施設 7

基準となる細胞浮遊液の容量が記載されていないので，原液を 15ml と仮定して記載する．

第 1 期	1 回目		再測定	再々測定	データ採否 (1)	データ採否 (2)
陽	0.089	→	非実施		△	○
	20ml(4/3)					
被	0.120				○	○
H B	20ml(4/3)				(1 回目)	

第 2 期	1 回目		再測定	データ採否 (1)	データ採否 (2)
陽	0.085	→	非実施	△	○
	15ml(1)				
被	0.123			○	○
C E	15ml(1)			(1 回目)	

第 3 期	1 回目	データ採否 (1)	データ採否 (2)
陽	0.121	○	○
	15ml(1)	(1 回目)	
被	0.145	○	○
I A	15ml(1)	(1 回目)	

2．副次解析

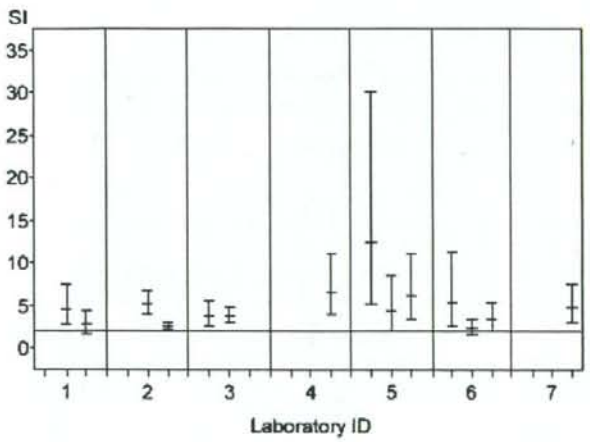


図 A-1　副次解析　陽性対照物質の SI 値とその 95％信頼区間

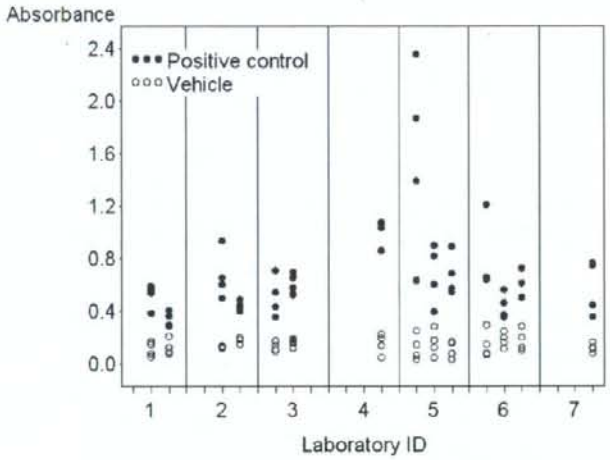
表 A-2　副次解析　陽性対照における施設・時期毎の吸光度の群内平均，SI 値とその 95%信頼区間

17JUL2008:17:36:17

Mean abosorbency of the positive control (SI_PC1.txt)
Data criterion 1

Labo. ID	Term	Vehicle mean abosorbency	PC mean abosorbency	SI	95%CI lower	95%CI upper
1	2	0.115	0.521	4.53	2.75	7.48
	3	0.128	0.341	2.67	1.65	4.31
2	2	0.131	0.677	5.15	3.91	6.79
	3	0.174	0.438	2.52	2.14	2.97
3	1	0.134	0.513	3.83	2.61	5.62
	2	0.164	0.616	3.77	3.05	4.65
4	3	0.154	1.012	6.58	3.96	10.91
5	1	0.126	1.569	12.46	5.14	30.17
	2	0.161	0.683	4.24	2.12	8.46
	3	0.112	0.678	6.07	3.34	11.05
6	1	0.150	0.793	5.30	2.48	11.30
	2	0.183	0.440	2.41	1.67	3.47
	3	0.178	0.589	3.32	2.02	5.45
7	3	0.122	0.581	4.78	3.05	7.50

図 A-2　副次解析　陽性対照群と溶媒群の吸光度のプロット



2）皮膚刺激性を調べるための LabCyte EPI-MODEL24 を用いた皮膚刺激性試験代替法のバリデーション研究

研究要旨

皮膚刺激性を調べるためのLabCyte EPI-MODEL24を用いた皮膚刺激性試験代替法の多施設バリデーションを日本動物実験代替法学会に依頼し、学会バリデーション委員会の下部組織としてバリデーション実行委員会を組織して、バリデーション研究が実施された。このバリデーション研究の結果、19 被験物質で得られた結果から、LabCyte EPI-MODEL24を用いた皮膚刺激性試験代替法はと全体として施設間再現性は良好であったとされた。代替の可能性はJ-TECが提唱した結果とほぼ同程度であり、再現性が高いと考察された。EPISKINとの同等性については、特異度は同程度であるが、感度がやや劣る可能性があると結論された。

A. 研究目的

LabCyte EPI-MODEL24 は、株式会社ジャパン・ティシュ・エンジニアリングが開発した培養皮膚モデルである。この試験は、皮膚刺激性試験代替試験法として開発が進められている。日本動物実験代替法学会では、バリデーション実行委員会を組織して、LabCyte EPI-MODEL24 を用いた皮膚刺激性試験で得られる皮膚刺激性の判定が、(1) 複数の施設間でどの程度一致するか（施設間再現性）、(2) 海外で先行して開発が進められた別の皮膚刺激性試験代替法である EPISKIN で得られた判定結果とどの程度一致するか（同等性）、(3) 動物実験結果とどの程度一致するか（代替可能性）、という 3 つの課題を検討するためにバリデーション研究を実施した。

B. 研究方法

日本動物実験代替法学会バリデーション委員会が皮膚刺激性試験代替法のバリデーション研究を公募した。結果として、多数の施設から応募があり、その中から厳しい基準をクリアした7施設の協力を得てバリデーション研究を行うことになった。ECVAM performance standardに記載されている20のうち、日本で入手可能な19被験物質をコード化して配布し、9月より開始し、2009年1月に終了した。被験物質処理後、MTTアッセイおよびインターロイキン（IL）-1αを指標とした。

判定方法は、プロトコールに従い

(1) OD_{NC} が 0.7 より小さい、または陽性対照の細胞生存率が 40%より大きい場合には「実験不成立」、そうでなければ「実験が成立」として (2) へ

(2) (1) で実験が成立した場合に、被験物質の細胞生存率が 50%以下の場合には「皮膚刺激性あり」、そうでなければ (3) へ、

(3) (2) で「皮膚刺激性あり」でなければ IL-1α産生量が 120pg/組織以上の場合には「皮膚刺激性あり」、そうでなければ「皮膚刺激性なし」と判定とした。

C. 結果と考察

これまでの解析結果から、IL-1αで判定が覆るデータは４点しかなく、ばらつきを大きくするだけであるとの見解で一致をみた。19 物質中、15 物質は判定に食い違いがなく、細胞生存率のみで判定した場合には、さらに 2 物質の判定には食い違いはなくなることから、全体として施設間再現性は良好であったとされた。代替の可能性は J-TEC が提唱した結果とほぼ同程度であり、再現性が高いと考察された。EPISKIN との同等性については、特異度は同程度であるが、感度がやや劣る可能性があるとされた。

in vitro 皮膚刺激性試験に関する OECD ガイドラインの動向といして、OECD ガイドラインでは①EPISKIN の指標が MTT アッセイのみになった、②in vivo データの分類が EU 分類から GHS 基準となった、③EPISKIN 以外にも EpiDerm や SkinEthics が提案されており、次回 6 月の専門家会議（資料９）で議論されると説明された。これを受け、ECVAM では EpiDerm や SkinEthics の認証を進め(資料８)、performance standard の変更について検討している（資料 10)。一方、日本でも本バリデーション研究の結果を受けて、資料 6 に示す LabCyte に関する提案書や添付資料を OECD に提出しており、OECD ガイドラインへの記載を目指している。今後の戦略として、可能であれば、ECVAM performance standard の変更を受け、追加バリデーション研究を実施したいと提案された。

D. 結論

本研究で実施した得られた結果から、LabCyte EPI-MODEL24を用いた皮膚刺激性試験代替法はと全体として施設間再現性は良好であったとされた。代替の可能性はJ-TECが提唱した結果とほぼ同程度であり、再現性が高いと考察された。EPISKINとの同等性については、特異度は同程度であるが、感度がやや劣る可能性があると結論された。

E. 資料

資料 2-1　培養皮膚モデル LabCyte EPI-MODEL24 バリデーション研究集計報告書

資料 2-2　バリデーション研究参加施設一覧

資料 2-3　培養皮膚モデル LabCyte EPI-MODEL24 を用いた皮膚刺激性試験のバリデーション研究計画書

資料 2-4　皮膚刺激性試験バリデーション予定被験物質

資料 2-5　培養皮膚モデル LabCyte EPI-MODEL24 を用いた皮膚刺激性試験プロトコール Ver. 5. 01

資料 2-6　LabCyte バリデーション研究の記録用紙についての質疑応答

資料 2-7　LabCyte バリデーションに関する昨今の動向と今後の予定

JSAAE VALIDATION REPORT:

VALIDATION STUDY OF *IN VITRO* SKIN IRRITATION TEST USING LABCYTE EPI-MODEL 24

APRIL 15, 2009

LABCYTE VALIDATION MANAGEMENT TEAM

Contents

This report describes the validation study for an *in vitro* skin irritation test using LabCyte™ EPI-MODEL 24 supported by the JSAAE (Japanese Society for the Alternative to Animal Experiments).

1. Goal Statement

- The aim of this study was to validate *in vitro* skin irritation tests in a formal inter-laboratory study. The ultimate goal of the test will be to replace the regulatory Draize skin irritation test according to OECD TG 404 (1).
- The primary goal of this validation study was to evaluate the ability of *in vitro* tests to reliably discriminate skin irritant (I) from non-irritant (NI) chemicals, as defined by the EU classification, or the classification and labelling of skin irritation (category 1/category 2; no category, as defined by the OECD and United Nations proposal for Globally Harmonised System (GHS; 2) .

2. Objective

In vitro test systems that use human reconstructed models were evaluated by JSAAE. The LabCyte™ model is comprised of normal, human-derived epidermal keratinocytes. This model has progressed through protocol optimisation and multi-laboratory assessments. The present objective was to perform a catch-up validation study to assess the relevance (predictive capacity) and reliability (reproducibility within and between laboratories) of this test system using a challenging set of coded test chemicals for which high quality *in vivo* data are available refer to the ESAC statement of EPISKIN (3) and the ECVAM performance standard (4).

The validation study was performed in accordance with the principles and criteria documented in the OECD *Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment* (5) and according to the Modular Approach to validation (6).

3. Test Methods

3-1. Reconstructed human cultured dermal model

LabCyte EPI-MODEL 24 is a new, commercially available reconstructed human cultured epidermal model produced by Japan Tissue Engineering Co., Ltd (7). It consists of normal human epidermal keratinocytes whose biological origin is neonate foreskin. In order to expand human keratinocytes while maintaining their phenotype, they were cultured with 3T3-J2 cells as a feeder layer (8 and 9). The human cultured epidermis is reconstructed by cultivating proliferating keratinocytes on an inert filter substrate (surface 0.3 cm^2) at the air-liquid interface for 13 days with optimized medium containing 5% fetal bovine serum. These cells construct a multilayer structure consisting of a fully differentiated epithelium with features of the normal human epidermis, including a stratum corneum. The LabCyte EPI-MODEL is embedded in an agarose gel containing a nutrient solution and shipped in 24-well plates at approximately 18°C.

3-2. Model supplier

According to OECD GLP Consensus Document No. 5 "*Compliance of Laboratory Suppliers with GLP Principles*", the management of the test facility is entirely responsible for the quality and reliability of both the equipment and materials (10).

Therefore, the acceptability of the equipment and materials in laboratories that comply with GLP-like principles should be guaranteed to any regulatory authority to whom studies are submitted. In Japan, GLP has been implemented and suppliers belong to national regulatory or voluntary accreditation schemes (for example, for laboratory animals), which can provide users with additional documentary evidence that a test system has a defined quality.

The audits focused on the procedures that were established to guarantee a defined quality of the tissue models.

4. Validation Management Structure

The management structure of the study is shown in Figure 1.

4-1. Validation Management Team

The Validation Management Team (VMT) plays a central role in overseeing the conduct of the validation study and is responsible for the following:

1) Project plan, including goal statement

2) Study protocol/amendments

3) Outcome of QC audits

4) Test chemicals

5) Data management procedures

6) Timeline/study progression

7) Study interpretation and conclusions

8) Reports and publications

The VMT makes the final decision about which laboratories will participate in the validation study.

Members:

Chair (Mr. Hajime Kojima, JaCVAM: Japanese Center for the Validation of ALternative Methods)
Sponsor representative: JSAAE representatives (Mr. Takashi Omori: Kyoto Univ., Mr. Kenji Idehara: Daicel Chemical Industries, LTD and Mr. Isao Yoshimura: Tokyo University of Science)
Sponsor representative: LabCyte™ suppliers and lead lab (Mr. Masakazu Kato: Japan Tissue Engineering Co., Ltd, J-TEC)

4-2. Chemical selection, acquisition, coding and distribution

1) Select chemicals
2) Liaise with suppliers
3) Final check of provided chemicals
4) Acquire chemicals
5) Code test chemicals
6) Distribute test chemicals

Member:

Mr. Hajime Kojima, JaCVAM

4-3. Independent biostatisticians

1) Approve spreadsheets
2) Collect data
3) Analyse data

Members:

Mr. Takashi Omori: Kyoto Univ.
Mr. Etsuyoshi Miyaoka and Mr. Kenya Ishiyama: Tokyo University of Science

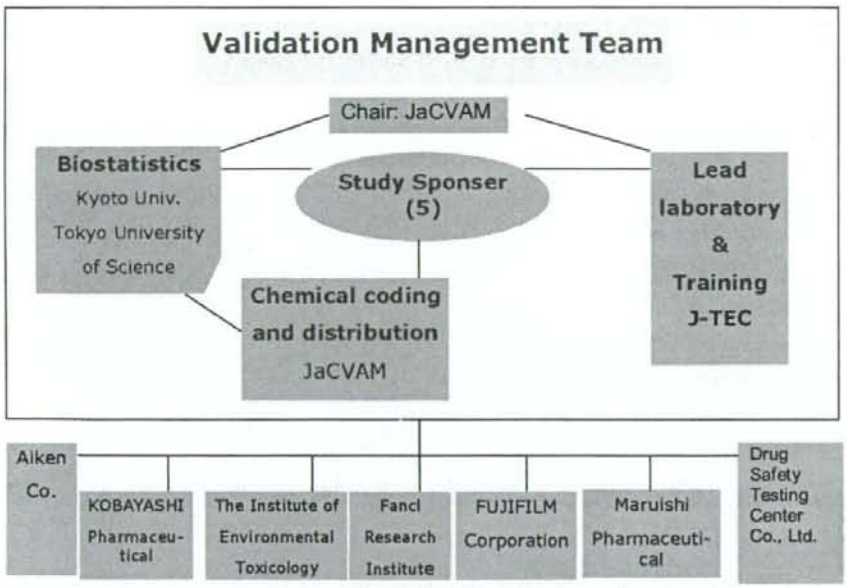


Figure 1. Management structure of the JSAAE skin irritation validation study

4-4. Participating laboratories

The laboratories participating in the study are defined as shown in Figure 1.

The following 7 laboratories participated in the validation study for the evaluation of the LabCyte assays:

- Laboratory 1 – Aiken Co., Ltd. (Ms Yoko Ando and Ms Yui Asako)
- Laboratory 2 – KOBAYASHI Pharmaceutical Co., Ltd. (Mr. Yoshihiro Yamaguchi and Ms Maki Nakamura)
- Laboratory 3 – The Institute of Environmental Toxicology (Mr. Tadashi Kosaka and Mr. Koichi hayashi)
- Laboratory 4 – Fancl Research Institute (Ms. Tamie Suzuki and Ms. Runa Izumi)
- Laboratory 5 – FUJIFILM Corporation (Ms. Atsuko Yuasa, and Mr. Shinichi Akimoto)
- Laboratory 6 – Maruishi Pharmaceutical Co., Ltd. (Mr. Yukihiko Watanabe and Osamu Mitani)
- Laboratory 7 – Drug Safety Testing Center Co., Ltd. (Mr. Shinsuke Shinoda and Ms Saori Hagiwara)

A lead laboratory is also identified as J-TEC (Mr. Masakazu Kato and Mr. Toshihiro Yokouchi). This laboratory was not participated in the validation study.

All of the laboratory responsibilities (particularly, the work programme and data submission deadlines) were specified in contracts between JaCVAM and the laboratories. Each

laboratory will also be responsible for complying with GLP-like principles and specifying QA aspects.

4-5. Sponsorship

The study was financed by JSAAE and J-TEC.

JSAAE finance:

- management of the study (VMT meetings)
- independent statistical support (biostatistician)
- independent laboratory responsible for purchasing, coding, and distributing chemicals to the participating laboratories
- purchasing and distributing IL-1α kits to the laboratories
- independent QC audit of the data
- publication of the study

J-TEC finance:

- lead laboratories for the test method
- training the participating laboratories
- independent QC audit of the LabCyte models
- financial assistance for the participating laboratories

5. Study Design

Before this validation study, a LabCyte training course was conducted by J-TEC in April 2008. All technicians from each laboratory participated in this training course.

Two phases of validation studies were performed. In Phase I, we confirmed the transferability of the test protocol and assessed its reproducibility using suitable statistical analyses by testing three coded chemicals (ethanol, glycerol and napthalen acetic acid) and a positive control (5% sodium lauryl sulfate solution) in seven laboratories between June and July of 2008. In Phase II, we confirmed the robustness of the intra- and inter-laboratory reproducibility and the correlation of the test using 19 new chemicals that were tested in reference to the EPISKIN Performance Standards(4). These tests were conducted by 7 laboratories between September 2008 and January 2009.

6. Test Chemicals

6-1. Chemical selection

According to the EPISKIN Performance Standards, we selected 19 new chemicals to test. One chemical on the chemical list reference for the EPISKIN Performance Standards cannot be purchased on the Japanese market. The VMT is responsible for the final approval of the chemicals proposed by JaCVAM. To avoid any potential bias in the final selection, the laboratory representatives on the VMT were not party to these discussions, nor were they informed of the final list of test chemicals for either phase of the validation study.

6-2. Chemical list

The reference chemicals described in the Performance Standards are shown in Table 1. Among them, tri-isobuthyl phosphate (No. 13) could not be used in the examination because it was not available in Japan. Therefore, a 5% SLS solution was used instead of tri-isobuthyl phosphate. The data obtained with the 5% SLS solution were not used for calculating the predictivity of the test.

Table 1. Reference test chemicals and codes

No.	Chemical	CAS number	EU label	In vivo score (PII)	Laboratory						
					a	b	c	d	e	f	g
01	1-bromo-4-chlorobutane	6940-78-9	no	0	A-01	B-099	C-077	D-115	E-133	F-031	G-049
02	diethyl phthalate	84-66-2	no	0	A-02	B-100	C-078	D-116	E-134	F-032	G-050
03	di-propylene glycol	25265-71-8	no	0	A-03	B-081	C-079	D-117	E-135	F-033	G-051
04	naphtalen acetic acid	86-87-3	no	0	A-04	B-082	C-080	D-118	E-136	F-034	G-052
05	allyl phenoxy-acetate	7493-74-5	no	0.3	A-05	B-083	C-061	D-119	E-137	F-035	G-053
06	isopropanol	67-63-0	no	0.3	A-06	B-084	C-062	D-120	E-138	F-036	G-054
07	4-methyl-thio-benzaldehyde	3446-89-7	no	1	A-07	B-085	C-063	D-101	E-139	F-037	G-055
08	methyl stearate	112-61-8	no	1	A-08	B-086	C-064	D-102	E-140	F-038	G-056
09	allyl heptanoate	142-19-8	no	1.7	A-09	B-087	C-065	D-103	E-121	F-039	G-057
10	heptyl butyrate	5870-93-9	no	1.7	A-10	B-088	C-066	D-104	E-122	F-040	G-058
11	hexyl salicylate	6259-76-3	R38	2	A-11	B-089	C-067	D-105	E-123	F-021	G-059
12	terpinyl acetate	80-26-2	R38	2	A-12	B-090	C-068	D-106	E-124	F-022	G-060
13	5(W/V %) SLS				A-13	B-091	C-069	D-107	E-125	F-023	G-041
14	1-decanol	112-30-1	R38	2.3	A-14	B-092	C-070	D-108	E-126	F-024	G-042
15	cyclamen aldehyde	103-95-7	R38	2.3	A-15	B-093	C-071	D-109	E-127	F-025	G-043
16	1-bromohexane	111-25-1	R38	2.7	A-16	B-094	C-072	D-110	E-128	F-026	G-044
17	α-terpineol	98-55-5	R38	2.7	A-17	B-095	C-073	D-111	E-129	F-027	G-045
18	di-n-propyl disulphide	629-19-6	R38	3	A-18	B-096	C-074	D-112	E-130	F-028	G-046
19	butyl methacrylate	97-88-1	R38	3	A-19	B-097	C-075	D-113	E-131	F-029	G-047
20	heptanal	111-71-7	R38	4	A-20	B-098	C-076	D-114	E-132	F-030	G-048

1) CAS No.: Chemical abstracts service registry number.

2) PII: Primary irritation index.

6-3. Chemical coding and distribution

The chemicals were coded and distributed by an independent company contracted by JaCVAM. The (company's name) is certified according to ISO 9001, EN 4500 and GLP and has an established record of reliable services. The codes were provided by JaCVAM.

7. Protocol

7-1. Protocol of the skin irritation test with LabCyte EPI-MODEL

LabCyte EPI-MODEL tissues were shipped from the supplier on Mondays and delivered to recipients on Tuesdays. Upon receipt, the tissues were aseptically removed from the transport agarose medium, transferred into 24-well plates (BD Biosciences, CA, USA) with the assay medium (0.5 mL), and incubated overnight (37°C, 5% CO_2 humidified atmosphere). On the following day, the tissues were topically exposed to the test chemicals. Liquids (25 µL) were applied with a micropipette, and solids (25 mg) were applied from microtubes and moistened with 25 µL sterile water. If necessary, the mixture was gently spread over the surface of the epidermis with a microspatula. Viscous liquids were applied using a cell-saver-type tip with a micropipette. Each test chemical was applied to three tissues. In addition, three tissues serving as negative controls were treated with 25 µL distilled water, and three tissues serving as positive controls were exposed to 5% SLS. After a 15-minute exposure, each tissue was carefully washed with PBS (Invitrogen, CA, USA) 10 times using a washing bottle to remove any remaining test chemical from the surface. The blotted tissues were then transferred to new 24-well plates containing 1 mL of fresh assay medium.

The treated and control tissues were incubated for 42 hours (37°C, 5% CO_2 humidified atmosphere). When the 42-hour post-incubation period was complete, blotted tissues were transferred to new 24-well plates containing 0.5 mL of freshly prepared MTT medium (1 mg/mL; Dojindo Co., Kumamoto, Japan) for the MTT assay and conditioned medium was collected to determine the interleukin-1 alpha (IL-1α) levels. Tissues were incubated for three hours (37°C, 5% CO_2 humidified atmosphere) and then transferred to microtubes containing 0.3 mL isopropanol, which completely immersed the tissue. Formazan extraction was performed at room temperature, and the tissues were allowed to stand overnight. Subsequently, 200-µL extracts were transferred to a 96-well plate. The optical density was measured at 570 nm and 650 nm as a reference absorbance, with isopropanol as a blank.

The tissue viability was calculated as a percentage relative to the viability of the negative controls. The mean of three values from identically treated tissues was used to classify a chemical according to the prediction model.

The amount of IL-1α released in the conditioned medium after 42 hours was determined using an IL-1α ELISA kit (Invitrogen, CA, USA), following the manufacturer's detailed instructions.

7-2. Prediction model of skin irritation

In this study, the prediction model of skin irritation potential with LabCyte EPI-MODEL was set to refer to the conditions for EPISKIN described in the ECVAM Performance Standards. This prediction model is described in Table 2. In the event that the three independent results within an individual batch were not consistent, the result that occurred twice was used.

Acceptance criteria

1) OD_{NC} of the negative control is greater than 0.7.
2) The viability of the positive control is less than 40%.

Table 2. Positive Criteria.

Tissue Viability (primary)	IL-1α ELISA (secondary)	Classification
Mean tissue viability ≤ 50%		Irritant
Mean tissue viability > 50%	Mean IL-1α release ≥ 120 pg/tissue	
Mean tissue viability > 50%	Mean IL-1α release < 120 pg/tissue	Non-irritant

7-3. Difference between LabCyte EPI-MODEL 24 protocol and EPISKIN protocol

The differences between the LabCyte EPI-MODEL 24 protocol and EPISKIN protocol are summarized in Table 3. Although the amount of medium (Table 3(A)), amount of test chemicals (Table 3(B)), and threshold of IL-1α content (Table 3(C)) for the LabCyte EPI-MODEL 24 protocol are different from the EPISKIN protocol, their conditions meet the descriptions of the Performance Standards.

Table 3. Differences between the LabCyte EPI-MODEL 24 protocol and EPISKIN protocol.

(A) Amount of medium.

	LabCyte EPI-MODEL 24 SOP	EPISKIN SOP	Reason
Pre-incubation	0.5 mL	2 mL	LabCyte EPI-MODEL 24 cultures are performed in 24-well culture plates. A medium volume of 0.5 mL to 1 mL is appropriate to add to the 24-well culture plate. A medium volume of 1 mL is necessary for a 42-hour culture.
Post-incubation	1 mL	2 mL	
MTT assay	0.5 mL	2 mL	

These conditions meet the descriptions of the Performance Standards.

(B) Amount of test chemicals.

Test chemical	LabCyte EPI-MODEL 24 SOP	EPISKIN SOP	Reason
Liquid	25 μL (75 μL/cm^2)	10 μL (25 μL/cm^2)	The lowest amount of the test chemical that spread uniformly was applied to the test model.
Solid	25 mg+25 μL DW (75 μL/cm^2)	10 mg+10 μL DW (25 μL/cm^2)	

These conditions meet the descriptions of the Performance Standards.

(C) Amount of test chemicals.

LabCyte EPI-MODEL 24 SOP	EPISKIN SOP	Performance Standards (EPISKIN)
IL-1α content ≥ 120 pg/tissue (IL-1α content ≥ 120 pg/mL)	IL-1α content ≥ 100 pg/tissue (IL-1αcontent ≥ 50 pg/mL)	IL-1α content ≥ 120 pg/tissue (IL-1α ≥ 60 pg/mL)

The threshold of IL-1α released in LabCyte EPI-MODEL was set based on the conditions for EPISKIN described in the Performance Standards.

7-4. Data collection, handling, and analysis

The independent biostatisticians for the study collected and organised the data using specific data collection software (Datasheet4.0:20080910.xls). They will work in close collaboration with the biostatisticians, (Mr. Takashi Omori, Mr.Etsuyoshi Miyaoka, and Mr. ken-

ya Ishiyama). After decoding the data, they will perform statistical analyses. The data management procedures and statistical tools applied will be approved by the VMT.

7-5. Quality assurance, GLP

Laboratories

All participating laboratories worked in the spirit of OECD GLP-like principles.

QA aspects

Mr. Kenya Ishiyama and Dr. Hajime Kojima assured the quality of all the data and records.

8. Results

8-1.Phase I

8-1-1 Negative control

In Phase I data, Table 4 shows the absorbance values for the negative control. All data for the negative control met the acceptance criteria.

Table 4. Absorbance of negative control by phase I.

	Exp.				
	1	2	3		
Lab.	Value	Value	Value	Mean	SD
a	1.073	0.928	1.007	1.003	0.073
b	0.93	1.245	1.042	1.072	0.16
c	0.96	0.869	0.761	0.863	0.1
d	0.987	0.928	0.939	0.951	0.031
e	0.84	0.884	0.973	0.899	0.068
f	1.049	0.934	0.968	0.984	0.059
g	1.147	1.159	1.074	1.127	0.046

8-1-2 Positive control and test chemicals

Table 5 shows the testing chemicals did not show any great score when the scores on tests were repeated in each laboratory. Furthermore, there was no significant inter-laboratory variation. These experiments suggested the feasibility of the LabCyte EPI-MODEL24 through the experiment. All laboratories were judged to participate at the Phase II by the validation management team.

Table 5. Viability of the positive control and three coded chemicals by phase I

		1	2	3		
Chem.	Lab.	Viability	Viability	Viability	Mean	SD
PC	a	6.35	27.55	15.67	16.52	10.63
	b	3.94	3.51	3.97	3.81	0.26
	c	5.45	4.81	3.49	4.58	1
	d	11.74	7.22	14.08	11.02	3.49
	e	31.6	9.76	38.61	26.66	15.05
	f	3.1	2.89	2.93	2.97	0.11
	g	4.46	7.17	2.62	4.75	2.29
P01	a	62.67	39.12	46.61	49.46	12.03
Ethanol	b	41.08	50.86	86.58	59.51	23.95
	c	68.13	34.13	67.31	56.53	19.4
	d	68.57	40.52	33.03	47.37	18.73
	e	54.19	72.08	60.55	62.27	9.07
	f	.	64.16	47.98	56.07	11.44
	g	4.68	5.23	6.67	5.53	1.03
P02	a	103.63	104.17	98.48	102.09	3.14
Glycerol	b	85.5	100.58	67.97	84.68	16.32
	c	101.24	99.41	104.84	101.83	2.76
	d	103.3	101.35	89.73	98.13	7.34
	e	101.75	98.06	99.04	99.62	1.91
	f	.	97.23	96	96.62	0.87
	g	94	98.16	103.6	98.59	4.82
P03	a	109.13	90.73	97.78	99.22	9.28
naphtalen acetic acid	b	93.96	103.91	103.96	100.61	5.76
	c	103.66	102.11	117.3	107.69	8.36
	d	102.28	98.15	94.56	98.33	3.86
	e	107.11	104.39	97.36	102.95	5.03
	f	.	101.34	102.07	101.7	0.52
	g	92.2	101.04	105.52	99.59	6.78

8-2. Phase II

8-2-1. Comments on the datasheets by phase II

Tables 6 and 7 show the comments from each laboratory. The 1st test from Lab. a did not meet the acceptance criteria. Therefore, an additional test was performed and then submitted to the biostatisticians.

Table 6. Comments on the datasheets (Viability) by phase II.

Lab ID	Exp.No.	Lot	Date	Comments
a	Main-2	LCE24-081013-B	2008/10/20	This test was recorded as the Main-1.
a	Main-3	LEC24-081117-B	2008/11/1	This test was recorded as the Main-2.
a	Main-4	LCE24-081117-B	2008/11/22	This test was recorded as the Main-3.
b	Main-1	LCE24-081013-B	2008/10/20	
b	Main-2	LCE24-081027-B	2008.11.04	
b	Main-3	LCE24-081117-B	2008/11/25	
c	1	LCE24-080929-B	2008.10.6	
c	2	LCE24-081020-B	2008/10/27	
c	3	LCE24-081027-B	2008.11.3	
d	81021	LCE24-081020-B	2008/10/27	
d	81028	LCE24-081027-B	2008/11/4	
d	81118	LCE24-081117-B	2008/11/25	
e	Main-1	LCE24-081006-B	2008/10/14	
e	Main-2	LCE24-081013-B	2008/10/20	
e	Main-3	LCE24-081020-B	2008/10/27	
f	LAB-08VAL	LCE24-080929-B	2008/10/6	
f	Maruishi	LCE24-081013-B	2008/10/20	
f	LAB-08VAL	LCE24-081103-B	2008/11/10	
g	Main-1	LCE24-080929-B	2008.10.06	By an application of G49,G53,G55, the model's cap was discolored.
g	Main-2	LCE24-081013-B	2008.10.20	By an application of G49,G53,G55, the model's cap was discolored.
g	Main-3	LCE24-081027-B	2008.11.03	By an application of G49,G53,G55, the model's cap was discolored.

Table 7. Comments on the datasheets (ELISA) by phase II.

Lab ID	Exp.No.	Lot	Date	Comments
a	Main-2	LCE24-081013-B	2008/10/20	This test was recorded as the Main-1.
a	Main-3	LEC24-081117-B	2008/11/1	This test was recorded as the Main-2.
a	Main-4	LCE24-081117-B	2008/11/22	This test was recorded as the Main-3.
b	Main-1	LCE24-081013-B	2008/12/12	
b	Main-2	LCE24-081027-B	2008/12/12	
b	Main-3	LCE24-081117-B	2008.12.26	
c	1	LCE24-080929-B	2008/10/7	
c	2	LCE24-081020-B	2008/10/30	
c	3	LCE24-081027-B	2008.11.3	
d	81021	LCE24-081020-B	2008/11/11	
d	81028	LCE24-081027-B	2008/11/26	
d	81118	LCE24-081117-B	2009/1/7	
e	Main-1	LCE24-081006-B	2008/12/2	
e	Main-2	LCE24-081013-B	2008/12/2	
e	Main-3	LCE24-081020-B	2008/12/19	
f	Maruishi	LCE24-081013-B	2008/11/25	
f	Maruishi	LCE24-081013-B	2008/11/27	
f	LAB-08VAL	LCE24-081103-B	2008/12/25	
g	Main-1	LCE24-080929-B	2008.10.09	
g	Main-2	LCE24-081013-B	2008.10.22	
g	Main-3	LCE24-081027-B	2008.11.05	

8-2-2. Negative control

Table 8 shows the absorbance values for the negative control. Excluding the results of Lab. a, 1st of 4th (data not shown), all data for the negative control met the acceptance criteria.

Table 8. Absorbance of negative control by phaseII.

	Lab.						
Exp.	a	b	c	d	e	f	g
1	0.75	0.93	0.91	0.82	0.80	0.84	1.13
2	0.86	0.85	1.01	0.90	0.90	0.79	1.18
3	0.82	0.84	0.93	0.96	0.91	0.83	1.05
Mean	0.81	0.88	0.95	0.89	0.87	0.82	1.12
Sd	0.06	0.05	0.05	0.07	0.06	0.02	0.06
Min	0.75	0.84	0.91	0.82	0.80	0.79	1.05
Max	0.86	0.93	1.01	0.96	0.91	0.84	1.18

8-2-3. Positive control

Table 9 and Figure 2 show three independent viabilities and summary statistics for the positive control from each laboratory. All data were sufficient and met the acceptance criteria for the positive control. In Table 10, the viabilities of No. 13, which is a positive control (5% SLS solution), are shown. These data were similar to that of positive control.

Table 9. Viability of the positive control by phase II.

	Lab.						
Exp.	a	b	c	d	e	f	g
1	5.9	5.2	4.1	5.7	4.1	3.5	3.1
2	8.8	12.3	5.4	2.6	12.6	2.9	10.8
3	2.5	7.8	3.8	3.3	5.6	3.2	4.2
Mean	5.7	8.4	4.4	3.9	7.4	3.2	6.0
Sd	3.1	3.6	0.8	1.6	4.5	0.3	4.1
Min	2.5	5.2	3.8	2.6	4.1	2.9	3.1
Max	8.8	12.3	5.4	5.7	12.6	3.5	10.7

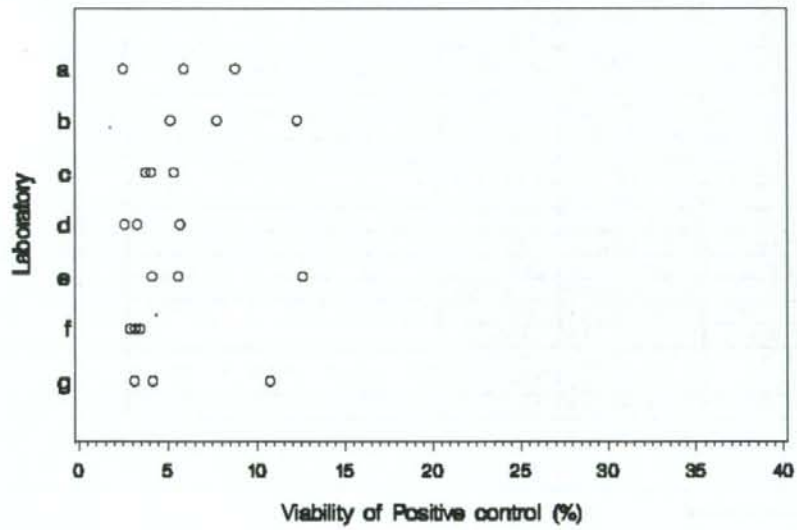


Figure 2. Viability of the positive control at each laboratory by phase II.

Table 10. Viability of chemical No. 13 (5% SLS solution).

			Lab.							
Chem.	Vivo	Score	Exp.	A	b	c	d	e	f	g
13			1	12.2	5.2	9.9	3.8	12.9	12.0	10.7
			2	3.6	3.2	5.0	3.6	6.7	3.1	8.0
			3	2.2	12.5	3.3	2.5	4.7	7.4	3.3

8-2-4. Skin irritation test by cell viability

The results of the LabCyte EPI-MODEL 24 skin irritation test when it was only evaluated based on cell viabilities as an indicator are shown in Table 11. A summary of the statistical analysis of the viability for each chemical is shown in Table 12 and Figure 3 .

Table 11. Viability of chemicals at each laboratory.

				Lab.						
Chem.	Vivo	Score	Exp.	a	b	c	d	e	f	g
01	no	0	1	31.0	47.1	10.6	14.3	38.1	14.3	10.6
			2	11.2	10.4	20.3	9.1	25.2	11.2	10.6
			3	11.6	16.1	12.4	9.6	32.3	10.4	14.0
02	no	0	1	79.8	66.9	88.1	102.3	101.8	75.3	96.0
			2	76.5	61.7	89.7	89.8	76.4	67.2	94.8
			3	65.2	88.7	85.8	67.6	85.8	75.7	103.3
03	no	0	1	109.1	93.3	94.6	105.1	129.6	94.2	100.5
			2	103.9	99.8	93.1	112.8	106.6	97.9	93.4
			3	100.9	102.3	95.7	101.4	103.9	92.5	111.1
04	no	0	1	106.3	94.4	97.1	106.1	127.1	100.1	104.8
			2	95.2	100.2	99.9	100.9	113.6	92.8	103.3
			3	96.5	98.6	97.8	98.4	105.2	92.7	109.8
05	no	0.3	1	78.5	61.7	91.4	79.4	103.0	71.9	96.8
			2	78.5	71.9	95.2	70.5	90.3	39.3	89.9
			3	74.1	84.5	89.2	66.1	89.6	55.1	88.4
06	no	0.3	1	92.5	77.9	81.0	91.3	97.0	87.8	87.2
			2	79.4	83.5	79.1	102.4	81.5	94.4	81.2
			3	82.4	80.5	83.6	82.7	90.7	81.1	54.1
07	no	1	1	24.1	10.8	20.8	21.7	17.5	15.8	31.5
			2	12.6	12.6	16.2	13.8	22.2	31.1	22.5
			3	17.8	13.2	15.2	19.8	21.3	15.6	19.9
08	no	1	1	111.9	86.7	75.3	109.4	114.9	89.7	101.1
			2	90.2	100.6	82.3	107.5	100.9	97.8	100.9
			3	95.3	104.8	77.2	103.0	100.9	96.5	109.0
09	no	1.7	1	112.8	96.7	106.6	105.0	115.8	98.8	102.3
			2	97.1	110.1	96.8	103.4	108.6	86.5	103.4
			3	101.1	109.5	93.5	98.1	103.9	97.7	112.1
10	no	1.7	1	115.9	115.4	107.5	114.3	132.0	104.0	107.9
			2	104.1	110.1	103.6	108.2	117.0	101.2	108.4
			3	86.5	111.3	103.7	105.5	107.5	101.2	113.1

Table 11. continued

				Lab.						
Chem.	Vivo	Score	Exp.	a	b	c	d	e	f	g
11	R38	2	1	113.7	105.0	101.0	102.4	123.1	103.1	102.8
			2	98.1	106.6	94.6	105.8	110.4	98.0	100.5
			3	112.6	103.7	94.1	102.7	105.5	94.6	109.0
12	R38	2	1	28.2	24.6	24.9	54.3	55.6	27.2	87.7
			2	18.4	24.6	44.8	76.2	57.8	65.2	98.0
			3	15.3	15.9	28.1	27.4	57.2	66.0	112.6
14	R38	2.3	1	11.1	12.1	14.7	10.7	14.2	13.1	13.5
			2	6.6	8.3	9.5	11.7	12.0	16.7	12.0
			3	6.8	8.8	9.1	10.2	10.4	17.0	10.6
15	R38	2.3	1	11.1	9.3	13.1	8.0	11.0	8.6	9.2
			2	7.1	10.2	19.3	8.6	11.3	5.9	24.7
			3	8.2	9.9	8.1	9.2	8.7	7.1	9.2
16	R38	2.7	1	67.9	92.0	51.5	18.1	98.2	59.6	64.9
			2	32.2	54.1	86.3	79.2	90.6	50.4	79.6
			3	59.8	98.3	81.7	37.7	78.7	67.5	86.5
17	R38	2.7	1	6.1	4.5	5.3	6.6	8.9	6.9	6.2
			2	4.8	4.7	6.0	5.3	6.3	5.5	5.3
			3	5.6	5.7	5.9	3.9	5.4	4.5	5.3
18	R38	3	1	82.1	46.5	91.2	83.7	98.9	69.2	92.4
			2	78.3	50.6	87.3	69.9	87.2	80.6	85.9
			3	25.3	100.0	87.5	59.0	69.1	71.9	94.4
19	R38	3	1	15.0	74.6	10.0	30.4	83.1	40.1	35.8
			2	19.9	10.9	22.4	28.3	26.1	87.0	44.7
			3	51.1	32.0	35.0	18.2	69.4	71.8	38.7
20	R38	4	1	31.1	24.8	10.4	9.6	10.7	8.1	8.8
			2	9.3	8.0	7.6	16.9	8.2	7.8	6.7
			3	29.5	9.3	7.6	30.9	6.2	8.2	8.6