Appendixes

1. LabCyte EPI-MODEL24 Round Robin Phase II Plan_ver.2.3
2. 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
3. LabCyte EPI-MODEL24 Round Robin Phase I study (Transferability Study) report
4. All data of all participating laboratories of LabCyte EPI-MODEL24 Round Robin Phase II
5. LabCyte EPI-MODEL24 Round Robin Phase II QC results report (in Japanese)

Appendix 1

LabCyte EPI-MODEL24 Round Robin Phase II Plan_ver.2.3

Appendix 1

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

Version 2.3 December 22, 2023
Version 2.2 April 5, 2023
Version 2.1 August 23, 2022

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

Contents

1. Background

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 guidance. LabCyte EPI-MODEL24, manufactured and marketed by J Japan Tissue Engineering Co., Ltd., 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 round robin study was conducted in 16 laboratories under non-Good Laboratory Practice conditions to verify the applicability of the model for assessing irritation caused by medical devices.

The RhE test method takes advantage of the fact that when keratinocytes are stimulated by an irritant, cells are damaged, cell viability is reduced, and cell viability is used as an indicator to assess irritation. The LabCyte EPI-MODEL24 tissues are incubated with either a positive control (1% SDS) or test sample extracts (saline or sesame oil extract). After 18 hours, the test solution is washed off and cell viability is determined by the MTT assay to determine whether the cells are stimulated or not.

In this study, 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.

The aim of the Phase I study is to transfer the technology of this method 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 study committee

3.1 Round robin study committee

The following organization will be established as a round robin study committee to carry out this round robin study. During the Round Robin Study, J-TEC and the participating laboratories will exchange a Memorandum of Understanding.

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

3.2 Participating laboratories

Participating laboratory 1: TRANS GENIC INC.

Principal Investigator: Akiko Yamaguchi

Participating laboratory 2: SB-KAWASUMI LABORATORIES, INC.

Test Director: Natsuki Yoshida

Participating laboratory 3: Mediford Corporation

Principal Investigator: Kinuko Zaizen

Participating laboratory 4: Olympus Medical Systems Corp.

Study Director: Kazunori Madate
Participating laboratory 5: Chemicals Evaluation and Research Institute, Japan
Principal Investigator: Masaru Suzuki
Participating laboratory 6: Safety Research Institute for Chemical Compounds Co., Ltd.
Study Director: Wataru Shimatani
Participating laboratory 7: Kamakura Techno-Science, Inc.
Principal Investigator: Yuko Naruke
Participating laboratory 8: CMIC Pharma Science Co.,Ltd.
Principal Investigator: Yoshiyuki Suzuki
Participating laboratory 9: Food and Drug Safety Center Hatano Research Institute
Study Director: Shigehiro Tachibana
Participating laboratory 10: Terumo Corporation
Study Director: Ryouhei Asou
Participating laboratory 11: Nissei Bilis Co.,Ltd.
Study Director: Hiroki Ohnaka
Participating laboratory 12: NIPRO CORPORATION
Principal Investigator: Yoichi Kato
Participating laboratory 13: Japan Food Research Laboratories r
Principal Investigator: Takashi Mishima
Participating laboratory 14: Nihon Bioresearch Inc.
Principal Investigator: Yasushi Yamada
Participating laboratory 15: BoZo Research Center Inc.
Principal Investigator: Takayuki Fukuda
Participating laboratory 16: Drug Safety Testing Center Co.,Ltd.
Principal Investigator: Koji Nakano

Of all participating laboratories, only representatives of the participating laboratories are included in the round robin study committee. The study will be conducted under non-GLP.

3.3 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 the LabCyte EPI-MODEL24 round robin study reports.

3.4 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 are organized within the committee.

3.5 Test sample management group

Test samples are selected, prepared, and distributed in consultation with the lead laboratory, test sample distributors, and experts, according to the objectives of Phase II.

3.6 Statistical analysis Group

The statistician and DMD will be responsible for data management and statistical.

3.7 Records management group

JaCVAM and DMD will review and archive all the test records.

3.8 Sponsor

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

4. Protocol

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 was prepared by the Lead lab will be used. It will have been revised in consultation with the committee as necessary.

5. Test sample

This Phase II study uses test samples of the sizes listed in Table 2, 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 are used as test samples. Irritant materials were selected based on their reactivity, balance of properties, and cost.

Test samples will be prepared by DMD, coded, and distributed to participating laboratories.

Table 2. Summary of the study design in each phase study in the LabCyte EPI-MODEL24 Round Robin Phase II Study

research	Test sample	Number of trails	Information to be obtained
Phase II	4 coded	3	Within and between laboratory reproducibility

6. Process and success criteria

6.1 Training

The lead laboratory will schedule a date for all participating sites to meet and conduct training on this assay. This training will be conducted using video.

6.2 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 4). The committee concluded that a series of technology transfers, including extraction operations, had been completed on the basis of these results.

6.3 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 set at 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 number of test solutions was used as the denominator for the concordance rate. The numerator of the concordance rate was calculated using the following method:

1) When all three judgements for each test solution were unanimous, the final judgement of the test solution was considered to be concordant.

2) The number of the test solutions judged as concordant by the procedure 1) was used as the numerator of the concordance rate.

6.4 Between laboratory reproducibility (BLR)

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

The number of test solutions was used as the denominator for the concordance rate. 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 concordant with the test solution if more than 85% of the final judgements of the participating laboratories matched (e.g., 14 out of 16 laboratories).

3) The number of the test solutions judged as concordant by the procedure 2) was used as the numerator of the concordance rate.

7. Records

The lead laboratory will provide the data and the recording forms to verify the process of the experiment. All raw data and completed record sheets must be submitted to JaCVAM.

8. Experiment duration: See Table 3.

Table 3. Overview of LabCyte EPI-MODEL24 Round Robin Phase II Study

months and years	activity
April 2022	Round Robin Study Committee established
May 2022	First Round Robin Study Committee Meeting / Phase II Research Plan
Phase II study for confirming within and between reproducibility	
June 2022	Phase II study briefing / Discussion, decision, and reading through of research plan proposal
Late July to early August 2022	The four test samples were coded and distributed.
August 2022	Start of Phase II study
December 2022	Phase II study completed
February 2023	Second Round Robin Study Committee Meeting / Phase II Results
August 2023	Completed Round Robin Research Report

Abbreviation

DMD: Division of Medical Devices

GLP: Good Laboratory Practice

JaCVAM: Japanese Centre for the Validation of Alternative Methods

J-TEC: Japan Tissue Engineering Co.

MTT: 3-[4, 5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide

OECD: Organisation for Economic Co-operation and Development

RhE: Reconstructed Human Epidermis

SDS: Sodium Dodecyl Sulfate

TG: Test Guideline

Appendix 1

Revision log

Rev.	Changes	Date
Ver. 2.1	First edition	August 23, 2022
Ver. 2.2	(1) Unified notation (2) Correction of errors	April 5, 2023
Ver. 2.3	Correct the date in (1) (2) Correct the ver. on the cover page. (3) Unified notation (4) Change in the person responsible for the study who was originally registered (Declared before the test was conducted)	December 22, 2023

Appendix 2

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

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

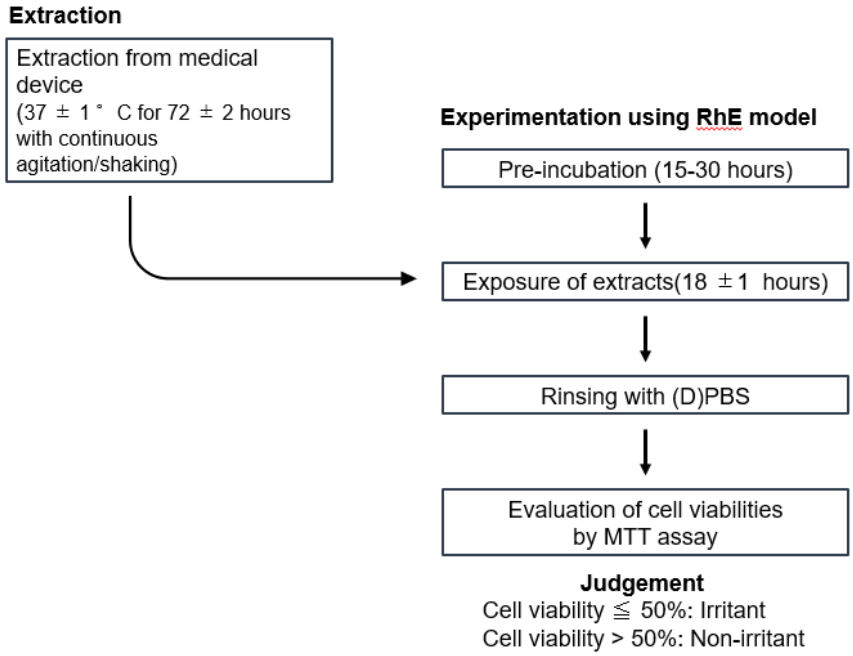
August 17th 2022

TABLE OF CONTENTS

1. OBJECTIVE

This SOP is for the protocol of Round Robin Study (RRS) in Japan for Skin Irritation Testing of Medical Device Extracts Using Cultured Epidermal Model LabCyte EPI MODEL24.

1.1 OUTOLINE OF PROTOCOL

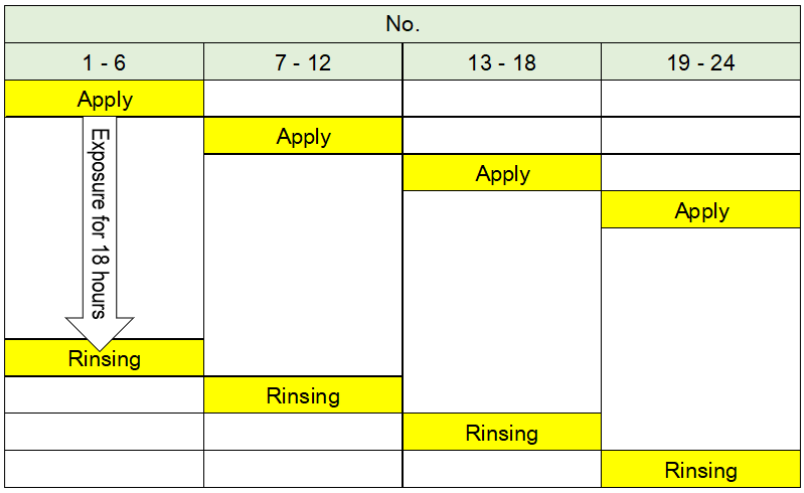


1.2 EXAMPLE WORK SCHEDULE

	Monday (Day 1)	Tuesday (Day 2)	Wednesday (Day 3)	Thursday (Day 4)	Friday (Day 5)
	Shipping of EPI-MODEL24	Delivery of EPI-MODEL24 (Store at 15-25℃)			Prepare MTT medium and pre-warm at 37 ± 1℃
10:00 10:30					Rinsing and collection of culture medium
11:00 11:30					Start MTT assay (3 hours ± 5 min)
12:00 12:30					
13:00 13:30	Place sesame oil at RT				
14:00 14:30			Pre-warm the assay medium at 37℃ ± 1℃	Prepare 0.3, 0.5 and 1 % of SDS	Formazan extraction (shaking at RT more than 2 hours)
15:00 15:30	Add the vehicle to the test substances				
16:00 16:30	Start extraction (37℃ ± 1℃, 72 ± 2hours)		Start pre-incubation (15-30 hours)	Application of extracts (18 ± 2hours)	$OD_{570-650}$
17:00 17:30					

1.3 RINSING PROCEDURE

A. Rinsing by pipetting



- Perform rinsing procedure per 6-well unit.
- Perform rinsing of 2nd plate as well as first plate.

B. Rinsing by wash bottle

Time	No.						
	1	2	3	・・・・・	22	23	24
0min	Apply						
1min		Apply					
2min			Apply				
・ ・ ・							
21min					Apply		
22min						Apply	
23min							Apply
24min							
	Exposure for 18 hours						
18h 0min	Rinsing						
18h 1min		Rinsing					
18h 2min			Rinsing				
・ ・ ・							
18h 21min					Rinsing		
18h 22min						Rinsing	
18h 23min							Rinsing

- Above indicates the rinsing schedule for one plate. Perform rinsing of 2nd plate as well as first plate.
- The time interval is set as 1-3 minutes.

2. MATERIALS

2.1 SKIN IRRITATION TEST KIT

Components of Skin Irritation Test kit are shown in Table 1.

Table 1 - LabCyte EPI-MODEL24 Kit Components

Component	Qty	Description
LabCyte EPI-MODEL24 plate	1 plate	Contains 24 culture inserts with tissues fixed in nutritive agar medium for transport (usable area: 0.3cm^2).
Assay Medium	2 bottles	Basic medium for incubation (30mL). Store at refrigeration temperature.
24-well plate	4 plates	Blank plate for use in assay. Store at room-temperature.

2.2 SHIPMENT OF LabCyte EPI-MODEL24

LabCyte EPI-MODEL24 is packed in a transportation box and delivered. After delivered, please check that all kit components (LabCyte EPI-MODEL24 plate, assay medium, and 24-well assay plates) are included in the transportation box. Verify lot numbers and expiration dates. Record details in the Methods Documentation Sheet (MDS) 3.

2.3 INSTRUCTIONS FOR USE OF LabCyte EPI-MODEL24

Start the incubation of the culture inserts soon after opening the package. Do not store the culture inserts again after opening the aluminum package.

The human epidermis cells used in LabCyte EPI-MODEL24 originate from a healthy donor and are HIV-, HBV-, and HCV-negative. However, handle them with enough care and in accordance with the laboratory biosafety guidelines since they contain raw materials of human origin.

2.4 CONSUMABLES

The following consumables are required.

Items	Usage and Recommended Quantity
Sesame oil	Approximately 30 mL (for one test) as the vehicle for medical device extraction. Use Sigma Aldrich #85067 or Japanese Pharmacopoeia
Saline	Approximately 30 mL (for one test) as the vehicle for medical device extraction. Use Otsuka Pharmaceutical 10095-3
SDS	Use as a positive control. 20% SDS solution will be provided to participating facilities.
DPBS (negative control)	Used as a negative control. The negative control DPBS will be delivered to participating facilities.
DPBS (rinsing solution)	Use for rinsing. Prepare approximately 2 liters (for 2 plates) at the participating facility.
Sterile cotton bud	Use for wiping the culture cups after rinsing. 48 pieces (for 2 plates)
MTT*	Use for measuring cell viability. 16 mg (for 2 plates).
Isopropanol	Use for extraction after the MTT reaction. Approximately 24 mL (for 2 plates)
96-well plate*	Use for absorbance measurement with a microplate reader.

* Available as separate items.

MTT reagent (25 mg), item number: 403026

96-well measurement plate (1 piece), item number: 406096

24-well plate (1 piece), item number: 353047

2.5 OTHERS

2.5.1 EQUIPMENT / INSTRUMENTS

- Safety cabinet (or clean bench)
- CO_2 incubator (37 ± 1 °C, 5% CO_2, capable of maintaining high humidity)

- Autoclave
- 96-well multi-plate reader (required filters: 570 nm, 650 nm)
- Precision balance (capable of accurately measuring 1 mg or more)
- Timer
- Adjustable micro-pipette (10-200 µL, 200-1000 µL)
- Adjustable positive displacement Pipette (10-200 µL, 200-1000 µL)
- Sharp-edged forceps (sterile)
- Micro spatula (sterile)
- Beaker (1~2L) Electronic pipette controller
- Sterilizable poly wash bottle (500~1000mL: sterile)

2.4.2 CONSUMMABLE ITEMS

- Micro-pipette tips for micro pipette and positive displacemnet pipette (sterile: 10-200 µL, 200-1000 µL)
- Serological pipettes (sterile: 10 mL, 25 mL)
- Micro-tubes (1.5mL)
- Scalpel (KEISEI MEDICAL INDUSTRIAL: Keisei Scalpel 11A)

3. TEST METHOD

*Perform operations in Section 3.1.1~3.1.6 and Section 3.2.1~3.2.2 aseptically in a safety cabinet (or clean bench).

*Operations other than above do not need to be performed with aseptic techniques. For these operations, refer to **Section 2.1.3 INSTRUCTIONS FOR USE OF LabCyte EPI-MODEL24**

3.1 PREPARATIONS

3.1.1 POSITIVE CONTROL SUBSTANCE (1% SDS (v/v) solution)

(1) Mix 100 µL of 20% SDS solution with 1.9 mL of saline and vortex thoroughly.

(2) Dilute 1 % of SDS as below to prepare 0.5 and 0.3 % solutions.

0.5 % SDS (v/v)

1 % SDS 500 μ L

Saline 500 μ L

0.3 % SDS (v/v)

1 % SDS 300 μ L

Saline 700 μ L

(3) Record details of steps (1) and (2) above in the MDS 4-1.

* Store in a dark, cold place and use it within 24 hours.

3.1.2 NEGATIVE CONTROL SUBSTANCE

(1) Use the provided DPBS as negative control.

3.1.3 VEHICLES SUBSTANCE

(1) The vehicle controls (saline and Sesame oil) should be kept in amber glass vial extraction vessels and subjected to the same extraction procedure (72 ± 2hours at 37 ± 1°C) adopted for the medical device extraction.

(2) Refer ISO 10993-12 extraction procedure.

3.1.4 PREPARATION OF MEDICAL DEVICE EXTRACTS

(1) Document the information on the test substances/medical devices in the table of **Annex 1**: Characterization of test substances.

(2) The preparation of device extracts is done according to ISO 10993-12 guidelines and is based on surface area to volume ratios described in this standard.

(3) Polar extracts are prepared in saline.

(4) Non-polar extracts are prepared in sesame oil.

(5) Extraction is conducted at (37 ± 1) °C for (72 ± 2) hours.

(6) Store extracted solution at room temperature and use within 24 hours after the extraction.

3.1.5 POLY WASH BOTTLE FILLED WITH (D)PBS FOR RINSING

(1) For rising with poly wash bottle, fill the poly wash bottle with (D)PBS.

3.1.6 MTT MEDIUM

(1) Dissolve MTT in the assay medium to prepare the MTT medium (final concentration: 1.0 mg/mL)

Use an ultrasonic cleaner equipment or a vortex mixer, as necessary, in order to completely dissolve the MTT powder. Keep the solution in a dark, cold place and use it within 24 hours in the case of ready-to use.

*Stocking condition: Under -20 °C for 6 months.

(2) Record details of step (1) above in the MDS 4-2.

3.2 EXECUTION OF THE TEST

3.2.1 PREPARATION OF LabCyte EPI-MODEL24

(1) Pre-warm the assay medium to 37 ± 1°C for 30 minutes using a water bath.

(2) Dispense 500 µL of pre-warmed assay medium into each well of 24-well assay plate(s).

(3) Take out the LabCyte EPI-MODEL24 from aluminum package.

(4) Take the plate lid of LabCyte EPI-MODEL24 out and pick up the culture inserts using sterile forceps.

*Do not touch the epidermis surface of culture inserts.

*Use forceps to remove agar medium sticking to the outside of the culture inserts.

(5) Using sterile forceps, transfer the culture inserts to the 24-well assay plate(s) pre-filled with assay medium.

*Avoid trapping air bubbles under the tissue inserts.

(6) Place the plate (lid on) in a CO_2 incubator.

(7) Incubate overnight (15-30 hours) before proceeding to Section 3.2.2 “APPLICATION OF EXTRACTS AND RINSING.”

(8) Record details of steps (1) – (7) above in the MDS 5-1.

3.2.2 APPLICATION OF EXTRACTS AND RINSING

3.2.2.1 PREPARATION FOR EXTRACTS

(1) Pre-warm the assay medium to 37 ± 1°C for 30 minutes using a water bath.

(2) Dispense 500 µL of pre-warmed assay medium into each well of 24-well assay plate(s).

(3) Put the lid on the 24-well assay plate(s) and proceed to Section **3.2.2.2 "APPLICATION OF TEST** EXTRACTS**"**.

(4) Record details of steps (1) – (3) above in the MDS 5-2.

3.2.2.2 APPLICATION OF EXTRACTS

(1) Take the pre-incubated plate(s) from the CO_2 incubator.

(2) Apply 100 µL of the undiluted medical device extracts, negative control, positive control and vehicle control on the surface of tissues (inside of culture inserts) as below plate layout. Use 3 tissues per extract (N=3). For concentration study of the positive control, apply 0.3 and 0.5 % of SDS to the corresponding wells.

*Check the extracts spread over entire of tissues.

*Tilt the plate to spread extracts throughout entire tissues if necessary.

【Plate layout】
Place the provided substances at each laboratories.

PLATE 1

1 ----------	2 NC (DPBS)	3 ----------	4 ----------	5 VC-1	6 ----------	Saline Extracts
7 ----------	8 A111-1	9 ----------	10 ----------	11 A222-1	12 ----------	
13 ----------	14 A333-1	15 ----------	16 ----------	17 A444-1	18 ----------	
19 ----------	20 PC (1 % SDS)	21 ----------	22 ----------	23 0.5 % SDS	24 ----------	

PLATE 2

1 ----------	2 NC (DPBS)	3 ----------	4 ----------	5 VC-2	6 ----------	Sesame oil Extracts
7 ----------	8 A555-2	9 ----------	10 ----------	11 A666-2	12 ----------	
13 ----------	14 A777-2	15 ----------	16 ----------	17 A888-2	18 ----------	
19 ----------	20 PC (1 % SDS)	21 ----------	22 ----------	23 0.3 % SDS	24 ----------	

NC: Negative control
PC: Positive control
VC: Vehicle control

As an example, code numbers are listed. On the MDS 5-2, please fill in the code names of the provided test substances.

(3) Transfer the culture inserts applied medical device extracts to each wells of the 24-well assay plate(s) prepared in section 3.2.2.1.

*Avoid trapping air bubbles under the culture inserts.

(4) Put on the lid of the 24-well assay plate(s) and place it in a CO_2 incubator.

(5) Incubate for 18 ±1 hours.

(6) Record details of steps (1) - (5) above in the MDS 5-2.

3.2.2.3 PREPARATION FOR AFTER-RINSE CONDITIONING

(1) Pre-warm the assay medium for 30 minutes to 37 ± 1°C using a water bath.

(2) Dispense 500 µL of pre-warmed assay medium into each well of 24-well assay plate(s) for transferring rinsed insert cups.

(3) Take out the plate with exposed test solution from the CO2 incubator after 18 ± 1 hours and transfer the culture inserts to the plate prepared in (2).

(4) After transferring the culture cups, place the 24-well plates on ice or in a refrigerator until the culture medium is collected.

(5) Put on the lid of 24 well assay plate(s) and proceed to Section **3.2.2.4 "REMOVAL OF THE EXTRACTS"**.

(6) Record details of steps (1) – (3) above in the MDS 5-3.

3.2.2.4 REMOVAL OF EXTRACTS

A. Rinsing by using wash bottle.

(1) After exposure of extracts for 18 ± 1 hours, pick up a culture insert with sterile forceps. Discard extracts onto the tissue by tilting and then tapping the insert on the beaker.

(2) Fill the culture insert with (D)PBS using a PBS filled poly wash bottle. Flow (D)PBS stream from the washing bottle against the side-wall of the culture insert and wash on the tissue surface by the PBS current (Photo 1).

Photo 1 - Rinsing by poly wash bottle

Attention: Avoid flowing the (D)PBS stream on the tissue surface directly. Be careful not to damage the tissue surface.

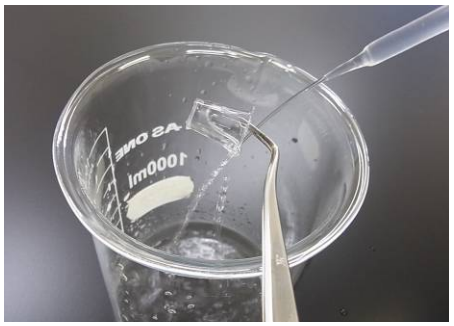
(3) Remove the PBS from inside of the culture inserts by tapping on the beaker (Photo 2).

(4) Repeat steps (2) and (3) at least 10 times or more as much as possible and remove all residual extracts on the tissue surface almost completely.

(5) Gently wipe the leftover PBS inside and outside of the culture inserts by using a sterile cotton bud (Photo 3).

*Wipe the inside of cell culture insert to remove PBS almost completely.

Photo 2 - Discarding the PBS



Transfer the rinsed culture inserts to the corresponding wells in the 24-well assay plate(s) prepared in section 3.2.2.3 (2).

*Avoid trapping air bubbles under the culture inserts.

Photo 3 - Wipe the cell culture insert

(6) Repeat steps (1) ~ (7) for all the culture inserts at 1~3-minute intervals.

(7) Record details of steps (1) – (4) above in the MDS 5-4.

(8) After the rinsing step, proceed to Section 3.2.3.2 “MTT ASSAY”.

B. Rinsing by pipetting

(1) After the substance application period (18 ± 1 hours), carefully remove the test substances from the culture inserts using micro pipette.

(2) Apply 0.5 mL/well of (D)PBS to the tissue surface.

(3) Remove (D)PBS from inside of cell culture insert by using micro pipette.

(4) Repeat steps (2) ~ (3) at least 10 times for all the culture inserts to remove extracts almost completely from tissues.

(5) Transfer the rinsed culture inserts to the corresponding wells in the 24-well assay plate(s)prepared in section 3.2.2.3 (2).

*Avoid trapping air bubbles under the culture inserts.

(6) Record details of steps (1) – (4) above in the MDS 5-4.

(7) After the rinsing step, proceed to Section 3.2.3.2 “MTT ASSAY”.

3.2.2.5 INFLAMATORY CYTOKINES MEASUREMENT (OPTIONAL)

For inflammatory cytokines measurement, collect medium after exposure of 18 ± 1 hours. Medium can be stored in a freezer at ≤ -20 °C. (If you have a medical freezer, please use that.) If you cannot proceed to the MTT reaction immediately, such as when only one operator is available, collect the samples during the 3-hour MTT reaction as described in section 3.2.3.2. Note that the samples can be stored for up to 12 months.

3.2.3 MTT ASSAY

3.2.3.1 PREPARATION FOR MTT ASSAY

(1) Pre-warm MTT medium to 37 ± 1°C for 30 minutes using a water bath.

(2) Dispense 300 µL of pre-warmed MTT medium into each well of 24-well assay plate(s).

(3) Put the lid on the assay plate and place it in the CO_2 incubator until the start of the MTT assay.

(4) Record details of steps (1) – (3) above in the MDS 4-2.

3.2.3.2 MTT ASSAY

(1) After rinsing procedure completed, transfer cell culture inserts to 24-well assay plate(s) dispensed with MTT medium. Before transfer cell culture insert, absorb the assay medium on the outside of cell culture inset into paper rag to remove remaining assay medium

*Avoid cross contamination of assay medium dropping from outside of cell culture inset when transferred cell culture inserts to 24-well assay plate(s).

*Avoid trapping air bubbles under the culture inserts after cell culture transferred.

(2) Put the lid on cell culture transferred 24-well assay plate(s) and place the plate(s) in the CO_2 incubator.

(3) Incubate for 3 hours ± 5min.

(4) Record details of steps (1) – (3) above in the MDS 5-5.

3.2.4 FORMAZAN EXTRACTION AND MEASUREMENT

3.2.4.1 FORMAZAN EXTRACTION

(1) After MTT reaction for 3 hours ± 5min, take out the 24-well assay plate(s) containing the culture inserts in MTT medium from the CO_2 incubator.

(2) Take off the lid of the 24-well assay plate(s) and pinch the cultured epidermis from each culture insert with forceps.

→ Photo 4

*Use a micro spatula to scratch up the epidermis, or a scalpel to cut the membrane filter on the base of the culture insert if the cultured epidermis cannot be pinched due to damage from a test substance.

Photo 4 - Detachment of epidermis

(3) Transfer the epidermis tissue into a 1.5mL micro-tube.

(4) Add 500 µL of isopropanol to the micro-tubes and immerse the entire epidermis tissue in the isopropanol.

(5) To extract formazan, shaking the micro-tube for at least 2 hours at room temperature or place in dark overnight in order to extract pigments completely.

*Tighten the micro-tube seal.

*Periodically shaking the micro-tubes will contribute to a more efficient extraction.

(6) Shake the micro-tubes to mix the solution.

*If epidermis tissue fragments are suspended, wait until they sediment or gently centrifuge them (if a centrifuge is available).

Record details of steps (1) – (6) above in the MDS 5-6.

(7) Fill two wells of a 96-well plate with 200 µL, each, of the solution from each micro-tube.

*Eight wells of 200 µL of isopropanol should be set as a blank.

*Figure 1 shows an example of sample allocation in a 96-well plate.

Record details of step (7) above in the MDS 5-7.

Figure 1 – Allocation for a 96-well plate

PLATE 1: Saline Extracts

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK				
B	NC ① (DPBS)	VC ① (Saline)	A111-1 ①	A222-1 ①	A333-1 ①	A444-1 ①	PC ① (1% SDS)	0.5 % SDS ①				
C												
D	NC ② (DPBS)	VC ② (Saline)	A111-1 ②	A222-1 ②	A333-1 ②	A444-1 ②	PC ② (1% SDS)	0.5 % SDS ②				
E												
F	NC ③ (DPBS)	VC ③ (Saline)	A111-1 ③	A222-1 ③	A333-1 ③	A444-1 ③	PC ③ (1% SDS)	0.5 % SDS ③				
G												
H												

PLATE 2: Sesame oil Extracts

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK				
B	NC ① (DPBS)	VC ① (Saline)	A555-2 ①	A666-2 ①	A777-2 ①	A888-2 ①	PC ① (1% SDS)	0.3 % SDS ①				
C												
D	NC ② (DPBS)	VC ② (Saline)	A555-2 ②	A666-2②	A777-2 ②	A888-2 ②	PC ② (1% SDS)	0.3 % SDS ②				
E												
F	NC ③ (DPBS)	VC ③ (Saline)	A555-2 ③	A666-2 ③	A777-2 ③	A888-2 ③	PC ③ (1% SDS)	0.3 % SDS ③				
G												
H												

(8) Measure the absorbance values at 570 nm and 650 nm using a plate reader.

(9) In this RRS, you can obtain the calculation results by entering the measured values into the provided Excel sheet.

(10) Record details of steps (1) – (7) above in the MDS 5.7

For your reference, the following is the method for calculating viability. Measure the absorbance at 570 nm and 650 nm using a 96-well multi-plate reader, and use the value obtained by subtracting the absorbance at 650 nm from the absorbance at 570 nm.

$$\text{Measured OD} = [570\text{ nm OD}_{sample} - 570\text{ nm OD}_{blank}] - [650\text{ nm OD}_{sample} - 650\text{ nm OD}_{blank}]$$

$$\text{Cell Viability (\%)} = \frac{\text{Mean of measured } OD_{sample}}{\text{Mean of measured } OD_{NC}} \times 100$$

4. ASSESSMENT

4.1 CONDITIONS FOR A SUCCESSFUL STUDY

The skin irritation test should be considered successful if all of the following criteria have been met.

- Tissue viability: 0.7 ≤ mean OD (A570/650) measured value for negative control ≤ 2.5.
- Positive control: mean tissue viability for 1% SDS (positive control) ≤ 40%.
- SD: SD (negative control, positive control and test substance) of tissue viability of 3 identically treated replicates ≤ 20%
- Vehicle control: 80% of tissue viability of negative control < mean tissue viability of vehicle control < 120% of tissue viability of negative control

4.2 ASSAY CRITERIA

The criteria for *in vitro* interpretation are shown below.

Tissue Viability (primary)	Classification
Tissue viability is ≤ 50%	Irritant
Tissue viability is > 50%	Non Irritant

The Methods Documentation Sheet

Test schedule (For 2 plates)

Procedure	Date (yy/mm/dd)	Plate 1 (Saline)		Plate 2 (Sesame oil)	
		Starting time	Ending time	Starting time	Ending time
Pre-incubation (15-30 hours)					
Exposure (18 ± 1 hours)					
Rinsing					
MTT assay (3 hours ± 5 min)					
Extraction (At least 2 hours)					
Measurement					

MDS 1. Equipment Check

Operator: _____

Date: _____

Manufacturer name of CO_2 incubator : _____ Model: _____

☐ The concentration of CO_2 is 5 ± 1%

☐ The temperature of incubator is 37 ± 1℃

☐ The torey in incubator is filled with water

Manufacturer name of refrigerator : _____ Model: _____

☐ The temperature of refrigerator is 5 ± 2℃

Check of micropipettes

1. Transfer each volume of distilled water to each tubes using micropipettes.
2. Confirm the consistency of the volume transferred by micropipette and weight measured by precision balance.

*Repeat 3 times above procedures and confirm the accuracy of the micropipettes

*Operator must confirm the accuracy of micropipettes before every experiments.

For micro pipette

	2 mL	900 µL	300 µL	200 µL	100 µL
1					
2					
3					
Average					
SD					
Tolerance	5%	5%	5%	5%	5%

Filled the name of micropipettes in gray columns

For positive displacement pipette

	2 mL	900 µL	300 µL	200 µL	100 µL
1					
2					
3					
Average					
SD					
Tolerance	5%	5%	5%	5%	5%

Filled the name of micropipettes in gray columns

MSD 2. The information of test samples

Operator: _____

Date: _____

- Saline

Manufacturer name of saline : _____

Lot. No. : _____

Expiration date: _____

- Sesame oil

Manufacturer name of saline : _____

Lot. No. : _____

Expiration date: _____

☐Extraction by shaker （Condition rpm）

Manufacturer name of shaker: _____ Model: _____

☐Extraction by hand mixing （Number of mixing ）

Manufacturer name of incubator: _____ Model: _____

Preparation of extracts

Name of samples	Vehicle	Volume of vehicle(ml)	Name of used incubator	Starting date _____	Ending date	Gross appearance after extraction

				Starting time	Ending time	
Vehicle control Saline	Saline					
	Saline					
	Saline					
	Saline					
	Saline					
Vehicle control Sesame oil	Sesame oil					
	Sesame oil					
	Sesame oil					
	Sesame oil					
	Sesame oil					

MSD 3. Receipt of EPI-MODEL24

Operator:

Date of receipt: _____

Lot No. _____

Expiration date: _____

Assay media: (Lot No. _____ Expired date: _____)

Defective appearance: ☐ No ☐ Yes (Outline of defective:)

MSD 4-1. Preparation of 1% SDS as positive control

Operator: _____

Preparation date:

- 1% SDS solution

20% SDS solution μL

Saline mL

MSD 4-2 .Preparation of MTT solution

Operator:

Preparation date:

Dissolve MTT in assay media to prepare 1.0mg/mL of MTT solution

(Recommend: Dissolve the 16 mg of MTT with 16 mL of assay media)

Supplier:

Lot No.:

Weight of MTT mg

Assay media mL

MSD 5-1. Pre-incubation (15-30 hr)

Operator: _____

☐ Pre-incubated the assay media at 37 ± 1 ℃ before use

Manufacturer name of incubator: _____ Model: _____

☐ Added 500 µL/well of assay media

Starting date (time): _____ (　　:　　)

Ending date (time): _____ (　　:　　)

MSD 5-2. Exposure of test sample (18 ± 1hr)

Operator: _____

☐Pre-incubated the assay media at 37 ± 1℃ before use

☐Added 500 μL/well of assay media to the 24-well assay plate prepared for exposure

☐Added 100 μL of test sample to the surface of tissues

☐ Starting incubation time of PLATE 1: _____

☐ Ending incubation time of PLATE 1: _____

☐ Starting incubation time of PLATE 2: _____

☐ Ending incubation time of PLATE 2: _____

☐Abnormal appearance is not occurred in the tissues after sample exposure for 18 ± 1 hr.

Fill in the remarks column if there is any abnormal appearance

Remarks.

MSD 5-3. Preparation before rinse

Operator: _____

☐Pre-incubated the assay media at 37 ± 1℃ before use

☐Added 500 μL/well of assay media to the assay plate prepared for rinsing

MSD 5-4. Rinsing

Operator: _____

Supplier of PBS: _____ Lot No: _____

.

☐ Rinsing by micropipette ☐ Rinsing by poly wash bottle

☐ Rinsing more than 10 times

Starting time of PLATE 1 (time): _____ (　　:　　～)

Ending time of PLATE 1 (time): _____ (～　　:　　)

Starting time of PLATE 2 (time): _____ (　　:　　～)

Ending time of PLATE 2 (time): （～ : ）

Fill in the remarks column if there are any issues. For example, the tissues are detached from culture inserts.

Remarks

5-5 MTT test （3 hours ± 5min）

Operator:

☐ Preincubated MTT solution at 37 ± 1℃ before use

☐ Added 300 μL/well of MTT solution to the 24-well assay plate

Starting time of PLATE 1 (time): （ : ～）

Ending time of PLATE 1 (time): （～ : ）

Starting time of PLATE 2 (time): （ : ～）

Ending time of PLATE 2 (time): （～ : ）

5-6 Extraction (at least 2 hours)

Operator:

☐ Soaked each tissues in 500 μL of iso-propanol

☐ Shaked during extraction (Not needed this item if extraction is preceded overnight)

Manufacturer name of shaker: Model:

☐Shaded during extraction

Starting date (time): (: ～)

Ending date (time): (～ :)

5-7 Measurement

Operator:

☐ OD values were measured at 570 nm and 650 nm

Manufacture name of microplate reader: Model:

Starting date (time): (: ～)

Ending date (time): (～ :)

Appendix 3

LabCyte EPI-MODEL24 Round Robin
Phase I study (Transferability Study) report

LabCyte EPI-MODEL24 Round Robin Study as an in vitro irritation test for detection of irritant activity in medical device extracts, Phase I (Transferability Study) report

Purpose: The aim of LabCyte EPI-MODEL24 Round Robin Phase I study was transferability 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.

Organizaton: A committee for LabCyte EPI-MODEL24 Round Robin Phase I Study

Coordinated Laboratory: Department of Medical Devices, National Institute of Health Sciences
Drs Reiko Kato and Atsuko Miyajima

Test method developer and Lead laboratory: Japan Tissue Engineering Co., Ltd. (J-TEC)

Participating laboratories:
Sixteen laboratories participated in Table 1.

Table 1. Participated 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.

Experiment duration: See Table 2

Materials and methods:

Test method: LabCyte EPI-MODEL24 Skin Irritation Test method (LabCyte EPI-MODEL24 SIT) for medical device Round Robin Study in Japan SOP for Transferability Study Ver.1.0

Test sample: Negative control (Dulbecco's phosphate-buffered saline: DPBS)

Positive control (1% Sodium Dodecyl Sulfate (SDS) solution)

Vehicle control (saline solution or sesame oil)

Test samples in the Phase I study included two materials:

1) Material classified as be non-irritant in both saline and sesame oil extracts (polyvinyl chloride (PVC) + 0.9% Genapol X-080 (Y-3)).

2) Material classified as be an irritant in both saline and sesame oil extracts (PVC + 5.8% Genapol X-080 (Y-4)).

*Send test samples with code name by the Coordinated Laboratory

Test acceptance criteria

Absorbance value: $0.7 \leq$ 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%

Positive criteria

Cell viability ≤ 50% Irritant

Cell viability > 50% Non-irritant

Results: The original results of the participating laboratories from which the Figure is derived are presented in Supplementary Material 1.

All 16 laboratories passed the test acceptance criteria of LabCyte EPI-MODEL24 SIT and were able to show the expected judgment results for the coded test samples A and B (Figure 1).

It should be noted that Y-3 was found to provide results equivalent to the negative control in both saline and sesame oil extracts in an irritation assessment based on cell viability using LabCyte EPI-MODEL24 in a previous study[1]. Therefore, Y-3 was used as a non-irritant material in both vehicle extractions.

1) Y. Nomura et al., J. Biomed. Mater. Res., Part B. 106, 2807-2814 (2018)

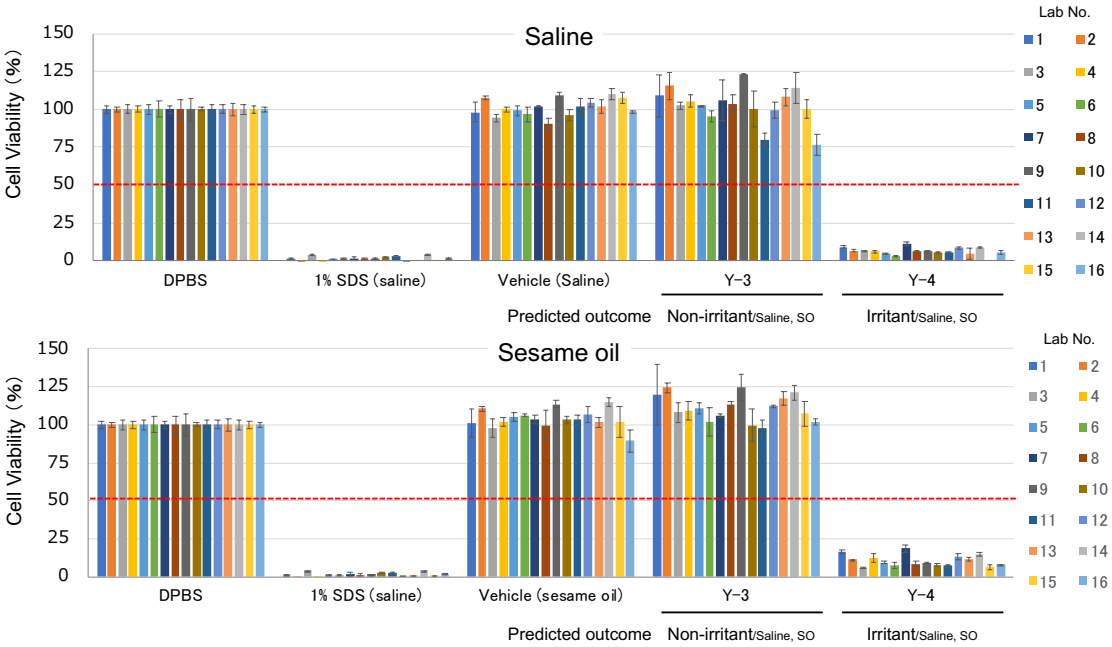


Figure 1. Cell viability obtained from LabCyte EPI-MODEL24 model exposed to extracts of polymer samples and controls.

Conclusion:

The Committee concluded that a series of technology transfers, including extraction operations, had been completed of LabCyte EPI-MODEL24 SIT as all the participating laboratories obtained the expected judgment results for each test sample.

Table 2. Overview of LabCyte EPI-MODEL24 Round Robin Phase I Study

Mm/yyyy	Activity
March 2020	Planning the round robin study
	Preparation of a (tentative) list of test samples
April 2020 ~May	Open call for participating laboratories
June 2020	Discussion, decision and reading through of proposed research plan
December 2020	Discussion and determination of protocols
	Online round robin study information sessions
January 2021	Online technical workshops
Late January to early April 2021	Transferability study with two coded test samples (Phase I)
April 2021	Reporting the results of the online transferability study and explaining the reproducibility test (Phase II)
June 2021	Status report on Phase II study sample preparation via email
January 2022	Current status meeting on online Phase II study sample preparation

All data of all participating laboratories of LabCyte EPI-MODEL24 Round Robin Phase I

	NC (DPBS)			PC			VC (saline)			VC (SO)			A-1:Y-3 (Saline)			A-2:Y-3 (SO)			B1:Y-4 (Saline)			B2:Y-4 (SO)		
Lab. No	cell viability	SD	prediction	cell viability	SD	prediction	cell viability	SD	prediction	cell viability	SD	prediction	cell viability	SD	prediction	cell viability	SD	prediction	cell viability	SD	prediction	cell viability	SD	prediction
1	100	± 2.29	N	1.16	± 0.17	I	98.03	± 6.33	N	101.26	± 9.58	N	108.83	± 13.54	N	119.74	± 19.86	N	9.06	± 0.89	I	16.17	± 1.13	I
2	100	± 1.67	N	0.01	± 0.01	I	107.56	± 1.09	N	110.71	± 1.79	N	115.50	± 9.09	N	124.46	± 3.47	N	6.45	± 0.69	I	11.10	± 0.25	I
3	100	± 3.02	N	3.87	± 0.12	I	94.17	± 2.76	N	97.88	± 6.11	N	102.27	± 2.21	N	108.11	± 6.30	N	6.36	± 0.12	I	5.49	± 0.49	I
4	100	± 2.21	N	0.00	± 0.00	I	99.84	± 1.38	N	101.62	± 2.79	N	105.38	± 4.24	N	109.31	± 6.20	N	5.98	± 0.70	I	12.06	± 2.73	I
5	100	± 3.18	N	1.24	± 0.03	I	99.04	± 3.14	N	105.44	± 2.86	N	102.29	± 0.20	N	110.79	± 3.41	N	4.34	± 0.32	I	9.42	± 0.57	I
6	100	± 5.29	N	1.27	± 0.07	I	96.70	± 5.03	N	106.38	± 1.06	N	95.27	± 3.77	N	102.03	± 9.42	N	3.12	± 0.51	I	7.44	± 1.81	I
7	100	± 2.62	N	1.33	± 1.51	I	101.94	± 0.42	N	103.41	± 3.05	N	105.78	± 13.75	N	105.96	± 1.04	N	11.09	± 1.15	I	18.57	± 2.36	I
8	100	± 5.95	N	1.17	± 0.72	I	90.33	± 3.79	N	99.55	± 9.88	N	103.78	± 6.00	N	112.95	± 2.45	N	5.91	± 0.46	I	8.33	± 1.60	I
9	100	± 7.32	N	1.41	± 0.16	I	109.00	± 2.47	N	113.46	± 2.36	N	122.96	± 0.64	N	124.46	± 8.63	N	6.49	± 0.15	I	8.83	± 0.59	I
10	100	± 1.14	N	2.40	± 0.18	I	96.14	± 3.33	N	103.38	± 2.38	N	100.44	± 11.81	N	99.79	± 10.89	N	5.38	± 0.59	I	7.61	± 0.75	I
11	100	± 3.62	N	2.01	± 0.27	I	98.31	± 2.72	N	99.70	± 2.93	N	102.64	± 3.03	N	108.59	± 4.57	N	5.03	± 0.95	I	8.19	± 0.49	I
12	100	± 2.80	N	0.00	± 0.00	I	104.06	± 2.85	N	106.80	± 4.93	N	99.17	± 5.36	N	112.32	± 0.85	N	8.42	± 0.92	I	13.05	± 2.07	I
13	100	± 4.23	N	0.35	± 0.16	I	101.78	± 4.85	N	101.59	± 3.38	N	108.09	± 5.76	N	117.37	± 4.51	N	4.41	± 3.88	I	11.50	± 1.35	I
14	100	± 3.20	N	3.90	± 0.21	I	109.98	± 3.70	N	115.05	± 2.68	N	114.26	± 10.34	N	121.11	± 4.70	N	8.49	± 0.57	I	14.66	± 1.27	I
15	100	± 2.41	N	0.08	± 0.04	I	107.37	± 3.53	N	101.97	± 9.85	N	100.02	± 6.14	N	107.39	± 8.05	N	0.24	± 0.12	I	6.17	± 1.39	I
16	100	± 2.55	N	1.13	± 0.26	I	100.45	± 3.73	N	106.79	± 1.65	N	106.57	± 1.79	N	113.73	± 1.92	N	4.57	± 1.06	I	10.37	± 0.94	I

cell viability: mean of viabilities [%]
SD: SD of viabilities
prediction:NI or I

Appendix 4

All data of all participating laboratories of LabCyte EPI-MODEL24 Round Robin Phase II

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
1	1Re	A1-1	DPBS	saline	102.80	94.59	102.61	100.00	4.69	NI	Qualified
1	1Re	A2-1	1% SDS	saline	0.71	2.62	0.12	1.15	1.30	I	Qualified
1	1Re	A3-1	Saline	saline	93.07	102.17	102.80	99.35	5.45	NI	Qualified
1	1Re	A059-1	Y-4	saline	8.83	9.22	7.56	8.54	0.87	I	Qualified
1	1Re	A695-1	PVC	saline	108.72	96.69	103.05	102.82	6.02	NI	Qualified
1	1Re	A748-1	PVC+SDS	saline	2.03	3.35	1.88	2.42	0.81	I	Qualified
1	1Re	A835-1	PVC+Heptanal	saline	101.09	97.23	104.71	101.01	3.74	NI	Qualified
1	1Re	A1-2	DPBS	SO	99.39	103.59	97.02	100.00	3.33	NI	Qualified
1	1Re	A2-2	1% SDS	SO	2.67	1.07	1.70	1.81	0.80	I	Qualified
1	1Re	A3-2	Sesame Oil	SO	99.24	100.02	101.66	100.31	1.23	NI	Qualified
1	1Re	A059-2	Y-4	SO	8.27	7.98	8.22	8.16	0.16	I	Qualified
1	1Re	A695-2	PVC	SO	100.55	106.73	97.60	101.63	4.66	NI	Qualified
1	1Re	A748-2	PVC+SDS	SO	99.53	94.61	102.33	98.82	3.91	NI	Qualified
1	1Re	A835-2	PVC+Heptanal	SO	30.96	23.29	29.23	27.83	4.03	I	Qualified
1	2	B1-1	DPBS	saline	98.87	98.74	102.39	100.00	2.07	NI	Qualified
1	2	B2-1	1% SDS	saline	1.38	-0.83	1.65	0.73	1.36	I	Qualified
1	2	B2-1	Saline	saline	97.97	100.05	101.98	100.00	2.01	NI	Qualified
1	2	B051-1	PVC+SDS	saline	1.20	2.05	1.60	1.62	0.43	I	Qualified
1	2	B103-1	PVC+Heptanal	saline	41.20	35.42	48.00	41.54	6.30	I	Qualified
1	2	B340-1	PVC	saline	102.84	104.74	108.57	105.38	2.92	NI	Qualified
1	2	B456-1	Y-4	saline	5.34	5.07	5.25	5.22	0.14	I	Qualified
1	2	B1-2	DPBS	SO	101.07	100.62	98.31	100.00	1.48	NI	Qualified
1	2	B2-2	1% SDS	SO	1.51	2.19	1.69	1.79	0.35	I	Qualified
1	2	B3-2	Sesame Oil	SO	106.87	105.20	109.59	107.22	2.22	NI	Qualified
1	2	B051-2	PVC+SDS	SO	103.52	104.24	101.89	103.22	1.21	NI	Qualified
1	2	B103-2	PVC+Heptanal	SO	13.65	10.48	9.57	11.24	2.14	I	Qualified
1	2	B340-2	PVC	SO	109.18	92.60	112.81	104.86	10.78	NI	Qualified
1	2	B456-2	Y-4	SO	8.89	8.49	7.53	8.30	0.70	I	Qualified
1	3	C1-1	DPBS	saline	95.16	100.81	104.03	100.00	4.49	NI	Qualified
1	3	C2-1	1% SDS	saline	1.70	3.33	1.75	2.26	0.93	I	Qualified
1	3	C3-1	Saline	saline	97.53	107.03	100.08	101.54	4.91	NI	Qualified
1	3	C248-1	Y-4	saline	8.59	6.27	8.02	7.63	1.21	I	Qualified
1	3	C309-1	PVC+Heptanal	saline	59.67	43.45	49.90	51.01	8.17	NI	Qualified
1	3	C491-1	PVC+SDS	saline	2.37	2.49	4.41	3.09	1.14	I	Qualified
1	3	C564-1	PVC	saline	112.68	109.74	111.55	111.32	1.48	NI	Qualified
1	3	C1-2	DPBS	SO	99.16	99.21	101.63	100.00	1.41	NI	Qualified
1	3	C2-2	1% SDS	SO	1.86	2.37	2.37	2.20	0.29	I	Qualified
1	3	C3-2	Sesame Oil	SO	112.90	111.94	104.60	109.81	4.54	NI	Qualified
1	3	C248-2	Y-4	SO	12.01	10.78	10.00	10.93	1.02	I	Qualified
1	3	C309-2	PVC+Heptanal	SO	19.30	20.93	18.41	19.55	1.28	I	Qualified
1	3	C491-2	PVC+SDS	SO	108.02	100.50	98.82	102.45	4.90	NI	Qualified
1	3	C564-2	PVC	SO	111.61	107.12	96.80	105.18	7.59	NI	Qualified

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
2	1Re	A1-1	DPBS	saline	104.00	90.36	105.64	100.00	8.39	NI	Qualified
2	1Re	A2-1	1% SDS	saline	-0.04	0.01	0.07	0.01	0.05	I	Qualified
2	1Re	A3-1	Saline	saline	103.79	110.72	114.90	109.80	5.61	NI	Qualified
2	1Re	A068-1	PVC+SDS	saline	-7.60	-6.55	-6.65	-6.93	0.58	I	Qualified
2	1Re	A234-1	PVC	saline	109.40	90.04	109.19	102.87	11.12	NI	Qualified
2	1Re	A796-1	Y-4	saline	-1.15	6.84	6.89	4.19	4.63	I	Qualified
2	1Re	A937-1	PVC+Heptanal	saline	105.59	97.23	102.68	101.83	4.24	NI	Qualified
2	1Re	A1-2	DPBS	SO	100.70	86.69	112.61	100.00	12.98	NI	Qualified
2	1Re	A2-2	1% SDS	SO	-0.11	-0.11	-0.17	-0.13	0.03	I	Qualified
2	1Re	A3-2	Sesame Oil	SO	115.96	106.26	115.17	112.46	5.39	NI	Qualified
2	1Re	A068-2	PVC+SDS	SO	115.17	99.91	107.79	107.62	7.63	NI	Qualified
2	1Re	A234-2	PVC	SO	116.02	107.39	106.37	109.93	5.30	NI	Qualified
2	1Re	A796-2	Y-4	SO	10.38	13.16	12.71	12.08	1.49	I	Qualified
2	1Re	A937-2	PVC+Heptanal	SO	27.63	30.18	28.31	28.71	1.32	I	Qualified
2	1	DPBS	DPBS	saline	92.78	101.89	105.33	100.00	6.48	NI	Qualified
2	1	1% SDS	1% SDS	saline	0.10	0.04	-0.01	0.04	0.06	I	Qualified
2	1	Saline	Saline	saline	104.64	106.47	103.21	104.77	1.64	NI	Qualified
2	1	B082-1	PVC+SDS	saline	0.04	0.10	-0.01	0.04	0.06	I	Qualified
2	1	B604-1	PVC	saline	104.30	105.27	105.04	104.87	0.51	NI	Qualified
2	1	B655-1	Y-4	saline	6.17	6.80	0.10	4.36	3.70	I	Qualified
2	1	B817-1	PVC+Heptanal	saline	91.41	96.79	98.80	95.67	3.82	NI	Qualified
2	1	DPBS	DPBS	SO	100.97	106.43	92.60	100.00	6.96	NI	Qualified
2	1	1% SDS	1% SDS	SO	-0.07	-0.01	-0.01	-0.03	0.03	I	Qualified
2	1	Sesame Oil	Sesame Oil	SO	97.47	106.13	92.84	98.81	6.75	NI	Qualified
2	1	B082-2	PVC+SDS	SO	95.33	107.26	111.65	104.75	8.44	NI	Qualified
2	1	B604-2	PVC	SO	94.44	102.63	112.60	103.22	9.09	NI	Qualified
2	1	B655-2	Y-4	SO	11.73	13.87	12.56	12.72	1.08	I	Qualified
2	1	B817-2	PVC+Heptanal	SO	22.12	23.42	23.72	23.09	0.85	I	Qualified
2	3	C1-1	DPBS	saline	98.72	98.17	103.11	100.00	2.71	NI	Qualified
2	3	C2-1	1% SDS	saline	0.08	0.03	-0.08	0.01	0.08	I	Qualified
2	3	C3-1	Saline	saline	106.04	111.42	106.32	107.93	3.03	NI	Qualified
2	3	C162-1	Y-4	saline	6.11	6.00	6.81	6.31	0.44	I	Qualified
2	3	C673-1	PVC+SDS	saline	0.03	0.03	0.03	0.03	0.00	I	Qualified
2	3	C772-1	PVC+Heptanal	saline	66.80	74.34	67.66	69.60	4.13	NI	Qualified
2	3	C906-1	PVC	saline	100.40	105.94	111.74	106.03	5.67	NI	Qualified
2	3	C1-2	DPBS	SO	96.46	103.17	100.36	100.00	3.37	NI	Qualified
2	3	C2-2	1% SDS	SO	0.00	0.00	0.05	0.02	0.03	I	Qualified
2	3	C3-2	Sesame Oil	SO	86.37	84.60	82.00	84.32	2.20	NI	Qualified
2	3	C162-2	Y-4	SO	13.58	14.15	16.49	14.74	1.54	I	Qualified
2	3	C673-2	PVC+SDS	SO	66.49	71.23	44.80	60.84	14.09	NI	Qualified
2	3	C772-2	PVC+Heptanal	SO	21.64	22.84	23.05	22.51	0.76	I	Qualified
2	3	C906-2	PVC	SO	91.57	79.24	105.05	91.95	12.91	NI	Qualified

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
3	1	A1-1	DPBS	saline	97.08	100.06	102.86	100.00	2.89	NI	Qualified
3	1	A2-1	1% SDS	saline	1.72	1.85	1.63	1.73	0.11	I	Qualified
3	1	A3-1	Saline	saline	100.29	105.11	97.71	101.04	3.75	NI	Qualified
3	1	A038-1	PVC+Heptanal	saline	99.78	102.85	105.02	102.55	2.63	NI	Qualified
3	1	A787-1	Y-4	saline	7.23	5.60	5.69	6.17	0.92	I	Qualified
3	1	A809-1	PVC+SDS	saline	1.78	1.80	2.55	2.04	0.44	I	Qualified
3	1	A954-1	PVC	saline	100.46	104.94	105.29	103.56	2.69	NI	Qualified
3	1	A1-2	DPBS	SO	105.96	98.44	95.60	100.00	5.35	NI	Qualified
3	1	A2-2	1% SDS	SO	1.73	2.01	2.28	2.01	0.28	I	Qualified
3	1	A3-2	Sesame Oil	SO	105.22	103.74	97.13	102.03	4.31	NI	Qualified
3	1	A038-2	PVC+Heptanal	SO	16.43	19.39	16.72	17.52	1.63	I	Qualified
3	1	A787-2	Y-4	SO	9.26	9.75	10.18	9.73	0.46	I	Qualified
3	1	A809-2	PVC+SDS	SO	110.68	107.10	103.05	106.94	3.82	NI	Qualified
3	1	A954-2	PVC	SO	102.69	100.65	103.44	102.26	1.44	NI	Qualified
3	2	B1-1	DPBS	saline	97.50	99.57	102.92	100.00	2.74	NI	Qualified
3	2	B2-1	1% SDS	saline	1.87	1.51	1.58	1.65	0.19	I	Qualified
3	2	B2-1	Saline	saline	98.86	99.14	105.94	101.31	4.01	NI	Qualified
3	2	B173-1	PVC+SDS	saline	2.49	2.47	2.71	2.56	0.13	I	Qualified
3	2	B622-1	PVC+Heptanal	saline	92.08	93.90	94.82	93.60	1.40	NI	Qualified
3	2	B667-1	PVC	saline	105.79	99.62	104.97	103.46	3.35	NI	Qualified
3	2	B948-1	Y-4	saline	5.75	5.47	5.19	5.47	0.28	I	Qualified
3	2	B1-2	DPBS	SO	101.33	97.68	100.99	100.00	2.02	NI	Qualified
3	2	B2-2	1% SDS	SO	2.23	0.82	1.92	1.66	0.74	I	Qualified
3	2	B3-2	Sesame Oil	SO	105.26	106.65	97.24	103.05	5.07	NI	Qualified
3	2	B173-2	PVC+SDS	SO	82.29	98.32	101.80	94.14	10.41	NI	Qualified
3	2	B622-2	PVC+Heptanal	SO	16.56	15.10	17.70	16.45	1.30	I	Qualified
3	2	B667-2	PVC	SO	102.38	109.12	107.58	106.36	3.53	NI	Qualified
3	2	B948-2	Y-4	SO	7.93	8.45	7.52	7.96	0.46	I	Qualified
3	3	C1-1	DPBS	saline	98.12	103.03	98.85	100.00	2.65	NI	Qualified
3	3	C2-1	1% SDS	saline	2.26	1.63	1.69	1.86	0.35	I	Qualified
3	3	C3-1	Saline	saline	95.63	98.59	102.06	98.76	3.22	NI	Qualified
3	3	C065-1	Y-4	saline	4.79	4.97	4.90	4.89	0.09	I	Qualified
3	3	C104-1	PVC	saline	98.19	96.67	102.78	99.22	3.18	NI	Qualified
3	3	C255-1	PVC+SDS	saline	3.38	2.04	1.91	2.44	0.81	I	Qualified
3	3	C811-1	PVC+Heptanal	saline	98.85	95.14	92.44	95.48	3.22	NI	Qualified
3	3	C1-2	DPBS	SO	102.76	99.84	97.40	100.00	2.68	NI	Qualified
3	3	C2-2	1% SDS	SO	1.72	2.14	1.75	1.87	0.23	I	Qualified
3	3	C3-2	Sesame Oil	SO	101.82	105.69	97.90	101.80	3.90	NI	Qualified
3	3	C065-2	Y-4	SO	8.20	8.51	8.07	8.26	0.22	I	Qualified
3	3	C104-2	PVC	SO	103.88	103.37	107.62	104.96	2.32	NI	Qualified
3	3	C255-2	PVC+SDS	SO	91.04	105.43	101.12	99.20	7.39	NI	Qualified
3	3	C811-2	PVC+Heptanal	SO	18.57	17.50	19.75	18.61	1.12	I	Qualified

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
4	1	A1-1	DPBS	saline	101.67	98.41	99.93	100.00	1.63	NI	Qualified
4	1	A2-1	1% SDS	saline	0.06	0.00	0.00	0.02	0.03	I	Qualified
4	1	A3-1	Saline	saline	105.88	100.04	104.36	103.42	3.03	NI	Qualified
4	1	A031-1	PVC+Heptanal	saline	92.23	95.32	98.86	95.47	3.31	NI	Qualified
4	1	A220-1	Y-4	saline	7.24	7.75	8.08	7.69	0.42	I	Qualified
4	1	A370-1	PVC	saline	109.24	107.39	108.18	108.27	0.93	NI	Qualified
4	1	A477-1	PVC+SDS	saline	-0.06	3.42	-0.06	1.14	1.98	I	Qualified
4	1	A1-2	DPBS	SO	98.95	100.19	100.86	100.00	0.97	NI	Qualified
4	1	A2-2	1% SDS	SO	-0.03	0.03	0.03	0.01	0.03	I	Qualified
4	1	A3-2	Sesame Oil	SO	99.98	103.02	105.29	102.76	2.66	NI	Qualified
4	1	A031-2	PVC+Heptanal	SO	12.13	11.82	13.11	12.35	0.67	I	Qualified
4	1	A220-2	Y-4	SO	11.25	9.66	9.71	10.21	0.91	I	Qualified
4	1	A370-2	PVC	SO	111.52	102.40	103.64	105.85	4.94	NI	Qualified
4	1	A477-2	PVC+SDS	SO	105.60	105.80	103.43	104.94	1.31	NI	Qualified
4	2	B1-1	DPBS	saline	97.56	99.32	103.13	100.00	2.85	NI	Qualified
4	2	B2-1	1% SDS	saline	0.01	0.01	-0.09	-0.02	0.06	I	Qualified
4	2	B2-1	Saline	saline	97.66	103.47	101.32	100.81	2.94	NI	Qualified
4	2	B131-1	Y-4	saline	5.87	6.66	6.17	6.23	0.39	I	Qualified
4	2	B182-1	PVC+Heptanal	saline	87.45	89.35	89.99	88.93	1.32	NI	Qualified
4	2	B672-1	PVC+SDS	saline	-0.09	-0.09	-0.09	-0.09	0.00	I	Qualified
4	2	B762-1	PVC	saline	104.30	103.66	99.76	102.57	2.46	NI	Qualified
4	2	B1-2	DPBS	SO	103.72	101.03	95.25	100.00	4.33	NI	Qualified
4	2	B2-2	1% SDS	SO	-0.02	-0.02	-0.02	-0.02	0.00	I	Qualified
4	2	B3-2	Sesame Oil	SO	99.39	101.53	100.23	100.38	1.08	NI	Qualified
4	2	B131-2	Y-4	SO	8.70	7.55	7.00	7.75	0.86	I	Qualified
4	2	B182-2	PVC+Heptanal	SO	24.60	18.62	21.31	21.51	3.00	I	Qualified
4	2	B672-2	PVC+SDS	SO	100.13	105.62	99.09	101.61	3.51	NI	Qualified
4	2	B762-2	PVC	SO	103.17	104.02	101.53	102.91	1.27	NI	Qualified
4	3	C1-1	DPBS	saline	100.34	100.65	99.00	100.00	0.88	NI	Qualified
4	3	C2-1	1% SDS	saline	-0.10	0.10	-0.05	-0.02	0.11	I	Qualified
4	3	C3-1	Saline	saline	100.50	98.48	90.21	96.40	5.45	NI	Qualified
4	3	C022-1	PVC	saline	97.66	108.93	105.05	103.88	5.73	NI	Qualified
4	3	C354-1	Y-4	saline	6.31	5.69	5.48	5.82	0.43	I	Qualified
4	3	C625-1	PVC+SDS	saline	0.00	0.00	-0.10	-0.03	0.06	I	Qualified
4	3	C892-1	PVC+Heptanal	saline	97.19	100.97	95.28	97.81	2.89	NI	Qualified
4	3	C1-2	DPBS	SO	98.73	95.02	106.25	100.00	5.72	NI	Qualified
4	3	C2-2	1% SDS	SO	-0.11	0.04	-0.16	-0.08	0.10	I	Qualified
4	3	C3-2	Sesame Oil	SO	95.78	101.24	96.68	97.90	2.93	NI	Qualified
4	3	C022-2	PVC	SO	107.10	94.37	102.04	101.17	6.41	NI	Qualified
4	3	C354-2	Y-4	SO	8.15	9.41	9.31	8.96	0.70	I	Qualified
4	3	C625-2	PVC+SDS	SO	96.48	103.04	100.23	99.92	3.29	NI	Qualified
4	3	C892-2	PVC+Heptanal	SO	15.97	16.77	18.17	16.97	1.12	I	Qualified

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
5	1	A1-1	DPBS	saline	101.58	94.88	103.54	100.00	4.54	NI	Qualified
5	1	A2-1	1% SDS	saline	3.35	3.30	1.99	2.88	0.77	I	Qualified
5	1	A3-1	Saline	saline	100.98	102.34	99.78	101.04	1.28	NI	Qualified
5	1	A239-1	PVC+Heptanal	saline	105.89	96.40	96.46	99.58	5.46	NI	Qualified
5	1	A324-1	Y-4	saline	7.17	10.55	8.37	8.69	1.71	I	Qualified
5	1	A602-1	PVC+SDS	saline	3.62	4.17	4.55	4.12	0.47	I	Qualified
5	1	A741-1	PVC	saline	101.64	103.82	98.58	101.34	2.63	NI	Qualified
5	1	A1-2	DPBS	SO	99.81	99.18	101.01	100.00	0.93	NI	Qualified
5	1	A2-2	1% SDS	SO	3.13	3.50	3.39	3.34	0.19	I	Qualified
5	1	A3-2	Sesame Oil	SO	100.54	97.30	98.82	98.89	1.62	NI	Qualified
5	1	A239-2	PVC+Heptanal	SO	18.95	23.23	18.43	20.20	2.63	I	Qualified
5	1	A324-2	Y-4	SO	11.48	12.37	12.63	12.16	0.60	I	Qualified
5	1	A602-2	PVC+SDS	SO	102.99	107.17	104.87	105.01	2.09	NI	Qualified
5	1	A741-2	PVC	SO	105.03	105.34	100.23	103.53	2.87	NI	Qualified
5	2	B1-1	DPBS	saline	98.43	100.61	100.96	100.00	1.37	NI	Qualified
5	2	B2-1	1% SDS	saline	2.73	2.63	2.58	2.65	0.08	I	Qualified
5	2	B2-1	Saline	saline	98.38	103.24	99.37	100.33	2.57	NI	Qualified
5	2	B331-1	PVC	saline	103.29	95.60	103.74	100.88	4.57	NI	Qualified
5	2	B478-1	Y-4	saline	7.84	8.09	8.43	8.12	0.30	I	Qualified
5	2	B823-1	PVC+SDS	saline	2.33	3.67	2.93	2.98	0.67	I	Qualified
5	2	B848-1	PVC+Heptanal	saline	103.44	106.42	105.42	105.09	1.52	NI	Qualified
5	2	B1-2	DPBS	SO	95.74	102.73	101.53	100.00	3.74	NI	Qualified
5	2	B2-2	1% SDS	SO	3.43	2.28	1.62	2.44	0.92	I	Qualified
5	2	B3-2	Sesame Oil	SO	106.76	105.65	102.63	105.01	2.14	NI	Qualified
5	2	B331-2	PVC	SO	103.69	102.63	99.21	101.84	2.34	NI	Qualified
5	2	B478-2	Y-4	SO	11.08	11.33	11.63	11.35	0.28	I	Qualified
5	2	B823-2	PVC+SDS	SO	106.05	103.19	105.10	104.78	1.46	NI	Qualified
5	2	B848-2	PVC+Heptanal	SO	14.50	14.55	16.51	15.19	1.15	I	Qualified
5	3	C1-1	DPBS	saline	99.97	102.73	97.30	100.00	2.72	NI	Qualified
5	3	C2-1	1% SDS	saline	0.81	3.37	2.32	2.17	1.29	I	Qualified
5	3	C3-1	Saline	saline	95.05	99.76	100.49	98.43	2.95	NI	Qualified
5	3	C720-1	PVC+Heptanal	saline	105.50	102.94	102.58	103.67	1.59	NI	Qualified
5	3	C837-1	Y-4	saline	8.86	7.97	9.59	8.80	0.81	I	Qualified
5	3	C911-1	PVC	saline	102.53	102.16	102.53	102.40	0.21	NI	Qualified
5	3	C991-1	PVC+SDS	saline	2.79	3.42	2.90	3.04	0.34	I	Qualified
5	3	C1-2	DPBS	SO	97.13	102.62	100.25	100.00	2.76	NI	Qualified
5	3	C2-2	1% SDS	SO	2.89	2.21	2.58	2.56	0.34	I	Qualified
5	3	C3-2	Sesame Oil	SO	109.76	99.08	102.31	103.72	5.48	NI	Qualified
5	3	C720-2	PVC+Heptanal	SO	19.49	17.90	16.69	18.03	1.40	I	Qualified
5	3	C837-2	Y-4	SO	11.77	12.20	10.35	11.44	0.97	I	Qualified
5	3	C911-2	PVC	SO	105.11	98.82	104.32	102.75	3.43	NI	Qualified
5	3	C991-2	PVC+SDS	SO	108.91	104.53	110.76	108.07	3.20	NI	Qualified

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
6	1	A1-1	DPBS	saline	101.09	98.34	100.57	100.00	1.46	NI	Qualified
6	1	A2-1	1% SDS	saline	1.29	2.05	1.43	1.59	0.40	I	Qualified
6	1	A3-1	Saline	saline	98.24	100.57	104.79	101.20	3.32	NI	Qualified
6	1	A172-1	PVC+Heptanal	saline	99.53	95.21	98.62	97.79	2.28	NI	Qualified
6	1	A312-1	Y-4	saline	5.42	5.13	5.94	5.50	0.41	I	Qualified
6	1	A632-1	PVC	saline	97.87	99.95	101.19	99.67	1.68	NI	Qualified
6	1	A999-1	PVC+SDS	saline	1.25	2.24	1.48	1.66	0.52	I	Qualified
6	1	A1-2	DPBS	SO	101.12	100.15	98.73	100.00	1.20	NI	Qualified
6	1	A2-2	1% SDS	SO	0.47	1.13	0.67	0.76	0.34	I	Qualified
6	1	A3-2	Sesame Oil	SO	102.44	104.83	103.51	103.59	1.20	NI	Qualified
6	1	A172-2	PVC+Heptanal	SO	12.36	14.40	12.36	13.04	1.17	I	Qualified
6	1	A312-2	Y-4	SO	9.16	11.50	10.79	10.48	1.20	I	Qualified
6	1	A632-2	PVC	SO	89.99	98.73	92.27	93.66	4.53	NI	Qualified
6	1	A999-2	PVC+SDS	SO	102.59	107.83	82.62	97.68	13.31	NI	Qualified
6	2	B1-1	DPBS	saline	99.84	101.59	98.58	100.00	1.51	NI	Qualified
6	2	B2-1	1% SDS	saline	1.73	2.15	1.94	1.94	0.21	I	Qualified
6	2	B2-1	Saline	saline	97.45	95.21	105.65	99.44	5.50	NI	Qualified
6	2	B540-1	Y-4	saline	5.80	7.76	6.71	6.76	0.98	I	Qualified
6	2	B723-1	PVC+Heptanal	saline	122.05	131.44	121.42	124.97	5.61	NI	Qualified
6	2	B731-1	PVC+SDS	saline	2.36	2.08	2.51	2.32	0.21	I	Qualified
6	2	B977-1	PVC	saline	109.37	113.36	107.26	110.00	3.10	NI	Qualified
6	2	B1-2	DPBS	SO	115.28	96.46	88.26	100.00	13.85	NI	Qualified
6	2	B2-2	1% SDS	SO	1.78	2.12	2.39	2.09	0.30	I	Qualified
6	2	B3-2	Sesame Oil	SO	103.52	107.62	110.77	107.30	3.64	NI	Qualified
6	2	B540-2	Y-4	SO	8.77	11.12	11.05	10.31	1.34	I	Qualified
6	2	B723-2	PVC+Heptanal	SO	14.08	12.94	22.41	16.47	5.17	I	Qualified
6	2	B731--	PVC+SDS	SO	104.59	113.13	119.11	112.27	7.29	NI	Qualified
6	2	B977-2	PVC	SO	108.83	117.83	115.28	113.98	4.64	NI	Qualified
6	3	C1-1	DPBS	saline	95.57	101.08	103.35	100.00	4.00	NI	Qualified
6	3	C2-1	1% SDS	saline	1.98	-0.16	2.53	1.45	1.42	I	Qualified
6	3	C3-1	Saline	saline	105.70	103.63	103.15	104.16	1.35	NI	Qualified
6	3	C044-1	PVC	saline	100.94	104.94	104.53	103.47	2.20	NI	Qualified
6	3	C552-1	Y-4	saline	8.05	7.08	7.22	7.45	0.52	I	Qualified
6	3	C803-1	PVC+SDS	saline	2.12	2.19	1.50	1.94	0.38	I	Qualified
6	3	C910-1	PVC+Heptanal	saline	103.01	113.83	109.49	108.78	5.44	NI	Qualified
6	3	C1-2	DPBS	SO	95.92	103.03	101.05	100.00	3.67	NI	Qualified
6	3	C2-2	1% SDS	SO	1.21	0.28	1.52	1.00	0.64	I	Qualified
6	3	C3-2	Sesame Oil	SO	92.39	92.39	95.11	93.30	1.57	NI	Qualified
6	3	C044-2	PVC	SO	97.28	87.88	78.66	87.94	9.31	NI	Qualified
6	3	C552-2	Y-4	SO	11.04	10.42	10.11	10.53	0.47	I	Qualified
6	3	C803-2	PVC+SDS	SO	93.26	104.76	106.37	101.46	7.15	NI	Qualified
6	3	C910-2	PVC+Heptanal	SO	18.96	21.68	27.31	22.65	4.26	I	Qualified

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
7	1Re	A1-1	DPBS	1	105.04	98.34	96.62	100.00	4.45	NI	Qualified
7	1Re	A2-1	1% SDS	1	5.95	4.99	8.72	6.55	1.94	I	Qualified
7	1Re	A3-1	Saline	1	107.11	99.28	102.36	102.91	3.94	NI	Qualified
7	1Re	A253-1	PVC+Heptanal	1	87.44	85.36	89.72	87.51	2.18	NI	Qualified
7	1Re	A591-1	PVC+SDS	1	8.16	7.59	7.91	7.88	0.29	I	Qualified
7	1Re	A785-1	PVC	1	109.25	110.85	101.87	107.32	4.79	NI	Qualified
7	1Re	A951-1	Y-4	1	16.00	12.12	10.82	12.98	2.69	I	Qualified
7	1Re	A1-2	DPBS	2	100.14	99.91	99.95	100.00	0.12	NI	Qualified
7	1Re	A2-2	1% SDS	2	1.85	6.71	6.66	5.08	2.79	I	Qualified
7	1Re	A3-2	Sesame Oil	2	103.85	101.79	100.60	102.08	1.64	NI	Qualified
7	1Re	A253-2	PVC+Heptanal	2	27.06	24.39	22.99	24.81	2.07	I	Qualified
7	1Re	A591-2	PVC+SDS	2	109.48	102.90	103.93	105.44	3.54	NI	Qualified
7	1Re	A785-2	PVC	2	120.01	100.57	97.93	106.17	12.05	NI	Qualified
7	1Re	A951-2	Y-4	2	17.44	17.43	24.00	19.62	3.79	I	Qualified
7	2	B1-1	DPBS	1	99.08	103.30	97.61	100.00	2.95	NI	Qualified
7	2	B2-1	1% SDS	1	3.97	4.36	4.02	4.11	0.22	I	Qualified
7	2	B2-1	Saline	1	100.15	102.39	104.59	102.38	2.22	NI	Qualified
7	2	B490-1	PVC+Heptanal	1	33.26	46.69	36.07	38.67	7.08	I	Qualified
7	2	B578-1	Y-4	1	12.66	13.91	12.22	12.93	0.87	I	Qualified
7	2	B715-1	PVC	1	108.36	107.58	102.83	106.25	2.99	NI	Qualified
7	2	B964-1	PVC+SDS	1	6.27	7.31	6.05	6.54	0.67	I	Qualified
7	2	B1-2	DPBS	2	102.07	94.28	103.65	100.00	5.01	NI	Qualified
7	2	B2-2	1% SDS	2	9.98	5.81	7.09	7.63	2.14	I	Qualified
7	2	B3-2	Sesame Oil	2	112.16	108.75	103.76	108.22	4.22	NI	Qualified
7	2	B490-2	PVC+Heptanal	2	23.07	21.45	23.60	22.71	1.12	I	Qualified
7	2	B578-2	Y-4	2	15.47	15.20	15.57	15.41	0.19	I	Qualified
7	2	B715-2	PVC	2	107.36	106.84	106.18	106.79	0.59	NI	Qualified
7	2	B964-2	PVC+SDS	2	107.85	108.21	111.82	109.29	2.20	NI	Qualified
7	3	C1-1	DPBS	1	103.27	95.55	101.19	100.00	3.99	NI	Qualified
7	3	C2-1	1% SDS	1	6.22	5.38	4.10	5.23	1.07	I	Qualified
7	3	C3-1	Saline	1	107.81	106.86	105.02	106.57	1.42	NI	Qualified
7	3	C056-1	PVC+Heptanal	1	62.36	53.54	53.24	56.38	5.18	NI	Qualified
7	3	C378-1	Y-4	1	8.53	11.36	11.51	10.47	1.68	I	Qualified
7	3	C391-1	PVC	1	102.89	99.84	98.61	100.45	2.20	NI	Qualified
7	3	C696-1	PVC+SDS	1	5.21	6.12	6.85	6.06	0.82	I	Qualified
7	3	C1-2	DPBS	2	96.34	101.26	102.40	100.00	3.22	NI	Qualified
7	3	C2-2	1% SDS	2	5.14	3.88	4.51	4.51	0.63	I	Qualified
7	3	C3-2	Sesame Oil	2	99.53	109.40	125.84	111.59	13.29	NI	Qualified
7	3	C056-2	PVC+Heptanal	2	23.45	23.67	21.87	23.00	0.98	I	Qualified
7	3	C378-2	Y-4	2	18.47	16.19	16.88	17.18	1.17	I	Qualified
7	3	C391-2	PVC	2	108.71	109.68	111.18	109.86	1.24	NI	Qualified
7	3	C696-2	PVC+SDS	2	118.19	113.02	115.15	115.45	2.60	NI	Qualified

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
8	1	A1-1	DPBS	saline	101.57	99.15	99.28	100.00	1.36	NI	Qualified
8	1	A2-1	1% SDS	saline	0.00	0.00	0.00	0.00	0.00	I	Qualified
8	1	A3-1	Saline	saline	102.47	94.48	93.72	96.89	4.84	NI	Qualified
8	1	A124-1	PVC+Heptanal	saline	106.77	98.92	96.14	100.61	5.51	NI	Qualified
8	1	A168-1	Y-4	saline	4.84	6.14	4.62	5.20	0.82	I	Qualified
8	1	A261-1	PVC	saline	94.22	95.47	106.32	98.67	6.66	NI	Qualified
8	1	A958-1	PVC+SDS	saline	0.00	0.00	0.04	0.01	0.03	I	Qualified
8	1	A1-2	DPBS	SO	107.26	95.62	97.12	100.00	6.33	NI	Qualified
8	1	A2-2	1% SDS	SO	0.19	0.14	0.19	0.18	0.03	I	Qualified
8	1	A3-2	Sesame Oil	SO	102.22	105.28	105.91	104.47	1.98	NI	Qualified
8	1	A124-2	PVC+Heptanal	SO	15.99	14.07	13.81	14.63	1.19	I	Qualified
8	1	A168-2	Y-4	SO	9.08	10.17	10.02	9.76	0.59	I	Qualified
8	1	A261-2	PVC	SO	112.14	108.77	102.17	107.69	5.08	NI	Qualified
8	1	A958-2	PVC+SDS	SO	106.48	108.97	109.49	108.32	1.61	NI	Qualified
8	2	B1-1	DPBS	saline	102.39	94.96	102.65	100.00	4.37	NI	Qualified
8	2	B2-1	1% SDS	saline	-0.04	0.01	0.06	0.01	0.05	I	Qualified
8	2	B2-1	Saline	saline	97.40	102.80	102.29	100.83	2.98	NI	Qualified
8	2	B132-1	PVC+Heptanal	saline	93.43	94.35	88.03	91.94	3.41	NI	Qualified
8	2	B197-1	PVC	saline	101.58	104.02	106.06	103.89	2.24	NI	Qualified
8	2	B303-1	PVC+SDS	saline	0.01	-0.04	-0.04	-0.02	0.03	I	Qualified
8	2	B397-1	Y-4	saline	7.40	6.28	6.58	6.75	0.58	I	Qualified
8	2	B1-2	DPBS	SO	98.11	101.50	100.39	100.00	1.73	NI	Qualified
8	2	B2-2	1% SDS	SO	0.00	0.00	0.06	0.02	0.03	I	Qualified
8	2	B3-2	Sesame Oil	SO	101.44	102.00	103.06	102.17	0.82	NI	Qualified
8	2	B132-2	PVC+Heptanal	SO	21.1	21.4	21.1	21.2	0.16	I	Qualified
8	2	B197-2	PVC	SO	94.17	99.72	99.72	97.87	3.21	NI	Qualified
8	2	B303-2	PVC+SDS	SO	96.06	98.22	100.22	98.17	2.08	NI	Qualified
8	2	B397-2	Y-4	SO	10.83	10.06	12.28	11.06	1.13	I	Qualified
8	3	C1-1	DPBS	saline	104.01	99.73	96.26	100.00	3.88	NI	Qualified
8	3	C2-1	1% SDS	saline	-0.05	0.00	0.00	-0.02	0.03	I	Qualified
8	3	C3-1	Saline	saline	97.92	99.13	91.58	96.21	4.05	NI	Qualified
8	3	C055-1	PVC	saline	101.64	98.02	103.96	101.21	2.99	NI	Qualified
8	3	C344-1	PVC+Heptanal	saline	94.80	94.55	97.67	95.67	1.73	NI	Qualified
8	3	C701-1	PVC+SDS	saline	-0.05	0.00	0.00	-0.02	0.03	I	Qualified
8	3	C766-1	Y-4	saline	6.49	7.29	6.29	6.69	0.53	I	Qualified
8	3	C1-2	DPBS	SO	99.36	100.35	100.29	100.00	0.55	NI	Qualified
8	3	C2-2	1% SDS	SO	-0.07	-0.01	-0.13	-0.07	0.06	I	Qualified
8	3	C3-2	Sesame Oil	SO	99.83	98.44	103.07	100.44	2.38	NI	Qualified
8	3	C055-2	PVC	SO	103.65	104.05	95.95	101.22	4.57	NI	Qualified
8	3	C344-2	PVC+Heptanal	SO	25.24	25.35	25.81	25.47	0.31	I	Qualified
8	3	C701-2	PVC+SDS	SO	97.92	100.69	105.85	101.49	4.03	NI	Qualified
8	3	C766-2	Y-4	SO	10.06	10.47	9.77	10.10	0.35	I	Qualified

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
9	1	A1-1	DPBS	saline	98.80	100.62	100.57	100.00	1.04	NI	Qualified
9	1	A2-1	1% SDS	saline	0.75	0.95	0.39	0.70	0.28	I	Qualified
9	1	A3-1	Saline	saline	98.60	97.94	106.95	101.16	5.02	NI	Qualified
9	1	A509-1	PVC+SDS	saline	1.56	1.40	1.20	1.39	0.18	I	Qualified
9	1	A732-1	Y-4	saline	2.31	3.48	3.48	3.09	0.67	I	Qualified
9	1	A825-1	PVC+Heptanal	saline	92.03	99.21	95.72	95.65	3.59	NI	Qualified
9	1	A971-1	PVC	saline	103.05	105.88	105.78	104.91	1.61	NI	Qualified
9	1	A1-2	DPBS	SO	97.09	101.92	100.99	100.00	2.56	NI	Qualified
9	1	A2-2	1% SDS	SO	1.22	1.64	1.22	1.36	0.24	I	Qualified
9	1	A3-2	Sesame Oil	SO	111.84	105.30	109.82	108.98	3.35	NI	Qualified
9	1	A509-2	PVC+SDS	SO	102.86	103.06	99.32	101.75	2.10	NI	Qualified
9	1	A732-2	Y-4	SO	6.73	7.45	7.09	7.09	0.36	I	Qualified
9	1	A825-2	PVC+Heptanal	SO	14.15	11.92	14.31	13.46	1.34	I	Qualified
9	1	A971-2	PVC	SO	111.48	108.73	113.55	111.25	2.42	NI	Qualified
9	2	B1-1	DPBS	saline	95.91	102.28	101.81	100.00	3.55	NI	Qualified
9	2	B2-1	1% SDS	saline	0.78	0.54	0.97	0.76	0.21	I	Qualified
9	2	B2-1	Saline	saline	101.62	100.20	100.25	100.69	0.80	NI	Qualified
9	2	B180-1	PVC+SDS	saline	1.44	1.06	1.30	1.27	0.19	I	Qualified
9	2	B323-1	PVC+Heptanal	saline	89.83	92.76	94.26	92.28	2.25	NI	Qualified
9	2	B658-1	Y-4	saline	5.02	4.74	4.27	4.68	0.38	I	Qualified
9	2	B759-1	PVC	saline	108.50	106.76	104.87	106.71	1.82	NI	Qualified
9	2	B1-2	DPBS	SO	93.89	101.13	104.98	100.00	5.63	NI	Qualified
9	2	B2-2	1% SDS	SO	0.86	1.63	1.17	1.22	0.39	I	Qualified
9	2	B3-2	Sesame Oil	SO	92.09	104.57	108.93	101.87	8.74	NI	Qualified
9	2	B180-2	PVC+SDS	SO	110.73	107.14	105.39	107.75	2.72	NI	Qualified
9	2	B323-2	PVC+Heptanal	SO	18.88	18.06	17.08	18.01	0.90	I	Qualified
9	2	B658-2	Y-4	SO	7.84	9.84	9.38	9.02	1.05	I	Qualified
9	2	B759-2	PVC	SO	119.30	115.04	110.83	115.06	4.24	NI	Qualified
9	3	C1-1	DPBS	saline	95.72	102.31	101.98	100.00	3.71	NI	Qualified
9	3	C2-1	1% SDS	saline	1.17	1.11	1.22	1.17	0.05	I	Qualified
9	3	C3-1	Saline	saline	100.60	106.81	101.04	102.82	3.46	NI	Qualified
9	3	C422-1	Y-4	saline	4.19	4.35	4.63	4.39	0.22	I	Qualified
9	3	C525-1	PVC	saline	102.20	107.52	102.42	104.04	3.01	NI	Qualified
9	3	C546-1	PVC+Heptanal	saline	114.17	113.07	111.26	112.83	1.47	NI	Qualified
9	3	C801-1	PVC+SDS	saline	1.66	1.94	1.72	1.77	0.15	I	Qualified
9	3	C1-2	DPBS	SO	100.05	100.83	99.12	100.00	0.86	NI	Qualified
9	3	C2-2	1% SDS	SO	1.13	0.61	0.56	0.77	0.32	I	Qualified
9	3	C3-2	Sesame Oil	SO	101.30	104.00	96.67	100.66	3.71	NI	Qualified
9	3	C422-2	Y-4	SO	8.26	9.24	9.14	8.88	0.54	I	Qualified
9	3	C525-2	PVC	SO	105.15	106.40	102.29	104.61	2.11	NI	Qualified
9	3	C546-2	PVC+Heptanal	SO	16.06	12.78	14.45	14.43	1.64	I	Qualified
9	3	C801-2	PVC+SDS	SO	103.64	112.48	108.48	108.20	4.43	NI	Qualified

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
10	1	A1-1	DPBS	saline	98.37	100.94	100.69	100.00	1.42	NI	Qualified
10	1	A2-1	1% SDS	saline	0.83	0.58	0.76	0.72	0.13	I	Qualified
10	1	A3-1	Saline	saline	99.29	101.24	96.72	99.08	2.27	NI	Qualified
10	1	A205-1	PVC+Heptanal	saline	83.68	67.53	57.80	69.67	13.07	NI	Qualified
10	1	A266-1	PVC+SDS	saline	0.15	2.05	1.87	1.36	1.05	I	Qualified
10	1	A365-1	PVC	saline	100.82	98.67	103.51	101.00	2.42	NI	Qualified
10	1	A918-1	Y-4	saline	5.17	4.62	5.23	5.01	0.34	I	Qualified
10	1	A1-2	DPBS	SO	98.06	99.80	102.14	100.00	2.05	NI	Qualified
10	1	A2-2	1% SDS	SO	1.20	1.56	0.54	1.10	0.52	I	Qualified
10	1	A3-2	Sesame Oil	SO	104.78	94.10	104.36	101.08	6.05	NI	Qualified
10	1	A205-2	PVC+Heptanal	SO	25.16	16.57	14.89	18.88	5.51	I	Qualified
10	1	A266-2	PVC+SDS	SO	100.88	103.28	106.35	103.50	2.74	NI	Qualified
10	1	A365-2	PVC	SO	98.72	100.64	90.13	96.50	5.60	NI	Qualified
10	1	A918-2	Y-4	SO	8.11	7.21	7.39	7.57	0.48	I	Qualified
10	2	B1-1	DPBS	saline	95.36	101.25	103.39	100.00	4.16	NI	Qualified
10	2	B2-1	1% SDS	saline	0.73	0.66	0.99	0.79	0.17	I	Qualified
10	2	B2-1	Saline	saline	98.01	110.78	101.84	103.54	6.55	NI	Qualified
10	2	B379-1	Y-4	saline	4.16	4.88	4.29	4.45	0.38	I	Qualified
10	2	B523-1	PVC	saline	95.81	95.48	103.00	98.10	4.25	NI	Qualified
10	2	B754-1	PVC+Heptanal	saline	87.06	86.80	88.55	87.47	0.94	NI	Qualified
10	2	B944-1	PVC+SDS	saline	1.90	2.28	1.77	1.98	0.27	I	Qualified
10	2	B1-2	DPBS	SO	100.13	99.62	100.25	100.00	0.33	NI	Qualified
10	2	B2-2	1% SDS	SO	0.53	0.78	0.41	0.57	0.19	I	Qualified
10	2	B3-2	Sesame Oil	SO	105.21	105.08	110.60	106.96	3.15	NI	Qualified
10	2	B379-2	Y-4	SO	7.75	8.37	8.31	8.14	0.35	I	Qualified
10	2	B523-2	PVC	SO	105.02	94.79	102.38	100.73	5.31	NI	Qualified
10	2	B754-2	PVC+Heptanal	SO	21.54	19.41	19.16	20.04	1.31	I	Qualified
10	2	B944-2	PVC+SDS	SO	102.13	109.16	104.77	105.35	3.55	NI	Qualified
10	3	C1-1	DPBS	saline	94.42	106.79	98.79	100.00	6.27	NI	Qualified
10	3	C2-1	1% SDS	saline	1.45	-0.17	1.58	0.95	0.98	I	Qualified
10	3	C3-1	Saline	saline	109.29	99.48	104.35	104.37	4.90	NI	Qualified
10	3	C053-1	Y-4	saline	4.76	4.45	4.14	4.45	0.31	I	Qualified
10	3	C069-1	PVC+SDS	saline	3.08	2.08	1.95	2.37	0.62	I	Qualified
10	3	C485-1	PVC	saline	104.35	101.29	106.48	104.04	2.61	NI	Qualified
10	3	C536-1	PVC+Heptanal	saline	55.18	73.61	84.11	70.97	14.64	NI	Qualified
10	3	C1-2	DPBS	SO	108.52	89.20	102.28	100.00	9.86	NI	Qualified
10	3	C2-2	1% SDS	SO	1.07	-0.03	1.56	0.87	0.81	I	Qualified
10	3	C3-2	Sesame Oil	SO	109.19	108.83	114.03	110.68	2.90	NI	Qualified
10	3	C053-2	Y-4	SO	5.35	6.33	7.06	6.25	0.86	I	Qualified
10	3	C069-2	PVC+SDS	SO	109.44	110.91	114.15	111.50	2.41	NI	Qualified
10	3	C485-2	PVC	SO	111.03	106.14	107.73	108.30	2.50	NI	Qualified
10	3	C536-2	PVC+Heptanal	SO	22.05	17.64	18.01	19.23	2.44	I	Qualified

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
11	1	A1-1	DPBS	saline	94.92	100.94	104.14	100.00	4.68	NI	Qualified
11	1	A2-1	1% SDS	saline	2.07	1.48	2.17	1.91	0.37	I	Qualified
11	1	A3-1	Saline	saline	98.47	99.75	95.32	97.85	2.28	NI	Qualified
11	1	A226-1	PVC+SDS	saline	2.07	1.87	2.07	2.01	0.11	I	Qualified
11	1	A388-1	PVC+Heptanal	saline	101.92	102.47	105.28	103.22	1.80	NI	Qualified
11	1	A519-1	PVC	saline	98.27	92.01	94.67	94.99	3.14	NI	Qualified
11	1	A686-1	Y-4	saline	5.72	6.21	6.51	6.15	0.40	I	Qualified
11	1	A1-2	DPBS	SO	94.52	102.36	103.12	100.00	4.76	NI	Qualified
11	1	A2-2	1% SDS	SO	1.68	1.53	1.73	1.65	0.11	I	Qualified
11	1	A3-2	Sesame Oil	SO	103.62	106.10	112.93	107.55	4.82	NI	Qualified
11	1	A226-2	PVC+SDS	SO	104.53	113.79	107.52	108.61	4.72	NI	Qualified
11	1	A388-2	PVC+Heptanal	SO	12.30	15.54	15.03	14.29	1.74	I	Qualified
11	1	A519-2	PVC	SO	103.73	102.76	100.84	102.44	1.47	NI	Qualified
11	1	A686-2	Y-4	SO	7.70	9.27	9.37	8.78	0.94	I	Qualified
11	2	B1-1	DPBS	saline	94.14	104.03	101.83	100.00	5.19	NI	Qualified
11	2	B2-1	1% SDS	saline	1.85	1.49	2.20	1.85	0.36	I	Qualified
11	2	B2-1	Saline	saline	98.93	108.41	106.93	104.76	5.10	NI	Qualified
11	2	B236-1	PVC	saline	101.43	100.15	102.09	101.22	0.98	NI	Qualified
11	2	B336-1	Y-4	saline	7.61	5.67	7.66	6.98	1.13	I	Qualified
11	2	B981-1	PVC+Heptanal	saline	107.08	105.86	102.24	105.06	2.52	NI	Qualified
11	2	B983-1	PVC+SDS	saline	2.61	5.77	2.97	3.78	1.73	I	Qualified
11	2	B1-2	DPBS	SO	97.72	103.34	98.94	100.00	2.96	NI	Qualified
11	2	B2-2	1% SDS	SO	4.45	2.40	1.18	2.67	1.65	I	Qualified
11	2	B3-2	Sesame Oil	SO	109.11	105.33	104.11	106.18	2.61	NI	Qualified
11	2	B236-2	PVC	SO	103.29	104.62	101.81	103.24	1.41	NI	Qualified
11	2	B336-2	Y-4	SO	9.20	12.52	8.89	10.20	2.01	I	Qualified
11	2	B981-2	PVC+Heptanal	SO	15.13	15.28	14.77	15.06	0.26	I	Qualified
11	2	B983-2	PVC+SDS	SO	101.04	106.15	103.44	103.54	2.56	NI	Qualified
11	3	C1-1	DPBS	saline	96.65	102.72	100.63	100.00	3.08	NI	Qualified
11	3	C2-1	1% SDS	saline	1.84	1.44	1.94	1.74	0.26	I	Qualified
11	3	C3-1	Saline	saline	94.61	103.91	95.81	98.11	5.06	NI	Qualified
11	3	C401-1	PVC+SDS	saline	3.33	2.39	3.78	3.17	0.71	I	Qualified
11	3	C556-1	Y-4	saline	5.42	6.02	4.62	5.35	0.70	I	Qualified
11	3	C679-1	PVC+Heptanal	saline	101.92	97.55	98.69	99.39	2.27	NI	Qualified
11	3	C738-1	PVC	saline	102.52	102.02	96.70	100.41	3.22	NI	Qualified
11	3	C1-2	DPBS	SO	100.68	98.39	100.93	100.00	1.40	NI	Qualified
11	3	C2-2	1% SDS	SO	1.66	1.61	2.11	1.80	0.27	I	Qualified
11	3	C3-2	Sesame Oil	SO	101.08	105.15	104.50	103.58	2.19	NI	Qualified
11	3	C401-2	PVC+SDS	SO	102.57	102.91	102.17	102.55	0.37	NI	Qualified
11	3	C556-2	Y-4	SO	8.57	10.85	8.52	9.31	1.33	I	Qualified
11	3	C679-2	PVC+Heptanal	SO	17.31	20.33	16.56	18.07	2.00	I	Qualified
11	3	C738-2	PVC	SO	102.91	102.07	95.46	100.15	4.08	NI	Qualified

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
12	1	A1-1	DPBS	saline	95.99	102.51	101.49	100.00	3.51	NI	Qualified
12	1	A2-1	1% SDS	saline	-0.17	-0.17	-0.21	-0.18	0.03	I	Qualified
12	1	A3-1	Saline	saline	101.54	109.53	100.70	103.92	4.87	NI	Qualified
12	1	A079-1	PVC	saline	103.18	101.23	103.31	102.57	1.17	NI	Qualified
12	1	A153-1	PVC+Heptanal	saline	109.44	109.13	109.53	109.36	0.21	NI	Qualified
12	1	A306-1	Y-4	saline	5.69	6.58	6.27	6.18	0.45	I	Qualified
12	1	A771-1	PVC+SDS	saline	-0.03	-0.26	-0.08	-0.12	0.12	I	Qualified
12	1	A1-2	DPBS	SO	98.97	102.54	98.49	100.00	2.22	NI	Qualified
12	1	A2-2	1% SDS	SO	-0.53	-0.31	-0.13	-0.32	0.20	I	Qualified
12	1	A3-2	Sesame Oil	SO	98.57	104.40	102.10	101.69	2.93	NI	Qualified
12	1	A079-2	PVC	SO	100.16	99.99	93.81	97.98	3.62	NI	Qualified
12	1	A153-2	PVC+Heptanal	SO	13.72	15.71	16.55	15.33	1.45	I	Qualified
12	1	A306-2	Y-4	SO	8.96	9.57	9.75	9.43	0.42	I	Qualified
12	1	A771-2	PVC+SDS	SO	110.35	99.19	105.15	104.90	5.59	NI	Qualified
12	2	B1-1	DPBS	saline	106.49	92.64	100.86	100.00	6.96	NI	Qualified
12	2	B2-1	1% SDS	saline	-0.25	-0.51	-0.20	-0.32	0.17	I	Qualified
12	2	B2-1	Saline	saline	94.91	95.65	88.22	92.93	4.09	NI	Qualified
12	2	B042-1	Y-4	saline	4.75	6.91	6.65	6.10	1.18	I	Qualified
12	2	B427-1	PVC+SDS	saline	0.12	-0.04	-0.25	-0.06	0.18	I	Qualified
12	2	B612-1	PVC	saline	109.50	99.49	103.02	104.00	5.07	NI	Qualified
12	2	B781-1	PVC+Heptanal	saline	117.98	120.82	117.98	118.92	1.64	NI	Qualified
12	2	B1-2	DPBS	SO	105.39	96.77	97.83	100.00	4.70	NI	Qualified
12	2	B2-2	1% SDS	SO	-0.36	-0.20	-0.25	-0.27	0.08	I	Qualified
12	2	B3-2	Sesame Oil	SO	105.29	108.83	105.87	106.66	1.90	NI	Qualified
12	2	B042-2	Y-4	SO	13.39	11.80	10.48	11.89	1.46	I	Qualified
12	2	B427-2	PVC+SDS	SO	107.77	106.61	100.85	105.08	3.71	NI	Qualified
12	2	B612-2	PVC	SO	114.70	112.80	104.60	110.70	5.37	NI	Qualified
12	2	B781-2	PVC+Heptanal	SO	16.99	19.42	20.48	18.96	1.79	I	Qualified
12	3	C1-1	DPBS	saline	98.82	103.36	97.81	100.00	2.96	NI	Qualified
12	3	C2-1	1% SDS	saline	0.07	-0.12	-0.20	-0.08	0.14	I	Qualified
12	3	C3-1	Saline	saline	100.59	102.80	101.19	101.53	1.14	NI	Qualified
12	3	C047-1	Y-4	saline	6.86	5.69	6.82	6.46	0.66	I	Qualified
12	3	C062-1	PVC+SDS	saline	-0.05	-0.01	-0.05	-0.03	0.02	I	Qualified
12	3	C080-1	PVC+Heptanal	saline	121.00	117.55	106.89	115.15	7.35	NI	Qualified
12	3	C468-1	PVC	saline	98.82	100.96	108.81	102.86	5.26	NI	Qualified
12	3	C1-2	DPBS	SO	99.60	98.51	101.88	100.00	1.72	NI	Qualified
12	3	C2-2	1% SDS	SO	-0.14	-0.25	-0.33	-0.24	0.09	I	Qualified
12	3	C3-2	Sesame Oil	SO	102.18	98.74	103.72	101.55	2.55	NI	Qualified
12	3	C047-2	Y-4	SO	9.14	9.56	8.85	9.18	0.36	I	Qualified
12	3	C062-2	PVC+SDS	SO	108.10	94.47	106.15	102.91	7.37	NI	Qualified
12	3	C080-2	PVC+Heptanal	SO	17.61	23.37	17.42	19.47	3.38	I	Qualified
12	3	C468-2	PVC	SO	113.00	105.22	101.88	106.70	5.71	NI	Qualified

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
13	1	A1-1	DPBS	saline	100.31	96.95	102.74	100.00	2.91	NI	Qualified
13	1	A2-1	1% SDS	saline	1.28	1.12	1.24	1.21	0.08	I	Qualified
13	1	A3-1	Saline	saline	102.55	101.66	104.40	102.87	1.40	NI	Qualified
13	1	A126-1	PVC	saline	110.41	100.19	104.35	104.98	5.14	NI	Qualified
13	1	A541-1	PVC+SDS	saline	1.54	2.48	1.67	1.90	0.51	I	Qualified
13	1	A606-1	PVC+Heptanal	saline	101.11	108.60	105.92	105.21	3.80	NI	Qualified
13	1	A995-1	Y-4	saline	3.91	3.98	4.56	4.15	0.36	I	Qualified
13	1	DPBS	DPBS	SO	96.08	100.53	103.39	100.00	3.69	NI	Qualified
13	1	1% SDS	1% SDS	SO	1.09	1.50	0.99	1.19	0.27	I	Qualified
13	1	Sesame Oil	Sesame Oil	SO	107.74	107.31	108.31	107.79	0.50	NI	Qualified
13	1	A126-2	PVC	SO	100.82	100.39	103.58	101.59	1.73	NI	Qualified
13	1	A541-2	PVC+SDS	SO	107.33	101.91	100.87	103.37	3.47	NI	Qualified
13	1	A606-2	PVC+Heptanal	SO	18.62	20.24	15.29	18.05	2.52	I	Qualified
13	1	A995-2	Y-4	SO	7.24	8.68	6.08	7.33	1.30	I	Qualified
13	2	B1-1	DPBS	saline	105.99	94.52	99.48	100.00	5.75	NI	Qualified
13	2	B2-1	1% SDS	saline	1.24	1.28	1.73	1.42	0.28	I	Qualified
13	2	B2-1	Saline	saline	101.63	103.28	99.56	101.49	1.86	NI	Qualified
13	2	B024-1	Y-4	saline	4.92	4.93	5.07	4.97	0.08	I	Qualified
13	2	B130-1	PVC+SDS	saline	1.88	2.42	2.17	2.16	0.27	I	Qualified
13	2	B156-1	PVC	saline	106.61	103.75	103.07	104.48	1.88	NI	Qualified
13	2	B579-1	PVC+Heptanal	saline	108.91	107.41	108.48	108.27	0.77	NI	Qualified
13	2	B1-2	DPBS	SO	104.21	98.87	96.91	100.00	3.78	NI	Qualified
13	2	B2-2	1% SDS	SO	1.30	1.25	1.28	1.28	0.02	I	Qualified
13	2	B3-2	Sesame Oil	SO	107.55	111.82	101.80	107.06	5.02	NI	Qualified
13	2	B024-2	Y-4	SO	8.20	9.29	8.09	8.53	0.67	I	Qualified
13	2	B130-2	PVC+SDS	SO	101.68	104.76	109.38	105.27	3.87	NI	Qualified
13	2	B156-2	PVC	SO	105.60	103.89	109.97	106.48	3.14	NI	Qualified
13	2	B579-2	PVC+Heptanal	SO	19.43	11.35	18.13	16.30	4.34	I	Qualified
13	3	C1-1	DPBS	saline	88.37	102.84	108.79	100.00	10.50	NI	Qualified
13	3	C2-1	1% SDS	saline	1.34	1.56	1.59	1.50	0.13	I	Qualified
13	3	C3-1	Saline	saline	94.47	101.95	103.83	100.08	4.95	NI	Qualified
13	3	C013-1	Y-4	saline	4.81	5.58	5.32	5.24	0.39	I	Qualified
13	3	C176-1	PVC	saline	104.90	100.71	104.47	103.36	2.31	NI	Qualified
13	3	C195-1	PVC+SDS	saline	1.81	1.92	2.16	1.96	0.18	I	Qualified
13	3	C522-1	PVC+Heptanal	saline	112.05	110.39	113.97	112.14	1.79	NI	Qualified
13	3	C1-2	DPBS	SO	100.62	102.42	96.97	100.00	2.78	NI	Qualified
13	3	C2-2	1% SDS	SO	1.34	0.74	1.27	1.12	0.33	I	Qualified
13	3	C3-2	Sesame Oil	SO	100.27	102.15	102.37	101.60	1.15	NI	Qualified
13	3	C013-2	Y-4	SO	7.78	9.23	9.34	8.78	0.87	I	Qualified
13	3	C176-2	PVC	SO	107.19	114.28	105.99	109.15	4.48	NI	Qualified
13	3	C195-2	PVC+SDS	SO	111.64	109.98	113.94	111.85	1.99	NI	Qualified
13	3	C522-2	PVC+Heptanal	SO	18.04	19.91	17.86	18.61	1.13	I	Qualified

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
14	1	A1-1	DPBS	saline	98.42	101.75	99.83	100.00	1.67	NI	Qualified
14	1	A2-1	1% SDS	saline	1.88	0.17	2.77	1.61	1.32	I	Qualified
14	1	A3-1	Saline	saline	99.74	89.64	96.00	95.13	5.11	NI	Qualified
14	1	A063-1	Y-4	saline	9.35	10.23	10.41	10.00	0.57	I	Qualified
14	1	A119-1	PVC+SDS	saline	2.15	3.30	1.81	2.42	0.78	I	Qualified
14	1	A681-1	PVC+Heptanal	saline	91.16	95.01	91.66	92.61	2.09	NI	Qualified
14	1	A839-1	PVC	saline	95.53	104.38	103.09	101.00	4.78	NI	Qualified
14	1	A1-2	DPBS	SO	98.07	100.02	101.91	100.00	1.92	NI	Qualified
14	1	A2-2	1% SDS	SO	3.49	3.70	1.31	2.83	1.32	I	Qualified
14	1	A3-2	Sesame Oil	SO	104.90	103.38	96.66	101.65	4.39	NI	Qualified
14	1	A063-2	Y-4	SO	11.37	12.49	13.09	12.32	0.87	I	Qualified
14	1	A119-2	PVC+SDS	SO	108.54	107.93	106.81	107.76	0.88	NI	Qualified
14	1	A681-2	PVC+Heptanal	SO	13.84	15.88	15.80	15.17	1.16	I	Qualified
14	1	A839-2	PVC	SO	109.95	105.75	107.91	107.87	2.10	NI	Qualified
14	2	B1-1	DPBS	saline	99.71	97.60	102.69	100.00	2.56	NI	Qualified
14	2	B2-1	1% SDS	saline	0.46	3.54	3.83	2.61	1.87	I	Qualified
14	2	B2-1	Saline	saline	105.72	102.52	109.59	105.94	3.54	NI	Qualified
14	2	B027-1	PVC+Heptanal	saline	107.51	106.39	96.94	103.61	5.81	NI	Qualified
14	2	B064-1	Y-4	saline	9.64	7.51	8.62	8.59	1.07	I	Qualified
14	2	B594-1	PVC	saline	106.26	101.83	99.71	102.60	3.34	NI	Qualified
14	2	B874-1	PVC+SDS	saline	2.82	2.81	3.25	2.96	0.25	I	Qualified
14	2	B1-2	DPBS	SO	102.69	96.21	101.10	100.00	3.38	NI	Qualified
14	2	B2-2	1% SDS	SO	1.63	2.60	0.38	1.54	1.11	I	Qualified
14	2	B3-2	Sesame Oil	SO	98.69	99.68	99.99	99.45	0.68	NI	Qualified
14	2	B027-2	PVC+Heptanal	SO	18.87	19.08	19.30	19.08	0.22	I	Qualified
14	2	B064-2	Y-4	SO	10.94	10.36	7.19	9.49	2.02	I	Qualified
14	2	B594-2	PVC	SO	110.53	101.85	104.81	105.73	4.41	NI	Qualified
14	2	B874-2	PVC+SDS	SO	104.60	106.14	102.89	104.54	1.62	NI	Qualified
14	3	C1-1	DPBS	saline	101.43	97.27	101.31	100.00	2.37	NI	Qualified
14	3	C2-1	1% SDS	saline	3.24	2.91	0.19	2.11	1.67	I	Qualified
14	3	C3-1	Saline	saline	97.74	102.77	93.90	98.14	4.45	NI	Qualified
14	3	C307-1	Y-4	saline	10.05	12.62	6.88	9.85	2.88	I	Qualified
14	3	C769-1	PVC+SDS	saline	2.93	3.65	2.79	3.12	0.46	I	Qualified
14	3	C914-1	PVC	saline	98.65	102.77	97.96	99.80	2.60	NI	Qualified
14	3	C978-1	PVC+Heptanal	saline	93.46	97.84	92.58	94.63	2.82	NI	Qualified
14	3	C1-2	DPBS	SO	96.34	101.69	101.96	100.00	3.17	NI	Qualified
14	3	C2-2	1% SDS	SO	2.48	3.19	3.68	3.12	0.60	I	Qualified
14	3	C3-2	Sesame Oil	SO	105.24	106.42	103.81	105.16	1.31	NI	Qualified
14	3	C307-2	Y-4	SO	9.72	11.60	9.22	10.18	1.26	I	Qualified
14	3	C769-2	PVC+SDS	SO	102.42	104.27	98.59	101.76	2.90	NI	Qualified
14	3	C914-2	PVC	SO	105.86	108.57	109.10	107.84	1.74	NI	Qualified
14	3	C978-2	PVC+Heptanal	SO	21.60	20.21	24.57	22.13	2.22	I	Qualified

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
15	1	A1-1	DPBS	saline	98.10	97.72	104.19	100.00	3.63	NI	Qualified
15	1	A2-1	1% SDS	saline	-0.14	-0.10	-0.14	-0.12	0.02	I	Qualified
15	1	A3-1	Saline	saline	101.22	106.71	104.06	103.99	2.75	NI	Qualified
15	1	A281-1	PVC+SDS	saline	0.23	0.01	0.16	0.13	0.11	I	Qualified
15	1	A576-1	PVC+Heptanal	saline	95.65	106.65	101.49	101.26	5.50	NI	Qualified
15	1	A583-1	PVC	saline	109.23	106.80	114.37	110.13	3.87	NI	Qualified
15	1	A938-1	Y-4	saline	0.15	0.18	0.13	0.15	0.03	I	Qualified
15	1	A1-2	DPBS	SO	96.91	98.34	104.74	100.00	4.17	NI	Qualified
15	1	A2-2	1% SDS	SO	-0.16	-0.22	-0.14	-0.18	0.04	I	Qualified
15	1	A3-2	Sesame Oil	SO	106.53	121.21	113.22	113.65	7.35	NI	Qualified
15	1	A281-2	PVC+SDS	SO	122.44	105.48	127.78	118.56	11.64	NI	Qualified
15	1	A576-2	PVC+Heptanal	SO	16.27	19.68	18.52	18.16	1.73	I	Qualified
15	1	A583-2	PVC	SO	118.25	123.69	100.58	114.17	12.08	NI	Qualified
15	1	A938-2	Y-4	SO	-0.24	-0.22	-0.27	-0.24	0.02	I	Qualified
15	2	B1-1	DPBS	saline	95.30	105.76	98.94	100.00	5.31	NI	Qualified
15	2	B2-1	1% SDS	saline	-0.09	-0.19	-0.08	-0.12	0.06	I	Qualified
15	2	B2-1	Saline	saline	83.88	90.46	87.79	87.38	3.31	NI	Qualified
15	2	B375-1	PVC+SDS	saline	-0.11	-0.12	-0.06	-0.10	0.03	I	Qualified
15	2	B442-1	PVC	saline	110.96	114.27	91.60	105.61	12.25	NI	Qualified
15	2	B537-1	PVC+Heptanal	saline	137.95	109.69	107.79	118.48	16.90	NI	Qualified
15	2	B972-1	Y-4	saline	-0.18	-0.13	-0.21	-0.17	0.04	I	Qualified
15	2	B1-2	DPBS	SO	102.14	97.48	100.38	100.00	2.35	NI	Qualified
15	2	B2-2	1% SDS	SO	-0.09	-0.19	-0.08	-0.12	0.06	I	Qualified
15	2	B3-2	Sesame Oil	SO	82.89	72.46	87.12	80.82	7.55	NI	Qualified
15	2	B375-2	PVC+SDS	SO	97.66	87.42	87.69	90.92	5.83	NI	Qualified
15	2	B442-2	PVC	SO	115.67	98.88	121.07	111.88	11.57	NI	Qualified
15	2	B537-2	PVC+Heptanal	SO	-0.19	-0.13	-0.18	-0.17	0.03	I	Qualified
15	2	B972-2	Y-4	SO	-0.20	-0.16	-0.18	-0.18	0.02	I	Qualified
15	3	DPBS	DPBS	saline	90.49	104.11	105.40	100.00	8.26	NI	Qualified
15	3	1% SDS	1% SDS	saline	0.15	0.07	-0.01	0.07	0.08	I	Qualified
15	3	Saline	Saline	saline	107.31	104.20	113.57	108.36	4.78	NI	Qualified
15	3	C137-1	PVC+SDS	saline	0.07	0.37	0.14	0.19	0.15	I	Qualified
15	3	C199-1	PVC+Heptanal	saline	101.98	104.30	99.38	101.89	2.46	NI	Qualified
15	3	C623-1	PVC	saline	112.36	75.86	108.63	98.95	20.08	NI	Failed
15	3	C908-1	Y-4	saline	-0.10	0.20	0.06	0.05	0.15	I	Qualified
15	3	DPBS	DPBS	SO	103.42	94.54	102.04	100.00	4.77	NI	Qualified
15	3	1% SDS	1% SDS	SO	0.03	-0.02	0.32	0.11	0.19	I	Qualified
15	3	Sesame Oil	Sesame Oil	SO	102.91	79.97	95.78	92.89	11.74	NI	Qualified
15	3	C137-2	PVC+SDS	SO	103.19	102.16	81.42	95.59	12.28	NI	Qualified
15	3	C199-2	PVC+Heptanal	SO	1.04	0.32	0.93	0.76	0.39	I	Qualified
15	3	C623-2	PVC	SO	99.78	98.30	96.05	98.04	1.88	NI	Qualified
15	3	C908-2	Y-4	SO	0.05	0.44	0.10	0.20	0.21	I	Qualified

					% viability (tripicalte)						
Lab No.	Exp. No.	Code No.	Sample name	Vehicle	1	2	3	Mean of viabilities [%]	SD of viabilities	Calssification	SD criterium
16	1	A1-1	DPBS	saline	95.56	101.21	103.23	100.00	3.98	NI	Qualified
16	1	A2-1	1% SDS	saline	4.81	4.97	4.97	4.92	0.09	I	Qualified
16	1	A3-1	Saline	saline	99.07	105.04	103.84	102.65	3.16	NI	Qualified
16	1	A007-1	PVC	saline	96.93	99.40	99.78	98.70	1.55	NI	Qualified
16	1	A330-1	PVC+SDS	saline	3.06	2.89	5.19	3.71	1.28	I	Qualified
16	1	A897-1	Y-4	saline	9.14	11.60	9.69	10.14	1.29	I	Qualified
16	1	A899-1	PVC+Heptanal	saline	91.83	91.34	100.77	94.65	5.31	NI	Qualified
16	1	A1-2	DPBS	SO	98.49	101.49	100.02	100.00	1.50	NI	Qualified
16	1	A2-2	1% SDS	SO	4.58	5.46	4.58	4.87	0.50	I	Qualified
16	1	A3-2	Sesame Oil	SO	101.55	100.89	104.00	102.15	1.64	NI	Qualified
16	1	A007-2	PVC	SO	104.22	98.93	104.17	102.44	3.04	NI	Qualified
16	1	A330-2	PVC+SDS	SO	97.13	99.31	100.24	98.89	1.60	NI	Qualified
16	1	A897-2	Y-4	SO	12.82	12.60	12.99	12.80	0.19	I	Qualified
16	1	A899-2	PVC+Heptanal	SO	28.37	26.46	29.47	28.10	1.52	I	Qualified
16	2	B1-1	DPBS	saline	94.83	105.39	99.78	100.00	5.28	NI	Qualified
16	2	B2-1	1% SDS	saline	3.72	5.39	5.17	4.76	0.91	I	Qualified
16	2	B2-1	Saline	saline	99.28	97.72	99.22	98.74	0.88	NI	Qualified
16	2	B221-1	PVC+Heptanal	saline	91.16	92.11	88.38	90.55	1.94	NI	Qualified
16	2	B407-1	Y-4	saline	10.51	11.40	11.78	11.23	0.66	I	Qualified
16	2	B511-1	PVC+SDS	saline	6.50	4.78	3.89	5.06	1.33	I	Qualified
16	2	B571-1	PVC	saline	99.56	100.67	97.83	99.35	1.43	NI	Qualified
16	2	B1-2	DPBS	SO	102.98	96.16	100.86	100.00	3.49	NI	Qualified
16	2	B2-2	1% SDS	SO	4.67	4.04	4.16	4.29	0.34	I	Qualified
16	2	B3-2	Sesame Oil	SO	95.47	96.56	107.11	99.71	6.43	NI	Qualified
16	2	B221-2	PVC+Heptanal	SO	16.42	17.51	17.97	17.30	0.79	I	Qualified
16	2	B407-2	Y-4	SO	13.61	14.76	13.27	13.88	0.78	I	Qualified
16	2	B511-2	PVC+SDS	SO	106.36	100.17	105.85	104.13	3.44	NI	Qualified
16	2	B571-2	PVC	SO	102.35	102.81	96.79	100.65	3.35	NI	Qualified
16	3	C1-1	DPBS	saline	92.83	104.55	102.62	100.00	6.28	NI	Qualified
16	3	C2-1	1% SDS	saline	4.12	3.95	3.66	3.91	0.23	I	Qualified
16	3	C3-1	Saline	saline	102.51	98.26	92.04	97.60	5.26	NI	Qualified
16	3	C109-1	PVC+SDS	saline	5.47	4.91	4.91	5.10	0.33	I	Qualified
16	3	C376-1	PVC+Heptanal	saline	92.55	92.72	96.45	93.91	2.21	NI	Qualified
16	3	C484-1	PVC	saline	100.02	100.58	101.21	100.60	0.59	NI	Qualified
16	3	C665-1	Y-4	saline	10.45	10.17	9.60	10.08	0.43	I	Qualified
16	3	C1-2	DPBS	SO	95.67	100.33	104.00	100.00	4.18	NI	Qualified
16	3	C2-2	1% SDS	SO	3.76	2.07	4.40	3.41	1.21	I	Qualified
16	3	C3-2	Sesame Oil	SO	105.17	100.91	109.02	105.03	4.05	NI	Qualified
16	3	C109-2	PVC+SDS	SO	109.02	105.29	104.12	106.14	2.56	NI	Qualified
16	3	C376-2	PVC+Heptanal	SO	31.17	32.10	36.01	33.09	2.57	I	Qualified
16	3	C484-2	PVC	SO	109.25	107.56	108.73	108.51	0.87	NI	Qualified
16	3	C665-2	Y-4	SO	13.44	13.21	13.62	13.42	0.20	I	Qualified

Appendix 5

LabCyte EPI-MODEL24 Round Robin
Phase II QC results report (in Japanese)

LabCyte EPI-MODEL24 ラウンドロビン研究

Phase II　QC 結果報告書
2023.02.10

実施者：山影康次

LabCyte EPI-MODEL24 ラウンドロビン研究に参加した 16 試験施設の phase II（施設内および施設間再現性確認）の試験資料について、「LabCyte EPI-MODEL24 ラウンドロビン研究計画」（ Version 2.0、July 19th 2022）および「ヒト三次元培養皮膚 LabCyte EPI-MODEL24 を用いた医療機器抽出物の皮膚刺激性試験　国内 Round Robin Study 本試験用プロトコル Ver.1.1」をもとに quality check (QC) を実施した。

研究計画書に「この試験は非 GLP で実施」と記載されていること、ラウンドロビン研究であることから、試験結果の評価が主題であり、各操作を実施した記録の有無が重要であるという観点から、QC は試験記録の記載不備およびプロトコルからの逸脱の有無等に焦点をあてて確認した。なお、参考までに、QC 実施者として GLP 上の視点から気になった点も記載した。

<このラウンドロビン研究の資料作成に対する QC 実施者からの感想>

1, 研究計画について

Phase II 実施前の 2022.07.19 付けで version 2.0 が発行されているが、内容的にはこの研究開始時に作成したと推定される記載となっており、Phase II の研究計画作成時における時系列が曖昧という印象を持った。なぜなら、Phase II の研究計画作成時に終了している phase I(2021 年 1 月下旬～4 月上旬)の結果が研究計画のスケジュール表に記載されているのみであり、それ以外は計画なのか実施済なのか不明であった。

2. プロトコルについて

各試験施設の記録を確認した結果、気になる点の多くがプロトコルに添付されている記録用紙に関連していると考えられた。以下に確認された具体的事項を示したが、最低限の操作は実施された記録であること、また、時間の許容幅が大きいことから、いずれもプロトコルからの逸脱には該当していないと推察された。しかしながら、各試験施設の記載内容を踏まえると試験施設へのプロトコルの説明が十分であったか疑問が持たれた。

<具体的事項 1>

すべての試験施設がプロトコルの記録用紙と異なる試験記録を使用していた。その差異は最初の記録用紙（プロトコルの 17 ページ）に「実験スケジュール表」追加されている点および前培養の記録用紙以外の操作について、プレート毎に開始時間および終了時間を記載するように変更されている点であった。

研究計画では 2022 年 8 月からこの研究が開始されているが、8 月に実験を開始した 2 施設ではプロトコル通りの記録用紙と上記の点が追加・変更された記録用紙が混在していたことから、試験開

始途中でプロトコルの改定が行われたと考えられる。しかし、その記録がなく、プロトコルの記録管理が十分であったか疑問が持たれた。

<具体的事項 2>

マイクロピペットの点検については、プロトコル 18 ページの記録用紙に「本作業は試験の開始前に毎回行ってください」と記載されているが、毎日、操作前に点検を実施した試験施設は 2 施設のみであり、殆どは、各実験の抽出開始前に 1 度実施するのみであった。

<具体的事項 3>

前培養（15~30 hrs)、曝露（18±1 hr）について、時間範囲がプロトコルに規定されているが、記録用紙に記載されている時刻が施設間で異なっていた。具体的には、実験スケジュール表にはプレート毎（Saline、Sesame oil）に開始時刻と終了時刻を記載する欄があるが、プロトコル 22 ページの記録用紙には「開始日（時間)」と「終了日（時間)」の記入欄が各 1 箇所のみのためか、22 ページの記録用紙にプレート毎の時刻を記載する試験施設もあれば、実験スケジュールに 2 プレートとも同じ時刻を記載する施設、実験スケジュール表と 22 ページの記録用紙の時刻が一致しない試験施設、アッセイ培地の添加を開始した時刻と添加が終了した時刻と勘違いしたと思われる施設などが認められた。曝露時間についても、モデルに抽出液添加を開始した時刻を記載した試験施設ではプレート毎に異なる開始時刻を記載しているが、プロトコル 23 ページの記録用紙にある培養開始時間を記載した試験施設では、2 プレートとも同じ曝露開始時間を記載していた。また、実験スケジュール表の各操作の実施日欄が 1 つであるため、2 日間にまたがる操作について、日付の記載が施設間でばらばらであった。

<具体的事項 4>

抽出温度については、プロトコル 20 ページの試験液の調製の表に CO_2 インキュベータの情報を記載した試験施設については、37℃で抽出したと推察できたが、振盪器を使用した試験施設(試験施設 1、2、3。4、6、7、8、11、12、13)については、抽出温度を記載する欄がなく、抽出温度を確認できなかった。なお、試験施設 5 では振盪器をインキュベータにセットした写真が添付され、試験施設 9、14 および 15 では振盪器による抽出温度データが添付され、試験施設 10 では振盪器の設定温度が記載されていた。試験施設 16 では CO_2 インキュベータを使用し、攪拌による抽出を実施していた。

<具体的事項 5>

Excel の集計表の「Date」が測定日なのか、表作成日(データ入力日)なのか不明確であった。生データである OD 測定結果印刷日よりも前の日付で結果表が作成されている試験施設があり、日付による試験操作のトレースができなかった。

3. GLP 上の観点から

・ピペットの点検について、秤量値を入力して作成した表を添付した試験施設があったが、その表の正確性が判断できなかった。また、実際の秤量値ではなく、計算結果である%を表示した試験施設についても結果の正確性が判断できなかった。

・洗浄操作について、2 プレートほぼ同時刻に実施されていた試験施設があり、2 人で操作したと考えられたが、実施者の記入欄がなく、プレート毎の洗浄時刻の記載内容の正確性を判断できなかった。GLP の観点から考えると、実施者の記入欄があれば、その判断も容易であると考えられる。

・曝露（18±1 hr）に関する記録用紙の記入欄は「○○○培養開始時間」および「○○○培養終了時間」となっていたが、実験スケジュール上 2 日間の作業となることは明確であるが、操作時間の記入欄だけではなく、日付記入欄も必要と思われた。殆どの試験施設は日付を記載していたが、日付の記載のない記録用紙も散見された。
・吸光度測定機器による測定結果の印刷データは生データと考えられることから、実行委員会側で作成された Excel 集計結果表以外に印刷データ(生データ)を試験資料に添付する必要があると思われる。添付されていない試験施設はわずかであったが、GLP 上は必須事項である。

<各施設の試験資料の QC 結果>

以下に試験施設毎の QC 結果を示した。なお、プロトコルの記録用紙の問題以外を記載した。

<試験施設 1>
・ 1 回目の再試験(2023.01.09 抽出開始)において、使用期限(2022.12)を過ぎたゴマ油が使用されていた。

<試験施設 2>
・1 回目の試験において、「5-1 前培養」の開始日と終了日が 2022.11.16 と同じ日であった。
・2 回目の試験の 2022.11.24 に実施したピペット点検において、ポジティブディスプレイスメントピペット用のピペット名が記載されているが、秤量値や計算値が記載されていなかった。
・2 回目の試験の「試験液配置図：Layout」の試験試料名の記載の修正（2022.12.12 付けで”A”を”B”に修正）が 3 回の試験すべてが終了（2022.12.09）してから行われていた。
・1 回目の試験の吸光度測定結果（生データ）が添付されていなかった(試験は不成立)。また、2 回目および 3 回目の測定結果（生データ）は添付されていたが、Saline の結果なのか、Sesame oil の結果なのか確認できなかった。

<試験施設 3>
・ピペット点検の表に測定した蒸留水の重量ではなく、%が記載されていた。

<試験施設 4>
・プロトコルの記録用紙の指摘以外は特になし。

<試験施設 5>
・ピペット点検結果が表にまとめられていた。

<試験施設 6>
・冷蔵庫の温度が記録用紙(プロトコル、22 ページ)では「5±2℃」となっているが、この施設では「1~10℃」と記載されていた。
・ピペット点検において、2 mL の点検が実施されていなかった。また、ポジティブディスプレイスメントピペットについては、900 µL および 300 µL 以外の 2 mL、200 µL、100 µL について点検が実施

されていなかった。
・2 回目の試験以外の陽性対照の調製に関する記録用紙(プロトコル、21 ページ)において、調製液量が印字のみとなっており、調製が実施されたれた記録であることが確認できなかった。
・MTT 試験の plate1 と plate2 のインキュベート時間が同じ開始時刻、同じ終了時刻であった。

<試験施設 7>
・1 回目の試験の記録用紙(プロトコル、23 ページ)の培養開始時刻および培養終了時刻のチェック欄「□」がチェックされていなかった。
・2 回目の試験の記録用紙(プロトコル、20 ページ)の抽出前後の外観に「細胞が半透明」と記載されており、抽出液調製後の変化ではなく、曝露後の変化と思われる内容が記載されていた。
・再試験(1 回目)の記録用紙(プロトコル、20 ページ)の試験試料名に「'」がついないが、他の記録用紙の試験試料名にはそれが記載されており整合性がとれていなかった。
・各プレートの吸光度測定値の印刷結果(生データ)には 3 つのデータが存在していたが、それらがどの結果(570 nm、650 nm、換算値)を示しているか判断できなかった。

<試験施設 8>
・1 回目の試験の記録用紙(プロトコル、26 ページ)の 96 ウェルプレートの配置図に試験試料名が記載されておらず、結果表の入力値の正確性が確認できなかった。
・2 回目の試験の記録用紙(プロトコル、20 ページ)の試験液の調製の表に試験試料名が記載されていなかった。
・2 回目の記録用紙(プロトコル、23 ページ)の PLATE2 培養終了時間のチェック欄「□」がチェックされていなかった。
・吸光度測定結果(生データ)が添付されていなかったが、後日、 3 回分の生データが提出された。

<試験施設 9>
・プロトコルの記録用紙の指摘以外は特になし

<試験施設 10>
・ポジティブディスプレイスメントピペット用のピペット点検が行われていなかった。
・MTT 試験の plate1 と plate2 のインキュベート時間が同じ開始時刻、同じ終了時刻であった。

<試験施設 11>
・試験液配置図の記録用紙(プロトコル、23 ページ)の培養開始時間および培養終了時間の日付および時刻は記載されていたが、チェック欄「□」のチェックがされていなかった。
・1 回目の記録用紙(プロトコル、24 ページ)の洗浄前の準備のチェック欄「□」がチェックされていなかった。
・3 回目の記録用紙(プロトコル、18 ページ)の B19 ピペットの 2 mL の秤量値の平均値に誤りがあった(2004.3 mg→2000.4 mg)。

<試験施設 12>

・1 回目の試験以外の MTT 試験の plate1 と plate2 のインキュベート時間が同じ開始時刻、同じ終了時刻であった。

<試験施設 13>

・ピペット点検結果が表にまとめられていた。
・2 回目の試験の記録用紙(プロトコル、24 ページ)の 18±1 時間曝露後の外観異常がないのチェック欄「□」がチェックされていたが、備考欄に外観異常が記載されていた。
・MTT 試験の plate1 と plate2 のインキュベート時間が同じ開始時刻、同じ終了時刻であった。

<試験施設 14>

・1 回目の試験の試験液配置図、PLATE2 の NC および VC のチェック欄「□」がチェックされていなかった。
・吸光度測定結果が添付されていたが、plate1(saline)と plate2(sesame oil)の識別ができなかった。

<試験施設 15>

・1 回目の試験において、実験スケジュール表の前培養の実施日が 2022.12.14 日のみの記載であり、かつ、前培養の記録用紙(プロトコル、22 ページ)の開始時間および終了時間とも異なり、前培養における操作時刻の確認・推定ができなかった。
・ポジティブディスプレイスメントピペット用のピペット点検が行われていなかった。
・1 回目の記録用紙(プロトコル、22 ページ)の試験液の暴露のチェック欄「□」がチェックされていなかった。
・1 回目の試験において、抽出時の Code name が「A583」であったが、試験液配置図および測定時のプレート配置図では「A538」となっていた。
・1 回目の試験において、PLATE 2 の試験液配置図に記載された Code name が「A576-1」、「A938-1」となっており、ゴマ油抽出時の試験試料名と異なる Code name が記載されていた。また、測定時の PLATE 2 の配置図においても Code name が「A576-1」、「A938-1」、「A538-1」となっていた。
・1 回目と 2 回目の試験において、MTT 試験の plate1 と plate2 のインキュベート時間が同じ開始時刻、同じ終了時刻であった。
・2 回目の試験において、抽出時の試験試料名の Code name が生理食塩液およびコマ油ともに「B442-1」、「B537-1」、「B972-2」であったが、生理食塩液の試験液の配置図では「B972-1」、ゴマ油の試験液の配置図では「B442-2」、「B537-2」、「B972-1」であった。さらに、測定時のプレート配置図では、「○○○○-1」、「○○○○-2」の記載ではなく、「○○○○」のみの記載であった。しかし、集計結果のエクセル表の配置図では、生理食塩液は「○○○○-1」、ゴマ油は「○○○○-2」と入力されていた。
・2 回目の試験において、アッセイ培地のロット No.および使用期限の記載がなかった。また、その外観の異常の有無のチェックも行われていなかった。
・3 回目の試験(2023.01.23 抽出開始)において、使用期限(2022.12)を過ぎたゴマ油が使用されていた。
・3 回目の試験の測定時のプレート配置図において、PLATE 1 の Code name が抽出時の「C137-1」で

はなく「C173-1」と記載されていた。

<試験施設 16>

・吸光度測定結果が添付されていたが、(570 nm の吸光度－650 nm 吸光度)と思われる計算値のみであった。

＜試験判定結果確認＞

試験施設番号	試験 1	試験 2	試験 3	再試験 1
1 (安評)	✖	○	○	ゴマ油使用期限切れ
2 (川澄)	✖	○	○	○
3 (LSIM)	○	○	○	
4 (OLYMPUS)	○	○	○	
5 (SERI)	○	○	○	
6 (化合物安研)	○	○	○	
7 (鎌倉テクノ)	✖	○	○	○
8 (シミック)	○	○	○	
9 (食薬センター)	○	○	○	
10(テルモ)	○	○	○	
11(日精バイリス)	○	○	○	
12(ニプロ)	○	○	○	
13(日本食品分析)	○	○	○	
14(日本バイオリ)	○	○	○	
15(ボゾリサーチ)	○	○	ゴマ油使用期限切れ	
16(薬物安全セ)	○	○	○	

以上