboratory reproducibility (WLR) for the three participating laboratories. WLR was 87–93% across the labs, averaging 91%. These fulfilled Evaluation Criterion 4. Exp. = experiment; N = negative; P = positive.

Between-laboratory Reproducibility

No.	Chemicals	Reference Categorization			Lab 1	Lab 2	Lab 3	Agreement Among Laboratories
		DASS LLNA*	DASS Human Ref. Data*	Basketter human potency†				
1	DNCB	1A	1A	Cat.1	P	P	P	Yes
2	Lauryl gallate	1A	NA	Cat.2	N	N	N	Yes
3	P-Phenylenediamine	1A	1A	Cat.1	P	P	P	Yes
4	Methyl heptane carbonate	1A	-	Cat.2	P	P	P	Yes
5	Glyoxal 40% solution in water	1A	1A	Cat.2	P	P	P	Yes
6	Benzisothiazolinone	1B	NA	Cat.2	N	P	P	No
7	Farnesol	1B	1B	Cat.3	P	P	P	Yes
8	Ethyl acrylate	1B	1B	Cat.4	P	P	P	Yes
9	Abietic acid	1B	NA	Cat.3	P	P	P	Yes
10	Sodium lauryl sulfate	1B	NC	Cat.6	P	P	P	Yes
11	Diethyl phthalate	NC	NA	Cat.6	P	P	P	Yes
12	Hexane	NC	NC	Cat.6	N	N	N	Yes
13	Dextran	NC	-	Cat,6	N	N	N	Yes
14	Tween80	NC	-	Cat.6	N	N	N	Yes
15	Lactic acid	NC	NA	Cat.6	N	N	N	Yes
16	Tetrachlorosalicylanilide	1A	1A	Cat.1	P	P	P	Yes
17	Glutaraldehyde	1A	1A	Cat.2	P	P	P	Yes
18	2-Aminophenol	1A	NA	Cat.2	P	P	P	Yes
19	Isoeugenol	1A	1B	Cat.2	P	P	P	Yes
20	Lilial	1B	1B	Cat.4	P	P	P	Yes
21	Methyl methacrylate	1B	NA	Cat.4	P	P	P	Yes
22	Amyl cinnamic aldehyde	1B	NA	Cat.4	P	P	P	Yes
23	Imidozolidinyl urea	1B	1B	Cat.3	P	P	P	Yes
24	Acetanisole	NC	NA	-	P	N	P	No
25	1-Iodohexane	NC	NA	-	P	P	P	Yes
26	Propylene glycol	NC	NC	Cat.5	N	P	P	No
27	Benzyl butyl phthalate	NC	NA	-	N	N	N	Yes

This table lists chemicals used to demonstrate between-laboratory reproducibility (BLR). Chemicals 16-27 (blue box) are the Phase 2 chemicals used only to assess BLR, while chemicals 1-15 were utilized for the WLR assessment and applied to BLR. BLR was 89%, which fulfilled Evaluation Criterion 4. The 27 chemicals tested include both sensitizers and non-sensitizers and represent a range of physicochemical parameters, fulfilling Evaluation Criterion 5. Reference classifications are provided alongside individual laboratory predictions.

DASS = Defined approach on skin sensitization; LLNA = local lymph node assay; P = positive, N = negative, N = Not applicable; Cat = Category; *OECD 2021b; †Basketter et al. 2014.

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Cumulative Predictive Capacity

Predictive Capacity against LLNA Reference Data (N=27 chemicals)		
Reference Result	**Positive**	**Negative**
Positive (N = 54 predictions)	50	4
Negative (N = 27 predictions)	10	17
Total Sensitivity (%)	93	
Total Specificity (%)	63	
Total Accuracy (%)	83	

Predictive Capacity against Human Data (N=24 chemicals)		
Reference Result	**Positive**	**Negative**
Positive (N = 51 predictions)	47	4
Negative (N = 21 predictions)	8	13
Total Sensitivity (%)	92	
Total Specificity (%)	62	
Total Accuracy(%)	83	

GHS Category*	No. of Chemicals (Test chemical Set)	Cumulative Predictive Capacity
1A	9	88%
1B	9	96%
Not Classified	9	63%

Tables summarize EpiSensA's predictive capacity against existing reference sets: LLNA (OECD 2021b) and human (OECD 2021b and Basketter et al. 2014).

*GHS Categorization for the 27 chemicals is based on a weight-of-evidence classification utilizing human data and accepted animal tests per the validation report. Data for each prediction are presented as the sum of the three test facility results. These data fulfilled Evaluation Criteria 6-8.

Applicability Domain – Predictive Performance for Pre-/Pro-Haptens and Lipophilic Chemicals

LLNA	Lipophilic Chemicals (N=69)	Hydrophilic chemicals (N=67)	Pre-/Pro-haptens (N=37)	Overall (N=136)
Sensitivity (%)	83	94	95*	88
Specificity (%)	65	67	--	66
Accuracy (%)	78	87	--	82

Human	Lipophilic Chemicals (N=25)	Hydrophilic chemicals (N=55)	Pre-/Pro-haptens (N=23)	Overall (N=80)
Sensitivity (%)	92	100	96*	98
Specificity (%)	17	67	--	48
Accuracy (%)	56	87	--	78

Separately from the reproducibility evaluations, the lead laboratory assessed 136 chemicals for sensitization hazard to define the EpiSensA's applicability domain. Predictive performance is compared to LLNA and human data. These data fulfilled Evaluation Criteria 5-8.

*Lauryl gallate was negative in the multi-laboratory validation study, but weakly positive at the lead laboratory. Additional testing showed that a longer incubation time resulted in a strong positive test.

Conclusion

- The PRP determined the EpiSensA has an appropriate rationale, mechanistic applicability, scientific need, transferability, reproducibility, and predictive capacity to perform as a test to detect skin sensitization hazard. All 13 evaluation criteria were met.
  - The assay performs well, with WLR of at least 87% and BLR of 89%.
- The overall conduct of the study was adequate, with testing conducted according to the principles of GLP.
- The EpiSensA was found to be able to:
  - Accurately detect pre/pro-haptens due to the metabolic capacity of the test system.
  - Accurately detect lipophilic chemicals with a non-aqueous exposure method.
- Both results fill gaps in the currently accepted test methods.
  - The test method developers were encouraged to further assess follow-on testing to capture test chemicals that are both highly lipophilic and predicted to be pre-/pro-haptens.
  - The test method developers were encouraged to determine appropriate ranges to classify results as borderline predictions for each of the genes in anticipation of inclusion in Test Guideline on Defined Approaches for Skin Sensitization (OECD 2021b).
- The test method protocol is well-written and transferable between facilities with the LabCyte EPI-MODEL 24 skin model.
  - Additional testing on other RhE systems is recommended to determine transferability across models for accessibility in all geographic regions.

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