Organisation for Economic Co-operation and Development

ENV/JM/MONO(2018)14

Unclassified

English - Or. English

11 December 2018

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

PEER REVIEW REPORT OF THE VALIDATION OF THE LABCYTE CORNEA-MODEL24 EYE IRRITATION TEST FOR INCLUSION IN TEST GUIDELINE 492

SERIES ON TESTING AND ASSESMENT
Number 282

JT03441157

OECD Environment, Health and Safety Publications

Series on Testing and Assessment

No. 282

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INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
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Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris 2018

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or contact:

OECD Environment Directorate,
Environment, Health and Safety Division
2, rue André-Pascal
75775 Paris cedex 16
France

Fax : (33-1) 44 30 61 80

E-mail : ehscont@oecd.org

FOREWORD

The LabCyte LabCyte CORNEA-MODEL24 Eye Irritation Test (LabCyte24 EIT) method has been proposed by Japan as a similar *in vitro* reconstructed human cornea-like epithelium (RHCE) test method for identifying chemicals not requiring classification and labelling for eye hazard, as described in the OECD Test Guideline 492.

In 2017, Japan undertook the peer-review of the validation of LabCyte24 EIT. The present report and a proposal for an updated version of OECD Test Guideline 492 were considered by the OECD Expert Group on Eye Irritation.

The present report was endorsed by the Working Group of the National Co-ordinators of the Test Guidelines Programme (WNT) at its 30th meeting in April 2018. The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology agreed to its declassification on 30 June 2018.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

Report of the Peer Review Panel on a JaCVAM co-ordinated study programme addressing the validation status of LabCyte CORNEA-MODEL24 EYE IRRITATION TEST for discriminating eye irritant from non-eye irritant substances June 30, 2017

EXECUTIVE SUMMARY

The commercially available LabCyte CORNEA-MODEL24 Eye Irritation Test (LabCyte24 EIT) method has been proposed as a similar in vitro reconstructed human cornea-like epithelium (RHCE) test method for identifying chemicals not requiring classification and labelling for eye hazard, as described in the OECD test guideline 492 (OECD, 2015a). It was validated in a performance standard (PS)-based study partly funded by the Japanese Society for the Alternative to Animal Experiments (JSAAE) and managed by a validation management team chaired by Satoshi Nakahara (Maruishilabo Corp.) and that included Japanese Center for the Validation of Alternative Methods (JaCVAM).

The Peer Review Panel (PRP), organised by JaCVAM, found that the Validation Management Team report and supporting material presented the necessary information for an independent review. The PRP concluded that the LabCyte24 EIT method adhered to the essential test method components as defined in the respective OECD performance standards, i.e. the actual version when the study was planned and conducted (OECD, 2015b). The PRP considered the use of the WST-8 instead of MTT to determine cell viability as sufficiently justified. The LabCyte24 EIT method met the PS criteria for within- and between-laboratory reproducibility (see Table below).

Also the predictive capacity described as specificity, sensitivity and accuracy clearly met the respective PS target values (see table below). In-house data on 139 chemicals showed similar predictive capacity values.

Table 1. Evaluation criteria guiding the review of the LabCyte24 EIT method

	LabCyte24 EIT performance-based validation	PS target values	LabCyte24 EIT test developer data
within-laboratory reproducibility	93%, 97%, 100%	= 90%	97% (135/139)
between-laboratory reproducibility	87% (26/30)	= 85%	not applicable
sensitivity	97.8%	= 90%	100.0% (76/76)
specificity	68.9%	= 60%	73.0% (46/63)
accuracy	83.5%	= 75%	87.8% (122/139)

1. Accordingly, the PRP concluded that it has been demonstrated that the LabCyte24 EIT method is a similar in vitro RhCE test methods for identifying chemicals not requiring classification and labelling for eye hazard, as described in the OECD test guideline 492. However, the PRP recommends to describe the impact of possible colour interference between test chemicals and WST-8 in more detail in the validation report.

Peer Review Panel

1. Sebastian Hoffmann (chair), seh consulting + services, Paderborn, Germany
2. Chantra Eskes, SeCAM, Magliaso, Switzerland
3. Pertti Hakkinen, National Institutes of Health, Bethesda, USA
4. Tae Cheon Jeong, Yeungnam University, Gyeongsan, South Korea
5. Jill Merrill, FDA, USA
6. Sanae Takeuchi, P&G Innovation Godo Kaisha, Kobe, Japan

Introduction

2. The LabCyte CORNEA-MODEL24 EIT (LabCyte24 EIT) method has been proposed as a similar in vitro RhCE test method for eye hazard, as described in the OECD test guideline 492 (OECD; 2015a). It has been submitted to a performance-based validation study partly funded by the Japanese Society for the Alternative to Animal Experiments (JSAAE) and managed by a validation management team (VMT) chaired by Satoshi Nakahara (Maruishilabo Corp.) and that included Japanese Center for the Validation of Alternative Methods (JaCVAM).

3. A peer review panel (PRP) was assembled by JaCVAM in the middle of 2016. It met in July 2016 to review a progress report on the LabCyte24 EIT method prepared by the VMT. The review was guided by 13 evaluation criteria (Table 2). Following the commentary on this work by the PRP, the VMT improved the documentation by refining the protocol, presentation of results and conducted additional work. The amendments were presented in a conference call in January 2017. Based on the comments of the PRP, the VMT carried out a final revision of the documentations addressing the open issues,

which were discussed by the PRP in May 2017. With the provision of the amended, updated and additional material, including the final VMT report and final protocol, this PRP Validation Report was prepared following the evaluation criteria, which were initially provided by JaCVAM and subsequently amended by the PRP (addition of criterion 1).

Table 2. Evaluation criteria guiding the review of the LabCyte24 EIT method

	Evaluation criteria
1	The adherence to the OECD PS should be demonstrated in terms of the essential test method components demonstration of reliability and accuracy using at least the recommended reference chemicals defined reliability and accuracy values
2	A rationale for the test method should be available, including a description of the human health effect, a clear statement of the scientific need, and the regulatory application
3	The toxicological mechanisms and the relationship between the test method endpoint(s) with the biological effect as well as the toxicity of interest should be addressed, describing limitations of the test methods
4	A detailed test method protocol should be available
5	Demonstration of the test method's performance should be based on testing of representative, preferably coded reference chemicals
6	All data should adequately support the assessment of the validity of the test method
7	All data from the validation study supporting the validity of a test method should have been obtained in accordance with the principles of Good Laboratory Practice (GLP)
8	Applicability domain of the test method should be defined
9	Proficiency chemicals should be set up in the proposed protocol
10	Performance standard should be set up with proposed protocol
11	Advantages in terms of time, cost and animal welfare
12	Completeness of all data and documents supporting the assessment of the validity of the test method
13	Validation Study Management and Conduct

Evaluation

Evaluation Criterion 1

4. The LabCyte24 EIT method underwent a performance-based validation study to assess its scientific validity to be used as an in vitro RhCE test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage similar to the test methods described in OECD Test Guideline 492 (OECD, 2015a). Therefore, the PRP thoroughly reviewed how the LabCyte24 EIT method met the requirements of the respective OECD performance standards (PS) Guidance Document 216 (OECD, 2015b).

1.1 Adherence to the essential test method components

5. The PRP considered that the LabCyte24 EIT method fulfilled the general conditions of PS. In particular, the LabCyte24 EIT test method uses human corneal epithelial cells that are neither cornified nor keratinized and the tissue forms a robust functional barrier (as demonstrated by the SLS concentration reducing cell viability by 50% one hour after application).

6. The PRP agreed that the functional conditions were sufficiently similar between the LabCyte24 EIT method and the validated reference method (as described within the OECD GD 216, 2015b) in terms of barrier function, morphology and tissue quality determination, although the quantification of tissue viability differed substantially. Instead of using the classical MTT, which is reduced to water-insoluble formazan), the LabCyte24 EIT method uses WST-8, which is reduced to a yellow, water-soluble formazan product. The principle of the WST-8 assay is the same as the MTT assay, with the exception that the WST-8 assay does not require an extraction step, simplifying the procedure as compared to the MTT assay. The PRP agreed that the justification for using the WST-8 and the comparison with MTT provided in the BRD is sufficient to conclude similarity of the functional conditions and mechanisms of action of WST-8 and MTT. Nevertheless, the PRP recommends including this justification and comparison also in the validation report.

7. The PRP also agreed that most of the procedural conditions of the PS have been fulfilled. Requirements on replicates, concurrent controls, suitability of the viability assay, acceptance criteria and interpretation of results and prediction model have been met. Information on the reproducibility of negative and positive control results over time presented in the Background Review Document (BRD) were considered sufficient. However, the PRP missed a justification (other than data-driven) that the lower limit of the negative control of 0.5 OD provides a sufficiently large dynamic response range.

1.2 Demonstration of reliability and accuracy using at least the recommended reference chemicals

8. The 30 reference chemicals proposed in the PS were tested in the validation study. It is important to note that due to the use of WST-8 chemical interference in the LabCyte24 EIT method, both colour interference and interference by direct reduction, will be different from those interferences specified in the PS for MTT-based test methods. The testing results appropriately address interference due to direct WST-8 reduction. Regarding colour interference, further information could have been provided in the validation report. However, the test developer informed the PRP, that out of 139 chemicals tested in-house only one chemical (2,2'-[[3-Methyl-4-[(4-nitrophenyl)azo]-phenyl]imino]bis-ethanol (CAS No. 3179-89-3)), which is one of the PS reference chemicals, directly stained the CORNEA-MODEL tissue. Based on in vivo data, this chemical is not classified for eye irritation/damage (according to UN GHS). However, it was clearly (false) positive in the LabCyte24 EIT method with cell viability close to zero. This suggests that the stained live tissues did not transfer the colour to the WST-8 medium. The test developer stated that they have not detected any other chemicals that directly stain the tissues, but are willing to further test other staining chemicals if and when required.

1.3 Performance meeting defined reliability and accuracy values

9. In the three participating laboratories the LabCyte24 EIT method's within-laboratory reproducibility (WLR) was 93%, 97%, and 100%, meeting the PS target value of ≥ 90%.

10. Also the between-laboratory reproducibility (BLR), which was 87% (=26/30), met the PS target value of ≥ 85%. Interference with WST-8 was consistently observed in the three laboratories for six of the chemicals.

11. In terms of predictive capacity, the LabCyte24 EIT method had a sensitivity of 97.8%, a specificity of 68.9% and an accuracy of 83.5%, all clearly meeting the respective PS target values (90%, 60% and 75%, respectively). This performance is supported by the results obtained by testing 139 chemicals (not coded, but with the same protocol) by the test developer's laboratory, as presented in the BRD (sensitivity: 100%; specificity: 73.0%; accuracy: 87.8%).

12. All values derived from the validation study data, i.e. the WLR, the BLR and the predictive capacity parameters, were determined according to the procedure specified in the PS.

13. For the reasons described under point 1.2 the performance for colour interfering chemical was not addressed. However, the PRP acknowledged that the impact of colour interfering chemicals can be expected to be minor based on the information provided by the test developer as described under point 1.2.

Evaluation Criterion 2: A rationale for the test method should be available, including a description of the human health effect, a clear statement of the scientific need, and the regulatory application

14. The PRP agreed that the rationale is similar to that of the validated reference methods (VRM) as described within the OECD GD 216 (2015b).

Evaluation Criterion 3: The toxicological mechanisms and the relationship between the test method endpoint(s) with the biological effect as well as the toxicity of interest should be addressed, describing limitations of the test methods

15. The toxicological mechanisms, the relationship between the test method endpoints and the biological effect, and the limitations of the LabCyte24 EIT method are considered similar to the VRM. In the LabCyte24 EIT method, human corneal cells are used which are considered more relevant for predicting human ocular effects as compared to keratinocytes as in the case of the VRM EpiOcularTM Eye Irritation Test described within the OECD GD 216 (2015b).

Evaluation Criterion 4: A detailed test method protocol should be available

16. A detailed test method protocol is available. The various protocol versions used in the validation study were available to the PRP. All data from the validation study as well as the in-house testing of chemicals were generated with protocol version 2.5.2, whereas version 2.5.6 was considered as the proposed final protocol. The PRP notes that the proposed final protocol has been amended to include screening for possible colour-interfering test chemicals using live tissues.

17. However, the PRP recommends that the possible colour interference between chemicals tested within the performance-based validation and WST-8 to be addressed in more details in the validation report, and that the possible impact of the colour interference when using the WST-8 assay on the final results be discussed.

Evaluation Criterion 5: Demonstration of the test method's performance should be based on testing of representative, preferably coded reference chemicals

18. In addition to the observations made under 1.2, the PRP notes that chemicals used in the validation study were coded per laboratory, but not per repeat experiment per laboratory.

Evaluation Criterion 6: All data should adequately support the assessment of the validity of the test method

19. The PRP agreed that the test method is adequately documented in a standardized protocol. Data and evidence provided, documented in validation report and the BRD as well as additional information provided following the PRP requests, were considered adequate for the assessment of the validity of the test method. In particular, quality control data for the tissue viability and barrier function were adequate for demonstrating reproducibility over time and across different FBS lots. However, the PRP notes a linear decrease within the acceptable range in tissue viability over time and/or FBS lots.

Evaluation Criterion 7: All data from the validation study supporting the validity of a test method should have been obtained in accordance with the principles of Good Laboratory Practice (GLP)

20. Based on the information provided, the study was conducted in the spirit of GLP.

Evaluation Criterion 8: Applicability domain of the test method should be defined

21. The PRP agreed that the applicability domain of the LabCyte24 EIT method has been defined in reference to the VRM in sufficient detail.

Evaluation Criterion 9: Proficiency chemicals should be set up in the proposed protocol

22. Proficiency chemicals have been defined in OECD TG 492. The PRP recommends however to investigate the possible interference between these proficiency chemicals and WST-8 either by direct reduction or by colour interference.

Evaluation Criterion 10: Performance standard should be set up with proposed protocol

23. This criterion is not applicable, as performance standard have already been defined in the OECD Guidance Document 216 (2015b).

Evaluation Criterion 11: Advantages in terms of time, cost and animal welfare

24. The PRP agreed that the LabCyte24 EIT method has the same advantages in terms of time, cost and animal welfare as the VRM. The PRP also notes that, as the LabCyte24 EIT method is produced in Japan, is likely to make RhCE test methods more readily available in the geographical region.

Evaluation Criterion 12: Completeness of all data and documents supporting the assessment of the validity of the test method

25. The PRP agreed that the data and documents provided were complete.

Evaluation Criterion 13: Validation Study Management and Conduct

26. The PRP considered the validation study to be conducted in accordance with internationally accepted principles (OECD Guidance Document 34). In particular, the absence of conflict of interests was stated. The PRP noted that laboratories were trained in a pre-validation, but that the selection of the laboratories for the validation study is not explained.

Conclusion

27. The PRP concluded that it has been demonstrated that the LabCyte24 EIT method is a similar in vitro RhCE for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage, as described in the OECD test guideline 492.

Acknowledgements

28. The PRP is grateful to: a) the members of the VMT for their hard work and patience, and b) JaCVAM for their support in setting up and hosting meetings in Japan, as well as for the setting up of several telephone conferences.

References

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