

Peer-Review of the Performance-based Validation Study on the MCTT HCE™ Eye Irritation Test (EIT) as a me-too test method according to OECD GD 216 and falling within the OECD TG 492

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Summary

The MCTT HCE™ Eye Irritation Test (EIT) method developed by Bio Solution (Korea) is proposed as a similar assay to the validated reference reconstructed human cornea-like epithelium (RhCE) test methods falling within the OECD TG 492. The method has undergone a performance standard (PS)-based validation study for eye hazard testing according to OECD GD 216. The assay underwent an independent peer-review coordinated by the Korean Centre for the Validation of Alternative Methods (KoCVAM), Keimyung University and SeCAM. 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:

- Els Adriaens (Adriaens consulting, Belgium)
- Chantra Eskes (SeCAM, Switzerland)
- Kristina Kejlova (National Institute of Public Health, Czech Republic)
- Bae-Hwan Kim (Keimyung University, South Korea)
- Hajime Kojima (JaCVAM, NIHS, Japan)
- Jill Merrill (US FDA, USA)
- Uwe Pfannenbecker (Beiersdorf, Germany)

The criteria for peer-review evaluation were prepared by SeCAM and were revised by the PRP members. The non-governmental PRP members provided a declaration of interest that can be made available upon request. The peer-review took place from March to July 2018, and a total of four teleconferences took place as follows:

- 13 April 2018: presentation of the method by the test method developer and preliminary questions from the PRP;
- 11 June 2018: PRP draft evaluation of the method in the absence of the test method developer;
- 12 July 2018: answers to additional questions raised by the PRP by the test method developer;
- 26 July 2018: finalization of the PRP evaluation of the test method.

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 MCTT HCE™ EIT to the validated reference methods both in terms of the essential test method components and of assay performance regarding its reproducibility and predictive capacity as described within the GD 216.

Evaluation criterion 1:

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 MCTT HCE™ EIT method makes use of the water soluble tetrazolium salt WST-1 to assess cell viability (instead of the MTT assay), which is spontaneously released into supernatant and therefore solvent extraction step is not necessary.

The model is produced and available in South Korea, and experiments were conducted in China showing appropriate responses to negative and positive controls as well as appropriate performance with the proficiency chemicals (Appendix 8 of the validation report sent to the PRP on 30 July 2018).

Finally, according to the information provided to the Peer Review Panel (PRP), the MCTT HCE™ EIT is not subject to intellectual property rights, and all components and reagents used for the MCTT HCE™ EIT are commercially available (Appendix 10 of the validation report sent to the PRP on 30 July 2018).

Evaluation criterion 2:

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

The MCTT HCE™ is reconstructed using cultured primary human corneal epithelial cells from residual limbus tissues remaining after corneal transplantation (cf. validation report chapters III and IV.1 sent to the PRP on 30 July 2018). A detailed protocol of the MCTT HCE™ EIT is available and was considered to be adequate by the PRP (revised protocol 1.6 provided to the PRP on 9 July 2018).

Evaluation criterion 3:

Adherence to the essential test method components as described in paragraphs 4 to 21 of GD 216 should be demonstrated for the similar or modified test methods regarding i.e., the general conditions, the functional conditions and the procedural conditions.

Adherence to the essential test method components was considered to be adequate by the PRP

Evaluation criterion 4:

In addition, for modified test methods, 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 method.

Testing of the 30 Performance Standard (PS) chemicals with WST-1 showed similar outcomes as MTT (answers to PRP questions provided to the PRP on 9 July 2018). In addition, corrections of coloured and reducing chemicals interfering with WST-1 have been characterized and the necessary procedures clearly described (Appendix 3 distributed to the PRP on 4 June 2018). Based on the information provided by the test method developer, the PRP considered the use of WST-1 to be similar to the use of MTT. In addition, it is noted that the recently introduced test method Labcyte CORNEA-MODEL24 EIT to TG 492 makes use of WST-8.

Evaluation criterion 5:

At least the 30 recommended reference chemicals within GD 216 should be tested with the similar or modified test method according to recommendations of paragraphs 22, 23, 24 and 29 of GD 216, to demonstrate reliability and accuracy.

Notes: The exclusive use of the Reference Chemicals for the development/optimisation of new similar test methods should be avoided to the extent possible and the identity of all additional chemicals used for test method development (e.g., for setting the prediction model or exposure times) should be reported when submitting a PS-based validation study.

For test methods to be used by several independent laboratories, all of the 30 Reference Chemicals should be tested in at least three laboratories. In each laboratory, all Reference Chemicals should be tested in three independent runs performed with different tissue batches and at sufficiently spaced time points. Each run should consist of at least two concurrently tested tissue replicates.

In case Reference Chemicals does/do not meet the test acceptance criteria or is/are not acceptable for technical reasons or because they were obtained in a non-qualified run, a maximum number of two additional tests/runs for each Reference Chemical is admissible per laboratory ("re-testing"). Non-qualified tests should be documented and reported. Excess production of data and subsequent data selection are regarded as not appropriate.

The test method developer tested the 30 PS reference chemicals and an additional set of 130 chemicals that were used initially to develop the prediction model of the assay (including 19 overlapping PS reference chemicals). The overall dataset tested in the MCTT HCE™ EIT comprised 141 non-overlapping chemicals including liquids (87) and solids (54). The PRP considers the number and distribution of chemicals tested to be sufficient and adequate.

Evaluation criterion 6:

The reliability obtained with the reference chemicals, calculated as described in paragraph 25.1 and 25.2, should be equal to or better than the defined minimum target values for the similar or modified test method as specified in paragraphs 26 and 27 of GD 216 (within-laboratory reproducibility $\geq 90\%$, between-laboratory reproducibility $\geq 85\%$).

The PRP considered the reliability of the MCTT HCE™ EIT as obtained with the reference chemicals and the new proposed cut-offs (60% for solids, 35% for liquids) as provided to the PRP in the answers to PRP questions on 9 July 2018 and summarized below to be sufficient and adequate.

Table 1: Within- and Between- laboratory reproducibility of the MCTT HCE™ EIT based on the 30 reference chemicals recommended within the OECD GD 216 (2017)) (extract from table 16 of the revised PS-based validation report sent on 30 July 2018 to the PRP)

Performance Standard (PS) chemicals from GD 216 (2017)	WLR			BLR	Minimum required PS	
	BS	KCL	BTT		WLR	BLR
New proposed Prediction Model (liquid 35%/ solid 60%)	96.7% (29/30)	90.0% (27/30)	90.0% (27/30)	93.3% (28/30)	90%	85%

In addition, only one out of the 30 reference chemicals required more than two test repetitions in two of the three participating laboratories (dipropyl disulphide) due to borderline results to the old cut-off of 45%. However, with the new proposed cut-off, this chemical would no longer be considered borderline, and the new borderline chemicals were not re-tested leading to the performances

described here above. Based on this, it is considered that study quality criteria as described within the OECD GD 216 were met.

Evaluation criterion 7:

The predictive capacity obtained with the reference chemicals, calculated as described in paragraph 25.3, should be equal to or better than the defined minimum target values for the similar or modified test method as specified in paragraph 27 of GD 220 (sensitivity \geq 90%, specificity \geq 60%, accuracy \geq 75%).

The predictive capacity obtained with the new proposed cut-offs (60% for solids, 35% for liquids) was considered to be sufficient and adequate by the PRP both for the PS reference chemicals and for the enlarged dataset of 141 chemicals. Below is a summary of the performances obtained.

Table 2: Performances of the MCTT HCE™ EIT based on the 30 reference chemicals recommended within the OECD GD 216 (2017) (extract from table 17 of the revised PS-based validation report sent on 30 July 2018 to the PRP)

	30 Reference Chemicals (GD 216, 2017)	PS target values
Sensitivity (n=15)	92.6%	\geq 90%
Specificity (n=15)	60.7%	\geq 60%
Accuracy (n=30)	76.7%	\geq 75%

Table 3: Performances of the MCTT HCE™ EIT based on the entire dataset of 141 non-overlapping tested chemicals (extract from table 15 of the revised PS-based validation report sent on 30 July 2018 to the PRP)

	Total	Liquids	Solids
Sensitivity	98.8% (n=80)	100% (n=49)	96.8% (n=31)
Specificity	68.9% (n=61)	63.2% (n=38)	78.3% (n=23)
Accuracy	85.8% (n=141)	83.9% (n=87)	88.9% (n=54)

Evaluation criterion 8:

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

A total of 111 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 MCTT HCE™ EIT was considered adequate and sufficient by the PRP.

Evaluation criterion 9:

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 GLP principles. In particular, two of the three participating laboratories are OECD GLP-accredited

contract research organizations from MFDS, Korea, and the third laboratory conducted the tests in the spirit of GLP.

Evaluation criterion 10:

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 MCTT HCE™ EIT.

Evaluation criterion 11:

PS-based validation study management and conduct.

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

Evaluation criterion 12:

Other considerations.

12.1. Quality control procedures for lot release

The PRP considered the quality control procedures for lot release sufficient provided that the tissue developer continues to assess the barrier function of the MCTT HCE™ tissue batches by calculating the ET_{50} by interpolating between two exposure times resulting in a viability above and a viability below 50%. This is due to the fact that the correlation between log-transformed (Log10) time of exposure and cell viability may not always be linear. Furthermore it is suggested that the tissue developer continues to report the figures based on cell viability rather than OD.

12.2. Audit of tissue production

Based on the information provided to the PRP, the manufacturing of the MCTT HCE™ tissues has been independently audited within the framework of an ISO 9001:2005 certification.

12.3. Colour interfering chemicals

The PRP is of the opinion that assessment of the colour interference with the WST-1 was appropriately conducted and reported (Appendix12 of revised validation report sent to the PRP on 30 July 2018).

Evaluation criterion 13:

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 MCTT HCE™ EIT to the validated reference methods both in terms of the essential test method components and of assay performance regarding its reproducibility and predictive capacity as described within the GD 216.