Additional validation of alternative skin irritation test method using LabCyte EPI-MODEL24 of cultured skin

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1.Introduction

From 2008 to 2009, the Validation Committee of the Japanese Society for Alternative to Animal Experiments twice implemented validation studies on an alternative skin irritation test method, to confirm the usefulness of LabCyte EPI-MODEL24 of cultured skin prepared in Japan. This multicenter validation study with LabCyte EPI-MODEL24 could address the following three issues related to judgment of skin irritation: consistency among study centers (inter-laboratory reproducibility), consistency with judgments obtained for EPISKIN™ authenticated by ECVAM (equivalence), and consistency with the results of animal experiments. (alternativity. The third-party accreditation of this test method was performed by the Skin Irritation Evaluation Committee of JACVAM and Third-party Accreditation Committee of OECD, and consequently, this model was judged to be insufficient for evaluation of skin irritation because of false-negative results for 1-bromohexane, etc. Then, the manufacturer of LabCyte EPI-MODEL24, Japan Tissue Engineering Co., Ltd. (J-TEC), reviewed the test method, leading to successful improvement. The Steering Committee of JACVAM considered this improvement to be critical for the protocol of the test method, and the improved test method was to be validated accordingly with the support of MHLW Grant-in-Aid for Scientific Research.

2. LabCyte EPI-MODEL

The LabCyte EPI-MODEL is produced by culturing human epidermal cells on a culture plate. After human epidermal cells have been cultured and proliferated, exposing their surface to the air causes it to keratinize, creating a cultured epidermis model similar to the human epidermis (Figures A and B).

QC batch release criteria: IC50 >1.4, OD/mL <2.575mL/mL, 18 treatment with SLS.

3. LabCyte EPI-MODEL Protocol

5. Plan of additional validation

Chairman : Hajime Kojima (NIHs)
Participating study center : KOBAYASHI Pharmaceutical Co., Ltd.
Fanci Corporation
Drug Safety Testing Center Co., Ltd.

Training : J-TEC gave technical guidance using the improved protocol at the National Institute of Health Sciences on July 27, 2010.

Preliminary test
At each study center, the preliminary test was repeated several times to master the protocol, which was improved for 1-bromohexane to yield positive results.

Implementation period
Validation was performed during the period between September and November, 2010.

6. Modification points of rinsing protocol between SOP ver.7.1 and SOP ver.8.2

1) The PBS stream from washing bottle
It was briefly defined. It was defined to avoid that the PBS stream hit directly to the tissue surface.

2) To remove PBS by tapping
It was not briefly defined. It was briefly defined.
It was defined to avoid that the cotton pad touched directly to the tissue surface.

11. Results and Discussion

The test was positive for 1-bromohexane at all study centers; judgments for other substances stayed unchanged.

The performance standards (acceptance criteria and success criteria) were satisfied at all participating study centers.

A summary report on the present validation results was sent to the OECD secretariat.