Additional validation of alternative skin irritation test method using LabCyte EPI-MODEL24 of cultured skin

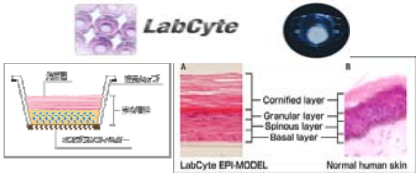
Nakamura, M. [1)], Suzuki, T. [2)], Shinoda, S. [3)], Kato, M. [4)] , Kojima, H. [5)]

KOBAYASHI Pharmaceutical Co., Ltd.[1)], Fancl Corporation[2)], Drug Safety Testing Center Co., Ltd.[3)], Japan Tissue Engineering Co.,Ltd.[4)], National Institute of Health Sciences [5)]

1.Introduction

From 2008 to 2009, the Validation Committee of the Japanese Society for Alternative to Animal Experiments twice implemented validation studies on an alternative skin irritation test method, to confirm the usefulness of the LabCyte EPI-MODEL24 of cultured skin prepared in Japan. This multicenter validation study with LabCyte EPI-MODEL24 could address the following three issues related to judgment of skin irritation: consistency among study centers (inter-laboratory reproducibility), consistency with judgments obtained for EPISKIN™ authenticated by ECVAM (equivalence), and consistency with the results of animal experiments.
(alternativity). The third-party accreditation of this test method was performed by the Skin Irritation Evaluation Committee of JaCVAM and Third-party Accreditation Committee of OECD, and consequently, this model was judged to be insufficient for evaluation of skin irritation because of false-negative results for 1-bromohexane, etc. Then, the manufacturer of LabCyte EPI-MODEL24, Japan Tissue Engineering Co., Ltd. (J-TEC), reviewed the test method, leading to successful improvement. The Steering Committee of JaCVAM considered this improvement to be critical for the protocol of the test method, and the improved test method was to be validated accordingly with the support of MHLW Grant-in-Aid for Scientific Research.

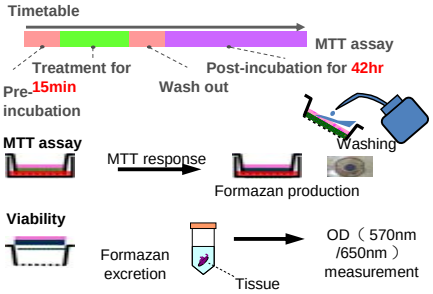
2.LabCyte EPI-MODEL



The LabCyte EPI-MODEL is produced by culturing human epidermal cells on a culture plate. After human epidermal cells have been cultured and proliferated, exposing their surface to the air causes it to keratinize*, creating a cultured epidermis model similar to the human epidermis (Figures A and B).

*QC batch release criteria
IC50=1.4-4.0mg/mL(mean 2.57mg/mL),
18 hr treatment with SLS.

3.LabCyte EPI-MODEL Protocol



4.History of LabCyte EPI-MODEL24 directed to comply with the OECD test guideline (since 2009)

Twenty substances listed in the performance standards in the "OECD Guidelines for the Testing of Chemicals Test No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method" were coded and delivered to 3 participating study centers where validation was performed using the improved protocol.

2009. 1 Phase3 Validation completed
Applied OECD TG
8 Validation report submitted to OECD
2010. 3 OECD peer review completed
Guideline for the skin irritation tests using EPISKIN™ established
8 Answer sent to OECD peer reviewer

5.Plan of additional validation

Chairman：Hajime Kojima (NIHS)
Committee：Masakazu Katou (J-TEC)、
Takashi Omori (Doshisha University）
Participating study center :
KOBAYASHI Pharmaceutical Co., Ltd.
Fancl Corporation
Drug Safety Testing Center Co., Ltd.
Training
J-TEC gave technical guidance using the improved protocol at the National Institute of Health Sciences on July 27, 2010.
Preliminary test
At each study center, the preliminary test was repeated several times to master the protocol, which was improved for 1-bromohexane to yield positive results.
Implementation period
Validation was performed during the period between September and November, 2010.

6.Modification points of rinsing protocol between SOP ver.7.1 and SOP ver.8.2

Modification points	SOP ver.7.1	SOPver.8.2
1.The PBS stream from washing bottle	It was not briefly defined.	It was defined to avoid that the PBS stream hit directly to the tissue surface.
2.To remove PBS by tapping	It was not briefly defined.	It was briefly defined.
3.How to use of the cotton pad	It was not briefly defined.	It was defined to avoid that the cotton pad touched directly to the tissue surface.

7.Test chemicals and coded No. used at an additional LabCyte validation study

No.	Name	CAS number	Storage	Chemical code Lab 1	Lab 2	Lab 3
1	1-bromo-4-chlorobutane	6940-78-9	RT	B-261	D-281	G-301
2	Diethyl phthalate	84-66-2	RT	B-262	D-282	G-302
3	naphtalen acetic acid	86-87-3	RT	B-263	D-283	G-303
4	allyl phenoxy-acetate	7493-74-5	RT	B-264	D-284	G-304
5	isopropanol	67-63-0	RT	B-265	D-285	G-305
6	4-methyl-thio-benzaldehyde	3446-89-7	RT	B-266	D-286	G-306
7	methyl stearate	112-61-8	RT	B-267	D-287	G-307
8	heptyl butyrate	5870-93-9	RT	B-268	D-288	G-308
9	hexyl salicylate	6259-76-3	RT	B-269	D-289	G-309
10	Cinnamaldehyde	104-55-2	2-8C	B-270	D-290	G-310
11	1-decanol	112-30-1	RT	B-271	D-291	G-311
12	Cyclamen aldehyde	103-95-7	RT	B-272	D-292	G-312
13	1-bromohexane	11-25-1	RT	B-273	D-293	G-313
14	2-chloromethyl-3,5-dimethyl-4-methoxypyridine HCl	86604-75-3	RT	B-274	D-294	G-314
15	di-n-propyl disulphide	629-19-6	RT	B-275	D-295	G-315
16	Potassium Hydroxide 5%	1310-58-3	RT	B-276	D-296	G-316
17	benzynethiol, 5-(1,1-dimethylethyl)-2-methyl	7340-90-1	RT	B-277	D-297	G-317
18	1-methyl-3-phenyl-1-piperazine	5271-27-2	RT	B-278	D-298	G-318
19	heptanal	111-71-7	RT	B-279	D-299	G-319
20	1,1,1 Trichloroethane	71-55-6	RT	B-280	D-300	G-320

8.Performance standard checklist

Acceptance criteria
・Negative control value
0.7≤ mean OD （A570/650）≤ 2.5
・Positive control value
5%SLS solution mean tissue viability ≤ 40%
・Standard Deviation
All substances standard deviation SD ≤ 18%

Substances not meeting the standards could be additionally tested up to twice for confirmation.

Success criteria
Results of intra-laboratory reproducibility:
Consistent for more than 90% of judgments.
Results of inter-laboratory reproducibility:
Consistent for more than 80% of judgments.
Sensitivity, Specificity, Accuracy:
Performance standards were met.

Sensitivity 90%,Specificity 70%,Accuracy 80%

9.Intra-and inter-laboratory reproducibility of negative and positive controls in the additional validation

Lab	Negative control OD value	Mean SD	Positive control Viability（%）	Mean SD
Lab1	0.88	0.91 ±0.05	2.29	2.65 ±0.68
	0.87		3.62	
	0.92		2.59	
	0.98		2.10	
Lab2	1.03	1.03 ±0.06	4.98	3.87 ±0.84
	1.02		3.22	
	1.13		4.50	
	0.98		3.02	
	0.98		3.62	
Lab3	1.09	1.07 ±0.09	2.87	2.69 ±0.49
	0.98		3.37	
	1.01		2.64	
	1.06		2.55	
	1.20		2.02	

10.Analyzed results in LabCyte additional validation study

No.	Name	Lab.1			Lab.2			Lab.3		
		1	2	3	1	2	3	1	2	3
1	1-bromo-4-chlorobutane	12.4	11.3	19.0	16.5	10.7	10.6	9.0	9.8	9.8
2	Diethyl phthalate	80.1	81.5	69.6	60.9	57.5	69.5	90.5	102.0	93.0
3	naphtalen acetic acid	108.0	113.0	105.0	96.5	96.7	90.2	89.4	106.0	98.9
4	allyl phenoxy-acetate	19.1	65.1	59.3	66.6	70.6	66.2	90.1	93.0	93.2
5	isopropanol	89.6	77.0	67.6	75.9	74.8	77.1	86.6	67.2	74.4
6	4-methyl-thio-benzaldehyde	16.2	15.9	17.0	17.3	13.5	11.4	15.5	16.1	12.0
7	methyl stearate	110.0	110.0	104.0	98.8	93.1	76.3	91.2	102.0	108.0
8	heptyl butyrate	109.0	122.0	111.0	93.1	106.0	86.6	95.5	106.0	119.0
9	hexyl salicylate	105.0	111.0	102.0	98.0	95.7	83.5	99.6	100.0	113.0
10	Cinnamaldehyde	15.7	20.3	16.0	11.5	15.9	11.4	17.3	14.1	14.9
11	1-decanol	14.2	16.5	9.4	12.4	17.3	16.2	22.1	15.1	14.1
12	Cyclamen aldehyde	8.89	15.9	10.0	11.0	7.8	9.0	6.0	7.4	5.7
13	1-bromohexane	16.2	16.1	15.5	6.6	17.2	19.0	17.5	17.0	16.2
14	2-chloromethyl-3,5-dimethyl-4-methoxypyridine HCl	2.1	4.3	4.1	4.9	5.2	9.1	2.8	3.4	3.2
15	di-n-propyl disulphide	19.9	95.9	83.5	17.5	18.5		61.1	83.2	86.3
16	Potassium Hydroxide 5%	0.9	1.7	1.6	4.6	2.0	3.3	0.9	3.1	1.0
17	benzynethiol, 5-(1,1-dimethylethyl)-2-methyl	6.9	46.6	30.8	10.6	21.0	11.6	6.3	5.0	6.6
18	1-methyl-3-phenyl-1-piperazine	6.7	4.5	3.6	9.8	10.9	11.0	1.3	1.8	2.2
19	heptanal	9.4	10.3	10.4	9.5	7.0	9.5	11.9	10.2	10.9
20	1,1,1 Trichloroethane	8.7	12.0	7.8	9.1	7.9	17.4	7.6	7.0	6.8

: Classification NI : Classification I

Lab 1 & 3

In vitro prediction	*In vivo* classification I	NI	Total
I	9	3	12
NI	1	7	8
Total	10	10	20

Sensitivity (%)	90.0
Specificity (%)	70.0
Accuracy (%)	80.0

Lab 2

In vitro prediction	*In vivo* classification I	NI	Total
I	10	3	13
NI	0	7	7
Total	10	10	20

Sensitivity (%)	100.0
Specificity (%)	70.0
Accuracy (%)	85.0

11.Results and Discussion

・The test was positive for 1-bromohexane at all study centers; judgments for other substances stayed unchanged.
・The performance standards (acceptance criteria and success criteria) were satisfied at all participating study centers.
・A summary report on the present validation results was sent to the OECD secretariat.