Round robin study report to evaluate LabCyte EPI-MODEL24 SIT as an in vitro irritation test for detection of irritant activity in medical device extracts

20 February 2024

The committee for the LabCyte EPI-MODEL24 round robin Phase II study

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Abbreviation

Between laboratory reproducibility (BLR)

International Organization for Standardization (ISO)

Japanese Centre for Validation of Alternative Methods (JaCVAM)

Organization for Economic Co-operation and Development (OECD)

Polyvinyl chloride (PVC)

Reconstructed Human Epidermis (RhE)

Skin Irritation Test (SIT)

Technical Committee (TC)

Test Guideline (TG)

Within laboratory reproducibility (WLR)

Working Group (WG)

Summary

In the biological safety evaluation of medical devices, irritancy evaluation is an essential item for all devices that come into direct or indirect contact with a living body. Traditionally, the irritancy of medical devices has been assessed by in vivo tests such as primary skin irritation tests, intradermal reaction tests, and eye irritation tests using rabbits. However, the usefulness of in vitro irritation tests using a reconstructed human epidermis (RhE) model has been demonstrated as an alternative method to animal testing for medical devices; therefore, the test method has been included in both domestic and international guidances. LabCyte EPI-MODEL24, manufactured and marketed by Japan Tissue Engineering Co., Ltd. (J-TEC), is an RhE model listed in the Organization for Economic Cooperation and Development Test Guidelines 439 (In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method). However, the applicability of this model to the evaluation of medical devices in which test solutions are prepared by polar and nonpolar solvent extraction has not been sufficiently verified. Therefore, a LabCyte EPI-MODEL24 round robin phase I study was conducted in 16 laboratories under non-Good Laboratory Practice conditions to transfer the technology of LabCyte EPI-MODEL24 Skin Irritation Test (SIT) and check whether participating laboratories could achieve results comparable to those of the lead laboratory (J-TEC).

As a next step, to confirm the reliability and accuracy of this method, the LabCyte EPI-MODEL24 round robin phase II study with 16 laboratories was conducted using four materials with properties equivalent to those of the test samples used in the international round robin study at the International Organization for Standardization (ISO) 10993-23 (1: material determined to be non-irritant in both saline and sesame oil extracts; 2: material determined to be irritant only in saline extracts; 3: material determined to be irritant only in sesame oil extract; and 4: material found to be irritant in both saline and sesame oil extracts). The tests were repeated three times per test sample in each laboratory. In this Phase II study, the within laboratory reproducibility (WLR) of LabCyte EPI-MODEL24 SIT was 100% in 13 of the 16 laboratories and 87.5% in one laboratory (two laboratories were excluded from the analysis). Moreover, the between laboratory reproducibility (BLR) was 100% for 14 laboratories that met the success criteria for WLR, and the results showed 100% sensitivity, 98.9% specificity, and 99.5% accuracy. These reproducibility and accuracy values satisfied the criteria set by the committee for the LabCyte EPI-MODEL24 round robin Phase II study. The LabCyte EPI-MODEL24 model was judged to have comparable performance to the two RhE models (EpiDerm™ and SkinEthic™ RHE) used in the international round robin study at ISO/TC 194/WG 8.

Based on the results obtained, the committee concluded that the LabCyte EPI-MODEL24 SIT for Medical Device Extracts is reproducible, accurate, and predictive for the assessment of irritant activity in medical device extracts.

1. Background

The reconstructed human epidermis (RhE) test method for medical devices was used to assess skin irritation using the cytotoxicity of test samples to RhE tissues as an indicator. The concept of the test method is described in the domestic guidance on biological safety test of medical devices, which was revised in 2020, attachment "Biological safety test methods for medical devices" Guidance, Part 5: Irritation Test" of "About revision of Basic Principles of Biological Safety Evaluation Required for Application for Approval to Market Medical Devices (MHLW Notification, Yakuseikishin-hatsu 0106 No. 1)"[1].

In contrast, the EpiDerm™ Skin Irritation Test for Medical Device Extracts (EpiDerm™ SIT) and the SkinEthic™ RHE Skin Irritation Test for Medical Device Extracts (SkinEthic™ RHE SIT) were evaluated in an international round robin study conducted by ISO/TC 194/WG 8 (irritation, sensitization) and listed in ISO 10993-23 as validated standard methods for the RhE test method[2,3]. The ISO/TC 194/WG 8 requirements for a new RhE model method to be an international standard are: 1) the RhE model already listed in OECD TG 439, 2) the inter laboratory study to be conducted at three or more laboratories, and 3) within laboratory reproducibility (WLR), between laboratory reproducibility (BLR), and predictability are equivalent to the international round robin study.[3]

LabCyte EPI-MODEL24 is a cultured epidermis model developed by the Japan Tissue Engineering Co., Ltd. (J-TEC) and was listed in OECD TG439 in 2013[4] .

In the case of medical devices, irritation is assessed using test solutions extracted with polar and non-polar solvents, which means that the TG 439 protocol, a chemical irritation test method, needs to be modified for the evaluation of medical devices. Therefore, in a previous study, the LabCyte EPI-MODEL24 Skin Irritation Test (SIT) protocol was also optimized for medical device evaluation with reference to the EpiDerm™ SIT and SkinEthic™ RHE SIT protocols already listed in the ISO 10993-23, and then the medical device protocol was then validated using test samples used in the international round robin study[5] . The study demonstrated that the LabCyte EPI-MODEL24 was a robust model for detecting of irritant activity in medical device extracts, similar to the EpiDerm™ and SkinEthic™ models. This validation study was conducted as a ‘me-too’ study for EpiDerm™ and SkinEthic™ skin irritation tests in ISO 10993-23.

Two optional investigations were carried out in parallel with this study. One was to verify the test application concentration of the positive control, sodium dodecyl sulfate (SDS). The other was a comparison of the performance of sesame oil listed in the European Pharmacopoeia and Japanese Pharmacopoeia.

2. Study objectives

The primary objective of this studies was to validate the LabCyte EPI-MODEL24 SIT as an in vitro skin irritation test for the detection of irritant activity in medical device extracts and to incorporate this test method as a me-too assay in 10993-23.

In a previous LabCyte EPI-MODEL24 round robin Phase I study was conducted in 16 laboratories under non-Good Laboratory Practice conditions to transfer the technology of LabCyte EPI-MODEL24 SIT and check whether participating laboratories could achieve results comparable to those of the lead laboratory (J-TEC).

As a next step, to confirm the reliability (reproducibility within and between laboratories) and relevance (predictive capacity) of this method , the LabCyte EPI-MODEL24 round robin Phase II study with 16 laboratories was conducted using four materials with properties equivalent to those of the test samples used in the international round robin study at the International Organization for Standardization (ISO) 10993-23.

3. Round robin Phase II study plan

This LabCyte EPI-MODEL24 round robin Phase II study was conducted according to the study plan described in Appendix 1. An overview of this is presented below.

3.1 Round robin study committee

The Phase II study was performed by the following structures (Table 1).

Table 1. Members of committee for the LabCyte EPI-MODEL24 round robin Phase II study

Name	Roles and expertise	Affiliation
Chair Reiko Kato	Round robin study manager Chairman	National Institute of Health Sciences (NIHS) Department of Medical Devices (DMD)
Lead Lab Mitsuko Hatanaka Hiromichi Mitake	Test method developers and study sponsors	Japan Tissue Engineering Co., Ltd. (J-TEC)
Hajime Kojima	Expert of irritation test	NIHS Japan Centre for the Validation of Alternative Methods (JaCVAM)
Takao Ashikaga	Records administrator	NIHS JaCVAM
Takashi Sozu	Biostatistician	Department of Information and Computer Technology, Faculty of

		Engineering, Tokyo University of Science
Atsuko Miyajima	Test sample manager	NIHS DMD
Eiichi Yamamoto	Study sponsor	NIHS DMD

Responsibilities of management members.

1) Chair of the committee for the LabCyte EPI-MODEL24 round robin Phase II study

The chair led and managed the operation of the Phase II study, and was responsible for planning, budgeting, scheduling, managing the committee and record-keeping, and prepared a LabCyte EPI-MODEL24 round robin study report.

2) Lead lab

The lead laboratory provided standard working papers and record forms for the test method and planned for each phase of the LabCyte EPI-MODEL24 round robin study.

Technical support was provided to the participating laboratories during the Phase II study.

The following sub-groups were organized within the committee.

3) Test sample management group

Test samples were selected, prepared, and distributed in consultation with the lead laboratory, test sample distributors, and experts, according to the objectives of each phase.

4) Statistical analysis Group

The statistician and DMD were responsible for data management and statistical analysis.

5) Records management group

JaCVAM and DMD reviewed and archived all the test records.

6) Sponsor

The costs of the Phase II study were covered by the DMD, lead laboratory, and participating laboratories, in consultation with these members.

7) Participating laboratories

Table 2 lists the participating laboratories. Of all participating laboratories, only representatives of the participating laboratories are included in the committee.

Table 2. Participating Laboratories

Lab No.	Laboratory
1	TRANSGENIC INC.
2	SB-KAWASUMI LABORATORIES, INC.
3	Mediford Corporation
4	Olympus Medical Systems Corp.
5	Chemicals Evaluation and Research Institute, Japan
6	Safety Research Institute for Chemical Compounds Co., Ltd.
7	Kamakura Techno-Science, Inc.
8	CMIC Pharma Science Co.,Ltd.
9	Food and Drug Safety Center Hatano Research Institute
10	Terumo Corporation
11	Nissei Bilis Co.,Ltd.
12	NIPRO CORPORATION
13	Japan Food Research Laboratories
14	Nihon Bioresearch Inc.
15	BoZo Research Center Inc.
16	Drug Safety Testing Center Co.,Ltd.

3.2 Protocol

The 'LabCyte EPI-MODEL24 Skin Irritation Test method (LabCyte EPI-MODEL24 SIT) for medical device_ Round Robin Study in Japan SOP for Main Study Ver.1.1 for this Phase II Study' (Appendix 2) was prepared by the Lead lab. It has been revised in consultation with the committee as necessary.

3.2.1 Role of the test method

The RhE test method is a test method used to detect irritants in the medical device extracts.

3.2.2 Principle of the test method

The RhE test method takes advantage of the fact that when keratinocytes are stimulated by irritants, cells are damaged, cell viability is reduced, and cell viability is used as an indicator to assess irritation. The RhE tissue used was commercially available (LabCyte EPI-MODEL24), which is a multilayer culture of normal human epidermal cells. Test solutions consisting of a positive control (1% SDS), a negative control (Dulbecco’s phosphate-buffered saline: DPBS), solvent controls (saline or sesame oil), and extracts of the test samples (saline or sesame oil extract) were exposed to LabCyte EPI-MODEL24, and the cell viability calculated by the MTT method was used as the endpoint. If the cell viability was >50% compared to that of the negative control, it was judged as a non-irritant.

3.2.3 Overview of the test procedures

The LabCyte EPI-MODEL24 SIT procedure is described below.

1) Application of test solutions (extracts of test samples) to RhE tissue

Test solutions were prepared by extracting the test samples with saline or sesame oil at 37°C (±1°C) for 72 h (±2 h). The test solutions were used within 24 h of extraction. 100 µL of each test solution was applied to the LabCyte EPI-MODEL24 tissue and incubated at 37°C (±1°C) for 18 h (±1 h), and then the tissue was washed at least 10 times with DPBS to remove the test solution.

1% SDS was used for the positive control and DPBS for the negative control.

2) Calculation of cell viability

The MTT method was used to calculate cell viability. After washing, RhE tissues were placed in 0.3 mL of MTT solution (1 mg/mL) and incubated at 37°C (±1°C) for 3 h (± 5 min) under 5% CO_2 and 95% humidity and then placed in 0.5 mL of isopropanol at least 2 h to extract formazan, which was quantified by OD measurement (OD 570 nm-OD 650 nm). Cell viability was calculated using the following equation.

Cell viability (%)

= ((OD 570 nm-OD 650 nm). treated tissue / (OD 570 nm-OD 650 nm). negative control tissue) × 100

3) Test acceptance criteria

The study was approved if all of the following conditions were fulfilled

Absorbance value: 0.7 ≤ mean absorbance of negative control ≤ 2.5

Positive control: 1% SDS (positive control) cell viability < 40% of negative control.

SD of cell viability of each test solution including negative and positive controls ≤ 20%

Vehicle control: 80% of negative control < average cell viability of vehicle control < 120% of negative control

4) Positive criteria

Cell viability ≤ 50% Irritant

Cell viability > 50% Non-irritant

4. Test sample

This Phase II study used test samples of the sizes listed in Table 3, in accordance with the objectives.

The test sample management group selected the test samples. Materials containing substances that have been reported to have irritant properties were used as test samples. Irritant materials were selected based on their reactivity, balance of properties, and cost. Test samples were prepared by DMD, coded, and distributed to the participating laboratories (Appendix 3).

Table 3. Summary of the study design in each phase study in the LabCyte EPI-MODEL24 Round Robin Phase II Study

Study Phase	Test samples	Number of trails	Information to be obtained.
Phase II	4 coded	3	Within and between laboratory reproducibility

The test samples in the Phase II study included four materials with properties comparable to those used in an international round-robin study:

1) material classified as be non-irritant in both saline and sesame oil extracts (PVC)
2) material classified as be irritant only in saline extract (PVC + 10% SDS)
3) material classified as be irritant only in sesame oil extract (PVC + 30% Heptanal)
4) material classified as be irritating in both saline extract and sesame oil extract (Y-4)

5. Process and success criteria

5.1 Training

The lead laboratory scheduled a date for all participating laboratories to meet and conduct training on the method. Training was conducted using videos.

5.2 Transferability

Phase I: Participating laboratories were judged based on whether they could achieve results comparable to those of the lead laboratory.

In the previous Phase I study, the test was conducted using two coded test samples, and was considered successful when the set criteria were fulfilled.

Transferability of technology was examined in a study involving 16 laboratories conducted prior to the Phase I study. The details of transferability study are presented in “LabCyte EPI-MODEL24 Round Robin Phase I study (Transferability) report” (Appendix 3). The committee concluded that a series of technology transfers, including extraction operations, had been completed on the basis of these results.

5.3 Within laboratory reproducibility (WLR)

In the Phase II study, eight solutions prepared by extracting four test samples in two different solvents were used for the test. The test was performed in triplicate and 24 test solutions were evaluated. The sample management group determined the study design.

The WLR criterion was a concordance rate of at least 87.5% (7/8) for judgements of the eight test solutions (irritant and non-irritant) in three independent tests.

The denominator of the concordance rate was the number of test solutions. The numerator of the concordance rate was the number of the test solutions in which all three judgements were consistent.

5.4 Between laboratory reproducibility (BLR)

BLR tests were performed in laboratories that met the WLR criteria. The BLR criterion was a concordance rate of at least 75% (6/8) for judgements of the eight test solutions (irritant or non-irritant).

The denominator of the concordance rate was the number of test solutions. The numerator of the concordance rate was calculated using the following method.

1) The most frequent result of three judgements for a test solution at each laboratory (more than twice) was adopted as the final judgement for the solution.
2) The judgement was adapted to be consistent with the test solution if at least 85% (i.e., at least 14 consistent results out of 16 laboratories) of the final judgements of the participating laboratories.
3) The number of the test solutions judged as consistent by the procedure 2) was used as the numerator of the concordance rate.

6. Data quality check

The laboratories participating in the study in accordance with GLP retained all data as far as possible, and each laboratory study director reviewed the record forms and submitted them to the committee chairperson and JaCVAM.

7. Round robin Phase II results

7.1 Overview of LabCyte EPI-MODEL24 round robin phase II study

The Phase II study of the LabCyte EPI-MODEL24 SIT was conducted in laboratories (12 contract research organizations and four medical device companies), as listed in Table 2. These 16 laboratories satisfied the criteria for technology transferability. All the test samples were coded and distributed in each laboratory. Trials were conducted three times at each of the 16 laboratories for all test samples.

7.2 Reliability of test methods

The original results of the participating laboratories are presented in Appendix 4.

7.2.1 Within laboratory reproducibility

The results from the 16 laboratories for the four materials are shown in Figure 1. The WLR values for each laboratory are listed in Table 4. The results of the third trial in Lab 1 and Lab 15 were excluded from the evaluation for the following two reasons: 1) it could not be completed within the stipulated test period (2022.12), and 2) The European Pharmacopoeia sesame oil distributed to both laboratories exceeded the quality assurance period (2022.12). All test results for sesame oil extraction in both laboratories were consistent with the expected judgments. The remaining 14 laboratories met the WLR

criteria (87.5 %, 7/8). The mean coincidence rate of the 14 laboratories was 99.1%. In contrast, the mean coincidence rates of EpiDerm™ and SkinEthic™ in international round robin studies were 92.2% and 94.7%, respectively[2)].

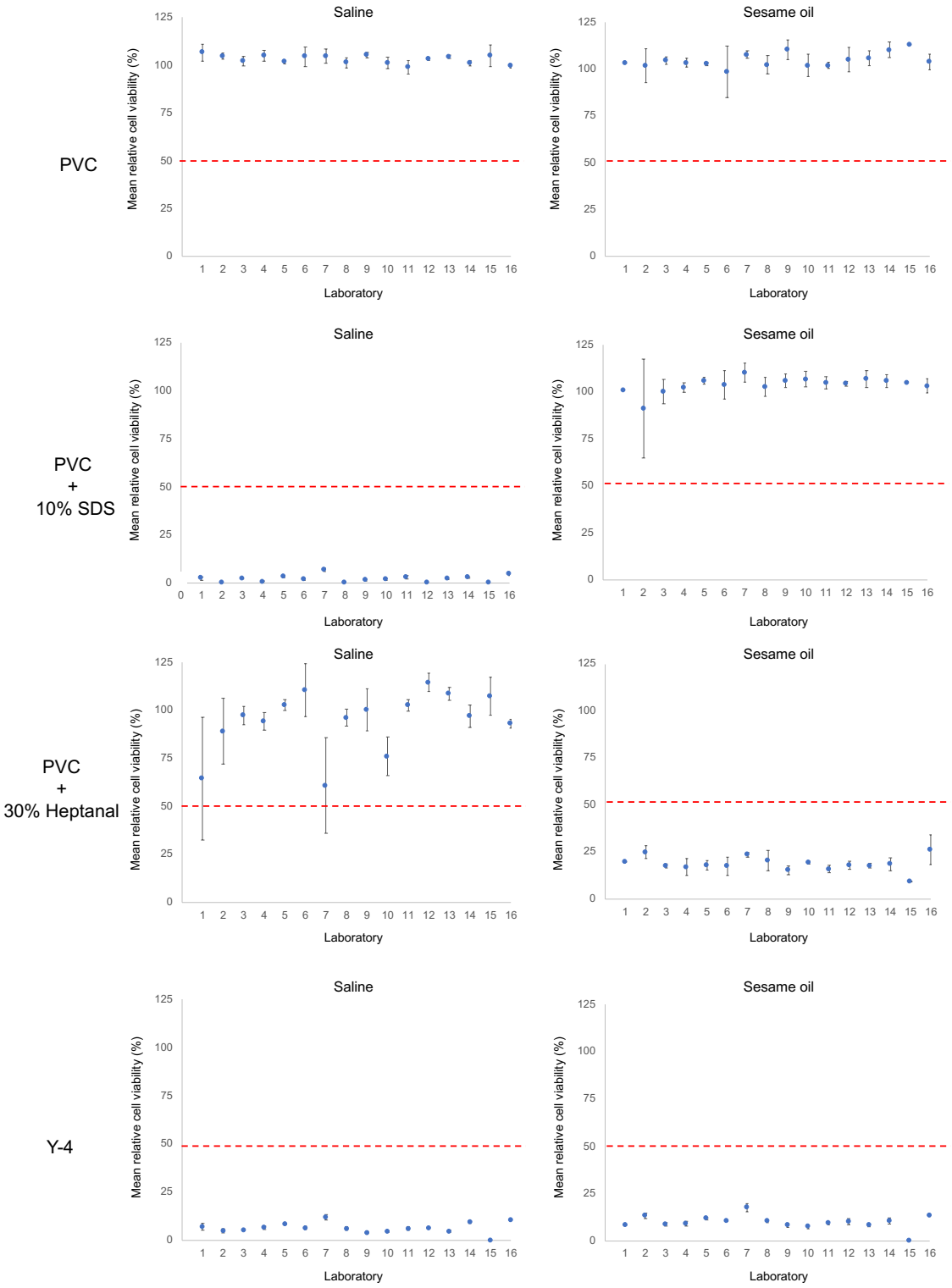

Figure 1. Cell viability obtained from LabCyte model exposed to extracts of polymer samples and controls.

The WLR values for each laboratory are listed in Table 4. The results of the third trial in Lab 1 and Lab 15 were excluded from the evaluation for the following two reasons: 1) it could not be completed within the stipulated test period (2022.12), and 2) The European Pharmacopoeia sesame oil distributed to both laboratories exceeded the quality assurance period (2022.12). All the test results of sesame oil extracts in both laboratories were consistent with the expected judgments. In the remaining 14 laboratories, 87.5% (7/8) of the criteria for within laboratory reproducibility were met. The mean coincidence rate of the 14 laboratories was 99.1%. In contrast, the mean coincidence rates of EpiDerm™ and SkinEthic™ in international round robin studies were 92.2% and 94.7%, respectively[2].

Table 4. Within laboratory reproducibility of the LabCyte EPI-MODEL24 SIT based on concordance of predictions for the four test materials

Lab.No.	Concordance of outcomes		
	Saline	Sesame Oil	Overall
1*	(3/4)	(4/4)	(7/8)
2	4/4 (100%)	4/4 (100%)	8/8 (100%)
3	4/4 (100%)	4/4 (100%)	8/8 (100%)
4	4/4 (100%)	4/4 (100%)	8/8 (100%)
5	4/4 (100%)	4/4 (100%)	8/8 (100%)
6	4/4 (100%)	4/4 (100%)	8/8 (100%)
7	3/4 (75%)	4/4 (100%)	7/8 (87.5%)
8	4/4 (100%)	4/4 (100%)	8/8 (100%)
9	4/4 (100%)	4/4 (100%)	8/8 (100%)
10	4/4 (100%)	4/4 (100%)	8/8 (100%)
11	4/4 (100%)	4/4 (100%)	8/8 (100%)
12	4/4 (100%)	4/4 (100%)	8/8 (100%)
13	4/4 (100%)	4/4 (100%)	8/8 (100%)
14	4/4 (100%)	4/4 (100%)	8/8 (100%)
15*	(3/4)	(4/4)	(7/8)
16	4/4 (100%)	4/4 (100%)	8/8 (100%)
average	55/56 (98.2%)	56/56 (100%)	111/112 (99.1%)

* Expired sesame oil was used in the third experiment in Lab 1 and Lab 15; therefore, the results of these two laboratories were excluded from the evaluation.

7.2.3 Between laboratory reproducibility

The BLR of the 14 laboratories that fulfilled the WLR criteria were 100% (8/8) (Table 5). On the other hand, the BLR of EpiDerm™ and SkinEthic™ RHE in the international round robin study was 92.8% and 94.6%, respectively[2] . The predictability of the LabCyte EPI-MODEL24 determination was 100% (Table 5). On the other hand, the predictability of the EpiDerm™ and SkinEthic™ RHE in the international round robin study was 95.5% and 100%, respectively.

Table 5. Between laboratory reproducibility of the LabCyte EPI-MODEL24 SIT for 16 laboratories per test material

Lab.No.	Saline															
	PVC				PVC + 10% SDS				PVC + 30% Heptanal				PVC + 5.8% Genapol X-080 (Y-4)			
	1st	2nd	3rd	prediction	1st	2nd	3rd	prediction	1st	2nd	3rd	prediction	1st	2nd	3rd	prediction
1	102.82	105.38	111.32	non-irritant	2.42	1.62	3.09	irritant	101.01	41.54	51.01	non-irritant	8.54	5.22	7.63	irritant
2	102.87	104.87	106.03	non-irritant	0.00	0.05	0.03	irritant	101.83	95.67	69.60	non-irritant	4.58	4.36	6.31	irritant
3	103.56	103.46	99.22	non-irritant	2.04	2.56	2.44	irritant	102.55	93.60	95.48	non-irritant	6.17	5.47	4.89	irritant
4	108.27	102.57	103.88	non-irritant	1.14	0.00	0.00	irritant	95.47	88.93	97.81	non-irritant	7.69	6.23	5.82	irritant
5	101.34	100.88	102.40	non-irritant	4.12	2.98	3.04	irritant	99.58	105.09	103.67	non-irritant	8.69	8.12	8.80	irritant
6	99.67	110.00	103.47	non-irritant	1.66	2.32	1.94	irritant	97.79	124.97	108.78	non-irritant	5.50	6.76	7.45	irritant
7	107.32	106.25	100.45	non-irritant	7.88	6.54	6.06	irritant	87.51	38.67	56.38	non-irritant	12.98	12.93	10.47	irritant
8	98.67	103.89	101.21	non-irritant	0.01	0.00	0.00	irritant	100.61	91.94	95.67	non-irritant	5.20	6.75	6.69	irritant
9	104.91	106.71	104.04	non-irritant	1.39	1.27	1.77	irritant	95.65	92.28	112.83	non-irritant	3.09	4.68	4.39	irritant
10	101.00	98.10	104.04	non-irritant	1.36	1.98	2.37	irritant	69.67	87.47	70.97	non-irritant	5.01	4.45	4.45	irritant
11	94.99	101.22	100.41	non-irritant	2.01	3.78	3.17	irritant	103.22	105.06	99.39	non-irritant	6.15	6.98	5.35	irritant
12	102.57	104.00	102.86	non-irritant	0.00	0.00	0.00	irritant	109.36	118.92	115.15	non-irritant	6.18	6.10	6.46	irritant
13	104.98	104.48	103.36	non-irritant	1.90	2.16	1.96	irritant	105.21	108.27	112.14	non-irritant	4.15	4.97	5.24	irritant
14	101.00	102.60	99.80	non-irritant	2.42	2.96	3.12	irritant	92.61	103.61	94.63	non-irritant	10.00	8.59	9.85	irritant
15	110.13	105.61	ND*	non-irritant	0.00	0.00	0.19	irritant	101.26	118.48	101.89	non-irritant	0.15	0.00	0.05	irritant
16	98.70	99.35	100.60	non-irritant	3.71	5.06	5.10	irritant	94.65	90.55	93.91	non-irritant	10.14	11.23	10.08	irritant
				16/16				16/16				16/16				16/16

Lab.No.	Sesame Oil															
	PVC				PVC + 10% SDS				PVC + 30% Heptanal				PVC + 5.8% Genapol X-080 (Y-4)			
	1st	2nd	3rd	prediction	1st	2nd	3rd	prediction	1st	2nd	3rd	prediction	1st	2nd	3rd	prediction
1	101.63	104.86	ND**	non-irritant	98.82	103.22	ND**	non-irritant	27.83	11.24	ND**	irritant	8.16	8.30	ND**	irritant
2	109.93	103.22	91.95	non-irritant	107.62	104.75	60.84	non-irritant	28.71	23.09	22.51	irritant	12.08	12.72	14.74	irritant
3	102.26	106.36	104.96	non-irritant	106.94	94.14	99.20	non-irritant	17.52	16.45	18.61	irritant	9.73	7.96	8.26	irritant
4	105.85	102.91	101.17	non-irritant	104.94	101.61	99.92	non-irritant	12.35	21.51	16.97	irritant	10.21	7.75	8.96	irritant
5	103.53	101.84	102.75	non-irritant	105.01	104.78	108.07	non-irritant	20.20	15.19	18.03	irritant	12.16	11.35	11.44	irritant
6	93.66	113.98	87.94	non-irritant	97.68	112.27	101.46	non-irritant	13.04	16.47	22.65	irritant	10.48	10.31	10.53	irritant
7	106.17	106.79	109.86	non-irritant	105.44	109.29	115.45	non-irritant	24.81	22.71	23.00	irritant	19.62	15.41	17.18	irritant
8	107.69	97.87	101.22	non-irritant	108.32	98.17	101.49	non-irritant	14.63	21.20	25.47	irritant	9.76	11.06	10.10	irritant
9	111.25	115.06	104.61	non-irritant	101.75	107.75	108.20	non-irritant	13.46	18.01	14.43	irritant	7.09	9.02	8.88	irritant
10	96.50	100.73	108.30	non-irritant	103.50	105.35	111.50	non-irritant	18.88	20.04	19.23	irritant	7.57	8.14	6.25	irritant
11	102.44	103.24	100.15	non-irritant	108.61	103.54	102.55	non-irritant	14.29	15.06	18.07	irritant	8.78	10.20	9.31	irritant
12	97.98	110.70	106.70	non-irritant	104.90	105.08	102.91	non-irritant	15.33	18.96	19.47	irritant	9.43	11.89	9.18	irritant
13	101.59	106.48	109.15	non-irritant	103.37	105.27	111.85	non-irritant	18.05	16.30	18.61	irritant	7.33	8.53	8.78	irritant
14	107.87	115.06	107.84	non-irritant	107.76	107.75	101.76	non-irritant	15.17	18.01	22.13	irritant	12.32	9.02	10.18	irritant
15	114.17	111.88	ND**	non-irritant	118.56	90.92	ND**	non-irritant	18.16	0.00	ND**	irritant	0.00	0.00	ND**	irritant
16	102.44	100.65	108.51	non-irritant	98.89	104.13	106.14	non-irritant	28.10	17.30	33.09	irritant	12.80	13.88	13.42	irritant
				16/16				16/16				16/16				16/16

Lab 1 and Lab 15 were excluded from the evaluation because they did not meet the within laboratory reproducibility criteria.

* SD of the third saline extract of PVC in Lab 15 was 20.08.

** Expired sesame oil was used in the third experiment in Lab 1 and Lab 15.

7.3 Reliability of test method

The assessment of trial reliability was based on data from all trials conducted in the 16 laboratories. The results showed a sensitivity, specificity, and accuracy of 100 %, 98.9%, and 99.5 %, respectively. In contrast, the overall accuracy of EpiDerm™ and SkinEthic™ RHE in the international round robin study was 97. 4%[2)].

Table 6. Sensitivity, specificity and accuracy on MTT assay vs classification

Lab.No.	Saline						Sesame Oil						Overall					
	Sensitivity		Specifity		Accuracy		Sensitivity		Specifity		Accuracy		Sensitivity		Specifity		Accuracy	
1*	6/6	100%	5/6	83.3%	11/12	91.7%	4/4	100%	4/4	100%	8/8	100%	10/10	100%	9/10	90.0%	19/20	100%
2	6/6	100%	6/6	100%	12/12	100%	6/6	100%	6/6	100%	12/12	100%	12/12	100%	12/12	100%	24/24	100%
3	6/6	100%	6/6	100%	12/12	100%	6/6	100%	6/6	100%	12/12	100%	12/12	100%	12/12	100%	24/24	100%
4	6/6	100%	6/6	100%	12/12	100%	6/6	100%	6/6	100%	12/12	100%	12/12	100%	12/12	100%	24/24	100%
5	6/6	100%	6/6	100%	12/12	100%	6/6	100%	6/6	100%	12/12	100%	12/12	100%	12/12	100%	24/24	100%
6	6/6	100%	6/6	100%	12/12	100%	6/6	100%	6/6	100%	12/12	100%	12/12	100%	12/12	100%	24/24	100%
7	6/6	100%	5/6	83.3%	11/12	91.7%	6/6	100%	6/6	100%	12/12	100%	12/12	100%	11/12	91.7%	23/24	100%
8	6/6	100%	6/6	100%	12/12	100%	6/6	100%	6/6	100%	12/12	100%	12/12	100%	12/12	100%	24/24	100%
9	6/6	100%	6/6	100%	12/12	100%	6/6	100%	6/6	100%	12/12	100%	12/12	100%	12/12	100%	24/24	100%
10	6/6	100%	6/6	100%	12/12	100%	6/6	100%	6/6	100%	12/12	100%	12/12	100%	12/12	100%	24/24	100%
11	6/6	100%	6/6	100%	12/12	100%	6/6	100%	6/6	100%	12/12	100%	12/12	100%	12/12	100%	24/24	100%
12	6/6	100%	6/6	100%	12/12	100%	6/6	100%	6/6	100%	12/12	100%	12/12	100%	12/12	100%	24/24	100%
13	6/6	100%	6/6	100%	12/12	100%	6/6	100%	6/6	100%	12/12	100%	12/12	100%	12/12	100%	24/24	100%
14	6/6	100%	6/6	100%	12/12	100%	6/6	100%	6/6	100%	12/12	100%	12/12	100%	12/12	100%	24/24	100%
15*,#	6/6	100%	5/5	100%	11/11	100%	4/4	100%	4/4	100%	8/8	100%	10/10	100%	9/9	100%	19/19	100%
16	6/6	100%	6/6	100%	12/12	100%	6/6	100%	6/6	100%	12/12	100%	12/12	100%	12/12	100%	24/24	100%
Mean	96/96	100%	93/95	97.9%	189/191	99.0%	92/92	100%	92/92	100%	184/184	100%	188/188	100%	185/187	98.9%	373/375	99.5%

* Lab 1 and Lab15 excluded the results of the third trial in which sesame oil had expired.

The test result of the third PVC saline extraction in Lab15 was excluded, as the standard deviation of cell viability was 20.08.

7.4 Additional investigations

7.4.1 Study of the concentration of SDS as a positive control

SDS (1%) was used as a positive control (average cell viability < 40%) in the international round robin study; therefore, 1% SDS is also listed as a positive control in ISO 10993-23. However, because the RhE tissue was excessively damaged and could not retain its morphology after 18 h of exposure using this method, in addition to 1%, 0.5%, and 0.3% SDS treatments were examined with the aim of exploring lower concentrations that would satisfy the criteria for a positive control. The results showed that 0.3% SDS had the same cell viability as 1% SDS, as shown in Figure 2. In conclusion, the committee considers that the LabCyte EPI-MODEL24 SIT is not hindered by changing the positive control to 0.3% SDS.

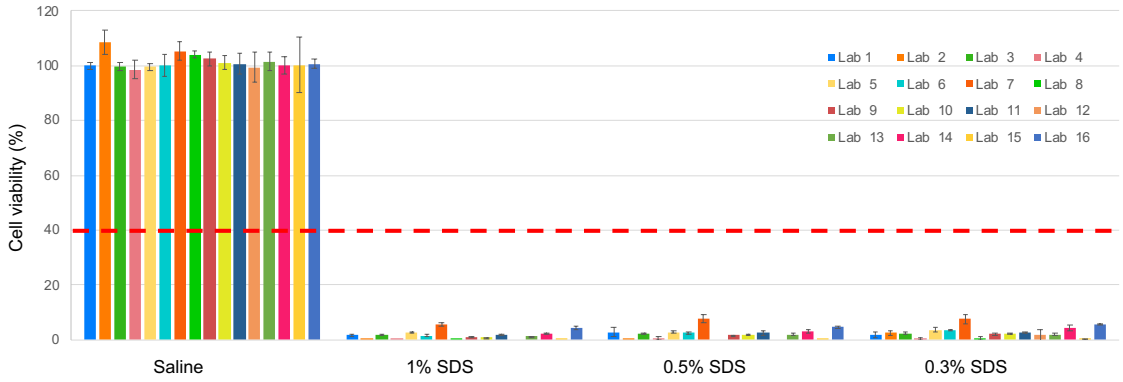

Figure 2. Cell viability obtained from LabCyte model exposed to various SDS solution

7.4.2 Comparison of sesame oil performance in European Pharmacopoeia and Japanese Pharmacopoeia

The international round robin study used sesame oil (Cat No. 850667) manufactured by Sigma, a European Pharmacopoeia (EP) product. However, as this was a domestic study, a comparative study was conducted with sesame oil manufactured by Kozakai Pharmaceutical Co., Ltd., a Japanese Pharmacopoeia (JP) product. As shown in Figure 3 and Table 7, there were no significant differences in cell viability between the test samples. Therefore, the committee considers that LabCyte EPI-MODEL24 SIT is not hindered by the use of either European or Japanese Pharmacopoeia sesame oil as the extraction solvent.

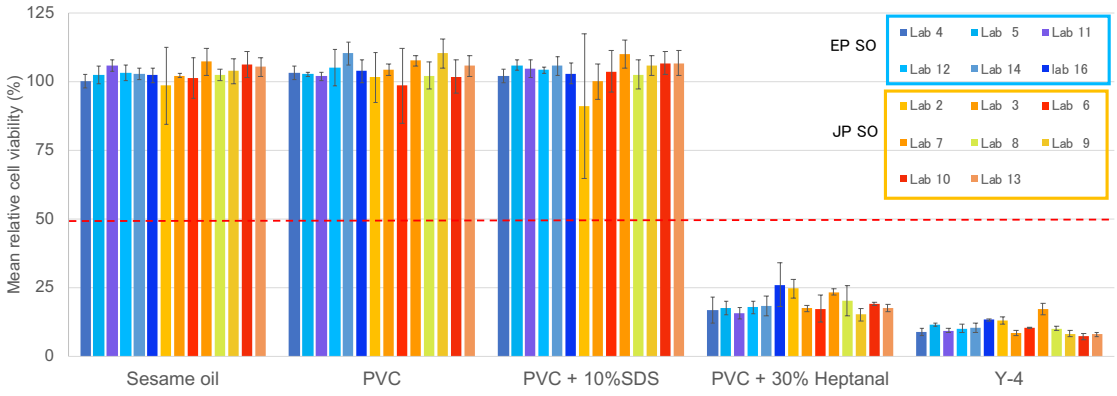

Figure 3. Cell viability obtained from LabCyte EPI-MODEL 24 exposed to extracts of polymer samples in EP or JP.

Table 7. Summary of mean relative cell viability, including the SD as a quantitative measurement

Test sample	Mean of cell viability (%)	
	EP SO	JP SO
Sesame oil	102.9 ± 1.8	103.4 ± 2.9
PVC	104.5 ± 3.0	104.1 ± 3.8
PVC + 10%SDS	104.4 ± 1.5	103.4 ± 5.8
PVC + 30% Heptanal	18.8 ± 3.7	19.5 ± 3.3
Y-4	10.7 ± 1.6	10.5 ± 3.3

7.5 Quality checks

The records manager reviewed all record forms, inquired about any uncertainties, and used only mutually confirmed data for analysis. The data quality was assured (Appendix 5).

8. Consideration

8.1. Views of the committee on the round robin Phase II study

The results of the LabCyte EPI-MODEL24 SIT round robin phase II study showed that the reproducibility, accuracy, and predictivity of the method fulfilled the performance criteria of the committee and were comparable to those of the international round robin study in ISO/TC 194/WG 8. Based on these results, the committee considers that the LabCyte EPI-MODEL24 SIT can be used to detect irritants in medical device extracts in the same manner as the other two methods using the RhE model.

8.2. Views of the committee on additional studies

This committee reaches the following conclusions:

1) 0.3% SDS can be used as a positive control for LabCyte EPI-MODEL24 SIT

2) Both European and Japanese Pharmacopoeia sesame oils are available for the extraction solvent.

8.3. Comparison with previous reports

The performance of LabCyte EPI-MODEL24 was comparable to that of EpiDerm™ and SkinEthic™ RHE, as validated in an international round robin study.

8.4 Overall conclusion

The objective of this Phase II study was to assess the within laboratory reproducibility, and between-laboratory reproducibility of the LabCyte EPI-MODEL24 SIT.

The committee for the LabCyte EPI-MODEL24 round robin Phase II study concluded that LabCyte EPI-MODEL24 SIT is a highly reproducible, accurate, and predictive method to assess irritant activity in medical device extracts.

Acknowledgements

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