

Poster: skin models as alternatives to animal testing

IL-1 α release quantification in culture media: how to ensure standardization?

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The reconstructed human epidermis EPISKIN™ model was involved in the formal ECVAM sponsored acute skin irritation validation (Draize test replacement). The model and the associated prediction model (PM) were validated by the ESAC as stated in April 2007 (Spielmann et al., 2007). The test is based on measurement cells viability by MTT test (ESAC validated) and the release of the pro-inflammatory mediator IL-1 α (recommended by ESAC as a useful adjunct) (ESAC statement, 2007). Indeed the combination of these two end points enabled a sensitivity shift from 75% to 91% (Spielmann et al., 2007). The validated PM relied on specific cut-off values for both viability and IL-1 α . IL-1 α was usually expressed in pg/ml and its quantification was performed by using the R&D Systems ELISA kit. The purpose of this study was to investigate the possibility to use other purchasable IL-1 α quantification systems. In this work, we compared commercially available kits from several suppliers. Dose response linearity of the assay systems was first assessed by using WHO/NIBSC IL-1 α international standard. Good linear responses were observed for all kits with associated specific sensitivities. IL-1 α quantification in test samples also revealed important sensitivity differences related to the kit used. Standardization of the assays and data expression can be obtained by using the common International Unit (IU) value. To make possible results expression in IU for further data comparisons between different laboratories using the validated test, we proposed a 3 steps procedure: 1- Check R&D Systems kit calibration with the reference NIBSC IL-1 α standard in the specific laboratory working conditions and convert pg/ml to IU/ml. 2- Measure IL-1 α in the test samples with the R&D Systems kit and the other ELISA kit to be used as a replacement. 3- Calculate the converting factor between both kits in order to express results in IU (R&D/IL-1 α standard calibration conditions). 3-D tissues being sensitive to mechanical stress during spreading steps, basal levels of IL-1 α in controls can be modulated by operator-dependent rubbing application strength during the process. In order to eliminate operator-dependent basal interleukin release levels, negative control values must be subtracted to test-sample value for calculations. This standardization process helps: 1) to clarify the use of IL-1 α by adjusting and normalizing calculations, 2) to open the possibility to use other supplier measurement kits while respecting the defined PM, 3) to normalize IL-1 α expression as common IU values.

References

ESAC statement. <http://ecvam.jrc.it/>

Spielmann et al. (2007). *ATLA* 35(6), 559-601.

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