LabCyte EPI-MODEL24 *In Vitro* Skin Corrosion Test Method

Peer Review Panel Evaluation

of

the Performance-based Validation Study on the LabCyte EPI-MODEL24 *in vitro* skin corrosion test method as a me-too test method according to OECD GD 219 and falling within the OECD TG 431

26 October 2018

Table of Contents

Summary

The LabCyte EPI-MODEL24 skin corrosion test (SCT) method, developed by Japanese Tissue Engineering Co., Ltd (J-TEC), aims at the identification of corrosive and non-corrosive substances, as well as to the sub-categorization of corrosive substances into sub-category 1A, according to the UN GHS, as well as a combination of subcategories 1B and 1C. The method has undergone a performance standard (PS)-based validation study for skin corrosion testing according to the OECD Guidance Document 219 (2015). The similarity of the me-too assay to the validated reference method, as well as its scientific validity was assessed by an international Peer Review Panel (PRP) composed of:

- Chantra Eskes (Swiss 3R Competence Centre, Switzerland)
- Bert Hakkinen (NIH/NLM, US)
- Sebastian Hoffman (seh consulting+services, Germany)
- Tae Cheon Jeong (Yeungnam University, Korea)
- Jill Merrill (US FDA, USA)
- Yuhji Taquahashi (NIHS, Japan)

The assay underwent an independent peer-review from July to October 2018. During this period three teleconferences, organized by JaCVAM, took place with the peer-review panel (3 July 2018, 21 August 2018 and 12 October 2018), the two first including also the test method developer.

In particular, the PRP notes that TG 431 has been updated in 2016 introducing a new prediction model for the EpiDerm™ SCT. However, the most recent version of GD 219 still dates from 2015, and is based on an earlier prediction model of the EpiDerm™ SCT. ***As a consequence, the required predictive capacities reported within GD 219 for EpiDerm™ SCT are no longer accurate, and cannot be compared with newly developed me-too or similar test methods.*** Furthermore, it is noted that the recommended reference chemicals within GD 219 derive from a post-validation exercise, in which a total of 80 test chemicals selected by an OECD Expert Group were tested in house by the different tissue developers in order to evaluate the performances of the test methods to distinguish Subcategory 1A from a combination of Subcategories 1B and 1C and from non-corrosives. However, as far as the PRP is aware of, ***not all of the 30 PS reference chemicals have been tested in three different laboratories by the Validated Reference Methods (VRMs)***.

Based on the above observations, the PRP considered therefore inappropriate to compare the results obtained with the LabCyte EPI-MODEL24 SCT in three laboratories with the predictive capacities required within GD 219 from 2015. In contrast, the PRP compared the overall within and between laboratory reproducibilities to the minimum performances required within GD 219. In addition, it compared the predictive capacity of the LabCyte EPI-MODEL24 SCT with the predictive capacities obtained for the 79 test chemicals tested with the LabCyte EPI-MODEL24 SCT (the 80 chemicals selected by the OECD expert group with the exception of one non-commercially available chemicals) with the predictive capacities obtained for these same chemicals tested in the two validated reference methods, i.e. EpiSkin™ and EpiDerm™ SCT (see point 6 below).

Based on its evaluation (see detailed criteria and evaluation below), the PRP is of the opinion that the information made available to them do support the scientific similarity of the LabCyte EPI-MODEL24 skin corrosion test (SCT) method to the validated reference methods both in terms of the essential test method components and of assay performance regarding its reproducibility and predictive capacity.

1. Rationale for the test method

Rationale for the test method, including a description of the advantages of the similar or modified test method in terms of i) mechanistic advantages, applicability, predictive capacity, technical advances, reduction in hazardous reagents, ii) IP rights, geographical availability and animal welfare, iii) costs, analysis time, sample amount, competitiveness, iv) others

The LabCyte EPI-MODEL24 SCT method offers the same mechanistic, technical and animal welfare advantages as the currently adopted test methods falling within TG 431. Also, if adopted, the LabCyte EPI-MODEL24 would be the only model falling within OECD TG 431 that is manufactured in Asia, benefiting of lower costs[1] and shorter transportation times as compared to current models adopted within TG 431 that are manufactured in Europe and in the US. The LabCyte EPI-MODEL underwent however long-distance shipments to China and USA, and was shown to maintain its cell viability and barrier functions within the acceptance criteria established by the manufacturer (cf. chapter 10.7 of the me-too validation report v. 2.1 from Aug. 2018). Finally, based on the information provided to the PRP[2], there are no IP rights for LabCyte EPI-MODEL24 SCT.

2. Test method protocol

A detailed protocol for the similar or modified test method should be available.

The LabCyte EPI-MODEL24 model is comprised of normal human skin-derived keratinocytes cultured on a feeder layer of 3T3-J2 cells that supports proliferation and maintenance of epithelial cells. Reconstruction of human cultured epithelial tissue is achieved by cultivating the keratinocytes on an inert filter substrate with a surface area of 0.3 cm^2 at the air-liquid interface for 13 days using an optimized medium containing 5% foetal bovine serum (FBS). This results in the formation of a multilayered structure comprised of fully differentiated stratified cells supported by a layer of proliferating basal cells that mimics that of normal skin (cf. chapter 4.1 of the me-too validation report v. 2.1 from Aug. 2018). A detailed protocol of the LabCyte EPI-MODEL24 SCT method is available and is considered to be adequate by the PRP (protocol 1.6).

3. Adherence to the essential test method components

Adherence to the essential test method components as described in paragraphs 6 to 26 of GD 219 should be demonstrated for the similar or modified test methods regarding i.e., the general conditions, the functional conditions, procedural conditions, acceptance criteria and interpretation of results.

Adherence to the essential test method components, including general conditions, functional conditions, (i.e., viability, barrier function, morphology, reproducibility and quality control) and procedural conditions (i.e. application of test chemicals, cell viability measurements, acceptability criteria, interpretation of results and prediction model) was considered to be sufficient and adequate by the PRP (cf. chapter 5 of the me-too validation report v. 2.1 from Aug. 2018).

[1] Based on comparative price lists provided to the PRP on 16 October 208 for EpiSkin (12 well/kit), SkinEthic RHE (24 well/kit) and LabCyte EPI-MOEL24 (24 well/kit).

[2] Based on responses from test developers received by the PRP on 12 October 2018.

4. Selection of test chemicals

At least the recommended reference chemicals within GD 219 should be tested with the similar or modified test method according to recommendations of paragraphs 27 to 30 of GD 219, to demonstrate reliability and accuracy.

The test method developer tested the 30 reference chemicals recommended within GD 219. In addition, a set of 79 chemicals was tested (including the 30 reference chemicals), which represent the 80 test chemicals recommended by the OECD expert group and used to evaluate the performances of RhE test methods to distinguish Subcategory 1A from a combination of Sub-category 1B and 1C (Desprez et al., 2015; OECD GD 190, 2013), except for one chemical, which is not commercially available (i.e., Glycol bromoacetate, CAS 3785-34-0). The overall dataset tested in the LabCyte EPI-MODEL24 SCT method comprised therefore 79 chemicals that were tested also in the RhE models currently included within TG 431. These chemicals comprise 37 *in vivo* non-corrosive chemicals (21 liquids and 16 solids), 30 chemicals classified *in vivo* UN GHS Sub-categories 1B and 1C (24 liquids, 5 solids, 1 viscous), and 12 chemicals classified *in vivo* UN GHS Sub-category 1A (9 liquids and 3 solids). The PRP considers the number and distribution of chemicals tested to be sufficient, adequate and to allow comparison of the LabCyte EPI-MODEL24 SCT method to the Validated Reference Methods (VRMs).

5. Within- and between-laboratory reproducibility

The reliability obtained with the reference chemicals, calculated as described in paragraph 30.1 and 30.2, should be equal to or better than the defined minimum target values for the similar or modified test method as specified in paragraphs 31 to 33 of GD 219 (within-laboratory reproducibility ≥ 80% for sub-categorization and ≥ 90% for identification of corrosives vs. non-corrosives; a between-laboratory reproducibility ≥ 70% for sub-categorization and ≥ 80% for identification of corrosives vs. non-corrosives).

Based on the results obtained for within- and between- laboratory reproducibility in three laboratories as summarized in Table 1, the LabCyte EPI-MODEL24 SCT method was shown to meet the WLR and BLR required performances for: i) the subcategorization of corrosive effects (i.e. ≥ 80% and ≥ 70%, respectively), and for ii) the discrimination of corrosives vs. non-corrosive test chemicals (i.e. ≥ 90% and ≥ 80%, respectively).

The LabCyte EPI-MODEL24 SCT method dataset comprised three complete test sequences for each of the 30 reference chemicals in all three participating laboratories. All participating laboratories achieved therefore 100% (30/30) complete test sequences, meeting the PS target (≥ 90% for each laboratory) for the study quality criteria. The frequency of invalid test runs was of 0% in Lab A, and of 1% (1/91) for Labs B and C.

Based on the above information, the PRP considered the reliability of the LabCyte EPI-MODEL24 SCT method as obtained with the reference chemicals to be sufficient and adequate.

Table 1: Within- and between- laboratory reproducibility of the LabCyte EPI-MODEL24 obtained with the 30 reference chemicals and based on the calculations recommended within the OECD GD 219 (2015) (values extracted from me-too validation report v. 2.1 from Aug. 2018)

Performance Standard chemicals from GD 219 (2015)	**WLR**			**Minimum required WLR**	**BLR**	**Minimum required BLR**
	Lab A	**Lab B**	**Lab C**			
Sub-categorization – 30 reference chemicals (non-corrosive / Subcat. 1B-1C / Subcat. 1A)	**90.0%** (27/30)	**96.7%** (29/30)	**90.0%** (27/30)	**≥ 80%**	**83.3%** (25/30)	**≥ 70 %**
Corrosives vs. non-corrosives – 20* reference chemicals	**95.0%** (19/20)	**100.0%** (20/20)	**95.0%** (19/20)	**≥ 90%**	**95.0%** (19/20)	**≥ 80%**

* GD 219 shows a discrepancy on the number of chemicals required to be tested for identifying corrosives vs. non-corrosive chemicals, whereas 20 chemicals are requested in paragraph 28 and in Table 5, 24 chemicals are requested in paragraphs 29, 31 and 33.

6. Predictive capacity

The predictive capacity obtained with the reference chemicals, calculated as described in paragraph 30.3, should be equal to or better than the defined minimum target values for the similar or modified test method as specified in paragraph 34 of GD 219.

The LabCyte EPI-MODEL24 SCT prediction model was established based on the EpiDerm™ SCT (EPI-200). The PRP notes that TG 431 has been updated in 2016 introducing a new prediction model for the EpiDerm™ SCT. However, the updated TG 431 still makes reference to GD 219 from 2015, which was based on an earlier prediction model of the EpiDerm™ SCT as reported in TG 431 from 2015. ***As a consequence, the required predictive capacities reported within Table 7 of GD 219 for EpiDerm™ SCT are no longer accurate, and cannot be compared with the present results.***

Furthermore, the recommended reference chemicals within GD 219 derive from a post-validation exercise, in which a total of 80 test chemicals selected by an OECD Expert Group were tested in house by the different tissue developers in order to evaluate the performances of the test methods to distinguish Subcategory 1A from a combination of Subcategories 1B and 1C and from non-corrosives. However, as far as the PRP is aware of, ***not all of the 30 PS reference chemicals have been tested in three different laboratories by the Validated Reference Methods (VRMs)***. The PRP therefore considered inappropriate to compare the results obtained with the LabCyte EPI-MODEL24 SCT in three laboratories with the predictive capacities required within GD 219 that were not all based on the outcome from three independent laboratories. The reason for this is that borderline chemicals could result in different predictions especially when more than one laboratory is involved.

Based on the above observations, the PRP did not compare the predictive capacity of the LabCyte EPI-MODEL24 SCT with the predictive capacities required within GD 219 from 2015, but to the predictive capacities obtained for the 79 test chemicals tested with the LabCyte EPI-MODEL24 SCT as well as with the two validated reference methods, i.e. EpiSkin™ and EpiDerm™ SCT (see Table 2 below).

Based on the results shown in Table 2, the PRP considers the predictive capacity obtained with the LabCyte EPI-MODEL24 SCT to be similar to the predictive capacities obtained with the two Validated Reference Methods EpiSkin™ and EpiDerm™ SCT.

Table 2: LabCyte EPI-MODEL24 SCT predictive capacity compared to the Validated Reference Methods (VRMs) (cf. table 22 from me-too validation report v. 2.1 from Aug. 2018)

	LabCyte EPI-MODEL24[(A)]	VRMs [(B)]	
		EpiSkin™	Epiderm™ SCT
1A correctly classified	86.1%	83.3%	83.3%
1A underclassified 1B-and-1C	13.9%	16.7%	16.7%
1A underclassified NC	0.0%	0.0%	0.0%
1B-and-/1C correctly classified	70.0%	76.3% (75.6%*)	71.0% (70%*)
1B-and-1C overclassified 1A	30.0%	21.5% (22.2%*)	29.0% (30%*)
1B-and-1C underclassified NC	0.0%	2.2% (2.2%*)	0.0% (0%*)
NC correctly classified (Specificity)	78.4%	79.3%	73.9%
NC overclassified 1A	2.7%	0.0%	2.7%
NC overclassified 1B-and-1C	18.9%	20.7%	23.4%
Overall Accuracy	76.4%	78.8% (78.5%*)	74.2% (73.8%*)
Global overclassification rate (all categories)	21.5%	17.9%	23.3%
Global underclassification rate (all categories)	2.1%	3.3%	2.5%

(A) Based on a set of 79 chemicals tested over three independent runs (which does not include Glycol bromoacetate (CAS 3785-34-0)).

(B) VRM results, according to Annex 3 of OECD TG431 (2016) and Desprez et al. (2015). *Results excluding Glycol bromoacetate (CAS 3785-34-0), an *in vivo* UN GHS Sub-Category 1B/1C correctly predicted as such by the two VRMs.

7. Applicability Domain

The applicability domain of the new or modified test method should be defined.

A total of 49 chemicals with clear identification and *in vivo* classification have been tested in addition to the 30 reference chemicals, so that the characterization of the applicability domain of the LabCyte EPI-MODEL24 SCT as compared to the VRMs was considered adequate and sufficient by the PRP.

8. Accordance with the principles of Good Laboratory Practices

All data from the PS-based validation study supporting the validity of the similar or modified test method should be obtained in accordance with the principles of Good Laboratory Practice (GLP).

According to the information provided to the PRP, the study was conducted according to the principles of GLP (cf. chapter 7.4 from the me-too validation report v. 2.1 from Aug. 2018).

9. Completeness of data and documents

Completeness of all data and documents supporting the assessment of the validity of the similar or modified test method.

The information provided by the test method developer was considered to be sufficient for the assessment of the similarity of the LabCyte EPI-MODEL24 SCT.

10. PS-based validation study management and conduct

The PRP considered the information provided on the study management conduct to be adequate and sufficient.

11. Other considerations

11.1. Audit of tissue production

The PRP was informed [3] that Japan Tissue Engineering Co. has been successfully inspected by the pharmaceutical and medical devices agency for GCTP compliance for manufacturing regenerative medicine products and medical devices in February 2017, in particular for the manufacturing of autologous cultured epidermis (JACE; Maeda et al., 2018; Morimoto et al., 2018) and others such as autologous cultured cartilage (JACC). According to the information provided by the test method developers, the same manufacturing personnel are involved in the production of both, JACE and LabCyte EPI-MODEL24, and therefore undergo the same training and education programs. Moreover, the raw materials (irradiated 3T3 feeder cells and culture media), and protocols for cell banking and cell culture used for JACE are also used for LabCyte EPI-MODEL24 production. Finally, internal documentation and record storage procedures (QMS) are the same for both products. JACE requires more strictly controlled surveillance, and quality control tests that are specific to regenerative medicine designed for clinical use (Maeda et al., 2018; Morimoto et al., 2018). LabCyte EPI-MODEL24 is produced in different cell culture rooms and undergoes different quality control procedures, which guarantee a more affordable product price for our target market. Based on the above, J-TEC believes that the periodical inspection above mentioned covers LabCyte EPI-MODEL manufacturing activities.

11.2. Serum batches

According to the test method developer, "*Serum batch to batch differences were not observed during the study as tissue lots were manufactured with the same serum batch".* According to the information provided to the PRP, in the period Jan. 2013 –Sept. 2018, at least three different serum lots were used with apparently no overall impact on the tissue viability and barrier function of the model[4]. In addition, the PRP was informed that *"J-TEC's quality management system is in place to ensure that when different serum lots are used in the manufacturing of LabCyte, the product quality and cell proliferation are not compromised*".

Table 3: Comparison of different serum lots based on batch quality control testing (mean ± standard deviation).

	Lot 1 (Jan. 2013-March 2014)	**Lot 2** (April 2014 – March 2018)	**Lot 3** (April – Sept. 2018)	**Lower and upper acceptance limits**
Tissue viability (OD)	1.128 ± 0.084	1.212 ± 0.058	1.177 ± 0.034	**0.7 – 2.5**
Barrier function IC_{50} (mg/ml)	2.55 ± 0.10	2.58 ± 0.08	2.67 ± 0.05	**1.4 – 4.0**

[3] The inspection report and its translation into English were sent to the PRP on 16 October 2018.

[4] Information sent to the PRP on the 12 October 2018.

12. Conclusions

All data should adequately support the peer review assessment that the proposed test method is structurally and mechanistically similar to the validated reference method, and demonstrate sufficient reliability and relevance for the proposed specific testing purpose i.e., that the proposed similar or modified test method is scientifically valid.

The data assessed by the peer-review panel supports the scientific similarity of the LabCyte EPI-MODEL24 SCT to the validated reference methods both in terms of the essential test method components and of assay performance regarding its reproducibility (as described within the GD 219) and in terms of its predictive capacity (based on a set of 79 commonly tested chemicals).

13. References

Desprez B., Barroso J., Griesinger C., Kandárová H., Alépée N., and Fuchs, H. (2015). Two Novel Prediction Models Improve Predictions of Skin Corrosive Sub-categories by Test Methods of OECD Test Guideline No. 431. Toxicol. In Vitro 29, 2055 - 2080.

LabCyte Validation Management Team (2018). Me-too validation report. Validation Study for *in vitro* skin corrosion test method using reconstructed human epidermal tissue LabCyte EPI-MODEL24. Version 2.1, August 2018.

Maeda T, Morimoto N, Kakudo N, Kusumoto K (2018). Efficacy of Cultured Epithelial Autograft after Curettage for Giant Melanocytic Nevus of the Head. Plast. Reconstr. Surg. Glob. Open 6, e1827.

Morimoto N, Kakudo N, Kako A, Nishimura K, Mitsui T, Miyake R, Kuro A, Hihara M, Kusomoto K (2018). Case report of the first application of culture epithelial autograft (JACE®) for giant congenital melanocytic nevus after its approval in Japan. J. Artif. Organs 21, 261-264.

OECD GD 190 (2013). Summary Document on the Statistical Performance of Methods in OECD Test Guideline 431 for Sub-Categorisation. OECD Serires on Testing and Assessment No. 190. Organisation for Economic Cooperation and Development, Paris.

OECD GD 219 (2015). Performance Standards for the Assessment of Proposed Similar or Modified *In Vitro* Reconstructed Human Epidermis (RhE) Test Methods for Skin Corrosion Testing as Described in TG 431. Environmental Health and Safety Publications, Series on Testing and Assessment (No. 219). Organisation for Economic Cooperation and Development, Paris.

OECD TG 431 (2016). Reconstructed human epidermis (RhE) method for skin corrosion testing. OECD Guideline for the Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris.