Me-Too Validation of the Epi2SensA Method Using EpiDerm™ Model for Skin Sensitization Testing Under OECD TG442D

MATTEK
Now Part of Sartorius

C. Pellevoisin[1,2], H. Kojima[3,4], S. Hoffmann[5], T. Ashikaga[6], T. Landry[1], C. Romero[1], K. Guntur[1], M. Klausner[1], J. Stadnicki[1], H. Gehrke[7], R. Mills-Goodlet[7], N. Panousi[7], V. J. Johnson[8], K. Narita[9], S. Tachibana[9], K. Kojima[9], and A. Armento[1]. [1]Mattek – Now Part of Sartorius, Ashland, MA; [2] Urbilateria, Saint Cyr sur Loire, France; [3]Sanyo-Onoda City University, Yamaguchi, Japan; [4]NIHS, Kawasaki, Japan; [5] seh consulting + services, Paderborn, Germany; [6]NIHS/JaCVAM, Kawasaki, Japan; [7] Eurofins, Munich, Germany; [8]Burleson Research Technologies, Morrisville, NC; [9] FDSC, Hadano, Japan.

Abstract ID #3255
Poster#E387

Abstract

Epi2SensA is similar to the validated EpiSensA assay for assessing the skin sensitization potential of chemicals. The Epi2SensA protocol includes adaptation (changes to exposure conditions and the controls) for using an alternative reconstructed human epidermis (RhE) model, the EpiDerm™ model. The interlaboratory validation study evaluated the reliability and predictive capacity of Epi2SensA according to OECD Performance Standards. Four laboratories (Mattek - Now Part of Sartorius, Eurofins Munich, Burleson Research Technologies, Inc., and Food and Drug Safety Center) conducted blinded testing of 20 coded reference substances representing various chemical categories and sensitization potencies. Statistical analysis using modified acceptance criteria (a 60% cell viability threshold) and a modified prediction model (requiring at least two genes induced for a positive prediction) demonstrated substantially improved performance compared to the original EpiSensA criteria. The between-laboratory reproducibility (BLR) was 85%, the average within-laboratory reproducibility (WLR) was 83.3%, and the average predictivity parameters were 88.1% for sensitivity, 88.9% for specificity, and 88.3% for accuracy. Epi2SensA achieved performance metrics comparable to the validated reference method (EpiSensA), supporting regulatory acceptance of the Epi2SensA assay using the EpiDerm™ model as an alternative RhE source for OECD TG 442D skin sensitization testing.

Epi2SensA Method

Solubility and dose finding: Chemicals are dissolved in Acetone:Olive Oil (AOO) 50:50, Distilled water (DW), or 50% Ethanol (EtOH) and tested for solubility. If insoluble at 50% w/v, serial dilutions are used to identify the highest soluble concentration. Then 4-fold dilutions are applied to EpiDerm tissues (1-hour exposure + 5-hour post-incubation) to select doses based on tissue viability measured by LDH.

Main study: Tissues are exposed for 1 hour + 5-hour post-incubation to at least 3 concentrations of the test articles; clotrimazole and 4NBB are used as positive controls, along with vehicle and non-treated negative controls. Viability is measured using LDH release versus 10% Triton-X 100–treated killed controls.

RNA extraction and RT-PCR: RNA is isolated (RNAqueous Kit), converted to cDNA (RT² First Strand Kit), and quantified by RT² SYBR Green PCR using ATF3, GCLM, DNAJB4, IL-8, and GAPDH primers over 40 cycles; fold induction is calculated and compared to gene-specific cut-offs for classification (ATF3>15, GCLM>2, DNAJB4>2, IL8>4).

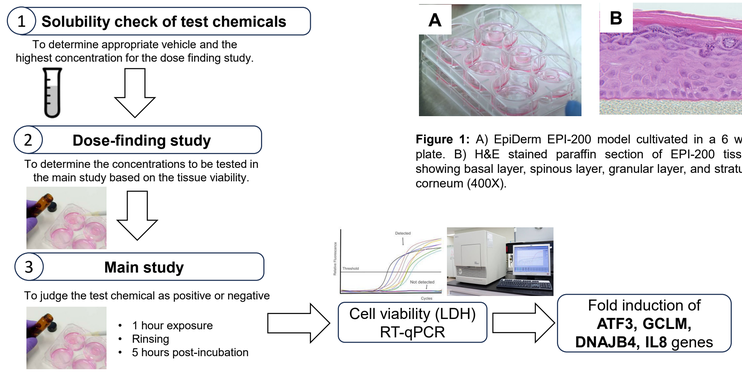

Figure 2: Different steps of the Epi2SensA method. For a given chemical, the first step is to identify the maximum concentration (solubility) in the appropriate solvent. Appropriate doses for the main study are determined in the Dose finding which measures cytotoxicity as a function of test article concentration, using the LDH assay. In the main study, the expression of the 4 genes involved in skin sensitization are measured by RT-qPCR.

Figure 1: A) EpiDerm EPI-200 model cultivated in a 6 well plate. B) H&E stained paraffin section of EPI-200 tissue showing basal layer, spinous layer, granular layer, and stratum corneum (400X).

Catch-Up Validation

The validation management team (VMT) supervised the testing of 20 blinded reference substances, including 14 UN GHS Category 1A/1B sensitizers and 6 non-sensitizers. Mattek, as the lead laboratory, provided the SOP and trained laboratories; Mattek, Eurofins, and BRT tested all 20 reference substances, while FDSC evaluated eight for between-laboratory reproducibility only.

Essential Test Method Component	VRM (EpiSensA)	Epi2SensA	Key Difference & Rationale
RhE Model	LabCyte EPI-MODEL24.	EpiDerm (EPI-200).	Different model. This was the fundamental difference requiring subsequent protocol adjustments.
Marker Genes	Quantifies expression of ATF3, GCLM, DNAJB4, and IL-8.	Quantifies expression of ATF3, GCLM, DNAJB4, and IL-8.	Identical. Both methods target the same four mechanistically relevant genes associated with keratinocyte activation.
Gene Cut-off Values	ATF3 > 15-fold; GCLM > 2-fold; DNAJB4 > 2-fold; IL-8 > 4-fold.	ATF3 > 15-fold; GCLM > 2-fold; DNAJB4 > 2-fold; IL-8 > 4-fold.	Identical. The gene-specific induction thresholds were retained.
Cytotoxicity Viability Threshold	Tissue viability must be > 80%.	Tissue viability must be > 60%.	Modified Criterion. The threshold was reduced from 80% to 60% based on preliminary data showing the LDH assay overestimated cytotoxicity for EpiDerm compared to the MTT assay, and to enhance test reproducibility.
Prediction Model	Prediction is positive if at least one marker gene exceeds its cut-off (Imax) at an acceptable concentration.	Prediction is positive if at least two marker genes exceed their respective cut-off values (Imax) at an acceptable concentration.	Modified Criterion. The requirement was increased to two positive genes to enhance robustness, a modification common when using different tissue models in similar method validation.
Exposure Time	6 hours.	1-hour topical exposure followed by a 5-hour post-incubation period.	Modified Procedure. The exposure duration was shortened to 1 hour to reduce unexpected cytotoxicity observed with the EpiDerm model, while maintaining the 6-hour time point for gene expression measurement.
Application Volume	5 µL applied to the epidermis surface.	10 µL applied to the epidermis surface.	Modified Procedure. The volume was doubled because the surface area of the EpiDerm model (0.63 cm2 is roughly double that of the LabCyte model (0.32 cm2) thus maintaining a similar volume/surface ratio.
Positive Control (Clotrimazole)	0.78%(w/v).	1.56% (w/v).	Modified Procedure. The concentration was increased to ensure the run acceptance criteria were consistently met for ATF3 and IL-8 fold induction
Killed Control Method	10 µL of 10% Triton X-100 applied topically.	50 µL of 10% Triton X-100 applied in the culture medium.	Modified Procedure. Changed the volume and application to ensure complete tissue death with maximum LDH release for the EpiDerm model.

Table 1. Comparison of Epi2SensA with the essential test method components and performance of the Validated Reference Method (VRM, EpiSensA).

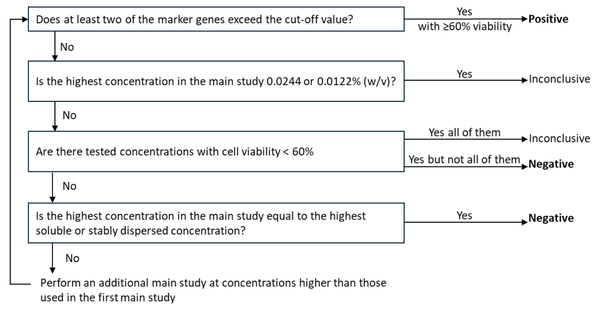

Figure 3. Flow-chart of the Epi2SensA prediction model.

Results

After data collection and unblinding, the dataset was analyzed according to the performance-standard (PS) criteria. Initial WLR (63.9%) and BLR (70%) were below the PS 80% threshold, with 66.6% specificity, 90.5% sensitivity, and 83.3% accuracy. Re-analysis of the data, using a tissue viability cutoff lowered to 60% and requiring induction of at least two genes for a positive classification, increased WLR and BLR to 83.3% and 85%, meeting the PS criteria. Predictive capacity improved to 88.9% specificity, 88.1% sensitivity, and 88.3% accuracy, all above PS minima.

Chemical	*CAS*	*Log P*	*Pre/Pro Hapten*	*Classification UN GHS in vivo*	*VRM classification*	*Epi2SensA classification*
2,4-Dinitrochlorobenzene	97-00-7	2,17		UN GHS Cat. 1A	Sensitizer	Sensitizer
p-Phenylenediamine	106-50-3	-0,39	**Pre**	UN GHS Cat. 1A	Sensitizer	Sensitizer
Metol	55-55-0	0.63	**Pre/Pro**	UN GHS Cat. 1A	Sensitizer	Sensitizer
Tetrachlorosalicylanilide	1154-59-2	**5,87**		UN GHS Cat. 1A	Sensitizer	Sensitizer
Lauryl gallate	1166-52-5	**6,9**	**Pre**	UN GHS Cat. 1A	Non-sensitizer	Non-sensitizer
Methyl heptine carbonate	111-12-6	2,79		UN GHS Cat. 1A	Sensitizer	Sensitizer
Isoeugenol	97-54-1	3,04	**Pre/Pro**	UN GHS Cat. 1B	Sensitizer	Sensitizer
Glyoxal	107-22-2	-0.08		UN GHS Cat. 1A	Sensitizer	Sensitizer
Abietic acid	514-10-3	**3,92**	**Pre**	UN GHS Cat. 1B	Sensitizer	Sensitizer
Dibutyl aniline	613-29-6	**4,7**	**Pro**	UN GHS Cat. 1B	Sensitizer	Sensitizer
Amyl cinnamic aldehyde	122-40-7	**3.99**		UN GHS Cat. 1B	Sensitizer	Sensitizer
Benzisothiazolinone	2634-33-5	0,8		UN GHS Cat. 1B	Sensitizer	Sensitizer
Imidazolidinyl urea	39236-46-9	-0,86		UN GHS Cat. 1B	Sensitizer	Sensitizer
Farnesol	4602-84-0	**4,91**		UN GHS Cat. 1B	Sensitizer	Sensitizer
Cetrimide	57-09-0	3,18		Not classified	Non-sensitizer	Non-sensitizer
Lactic acid	50-21-5	-0,72		Not classified	Non-sensitizer	Non-sensitizer
Benzyl butyl phthalate	85-68-7	**4,84**		Not classified	Non-sensitizer	Non-sensitizer
Diethyl phthalate	84-66-2	2,44		Not classified	Sensitizer	Non-sensitizer
Hexane	110-54-3	**3.9**		Not classified	Non-sensitizer	Non-sensitizer
1-Iodehexane	638-45-9	**3.99**		Not classified	Sensitizer	Sensitizer

Table 2: Set of 20 chemicals from the Performance standard used for the catch-up validation study with *in vivo* classification compared to Epi2SensA and VRM prediction.

	acceptance criterion	Average Epi2SensA	Mattek Epi2SensA	Eurofins Epi2SensA	BRT Epi2SensA
WLR	**≥ 80%**	**83,3%**	91,7% (11/12)	83,3% (10/12)	75,0% (9/12)
BLR	**≥ 80%**	**85%**			
Specificity	**≥ 65%**	**88,9%**	100% (6/6)	83,3% (5/6)	83,3% (5/6)
Sensitivity	**≥ 85%**	**88.1%**	92,9% (13/14)	78,6% (11/14)	92,9% (13/14)
Accuracy	**≥ 85%**	**88,3%**	95,0% (18/20)	80,0% (16/20)	90,0% (18/20)

Table 3: Comparison of Epi2SensA to performance standard acceptance criteria: WLR, BLR and predictive capacity.

Conclusion

The Epi2SensA method, optimized for the EpiDerm™ model, met the acceptance criteria for validation of a similar method to the validated reference method (VRM):

Interlaboratory validation across four labs and 20 reference chemicals yielded strong results: 85% between-laboratory reproducibility (exceeding the ≥80% threshold) and 83.3% average within-laboratory reproducibility (meeting the ≥80% requirement). Predictive performance was were 88.1% sensitivity, 88.9% specificity, and 88.3%, accuracy, all surpassing performance criteria (≥85% sensitivity/accuracy, ≥65% specificity).

The demonstrated performance using the EpiDerm™ model supports regulatory acceptance of Epi2SensA as a validated method. Epi2SensA integration in OECD TG442D will provide laboratories with multiple RhE supplier options and ensure worldwide supply chain reliability.