Peer Review Report of the EpiSensA Skin Sensitization Assay

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The EpiSensA skin sensitization assay was developed as an alternative method to address Key Event 2 (KE2; keratinocyte activation) in the skin sensitization adverse outcome pathway. The assay utilizes reconstructed human epidermis (RhE) and measures the gene expression of four markers of sensitization: (i) the encoding activating transcription factor 3 (*ATF3*), (ii) the glutamate-cysteine ligase, modifier subunit (*GCLM*), (iii) the DnaJ (*Hsp40*) homolog, subfamily B, member 4 (*DNAJB4*), and (iv) interleukin-8 (*IL-8*). These genes reflect the keratinocyte response to early induction of skin sensitization via cytoprotective and inflammatory pathways. Between 2018 and 2022, the EpiSensA underwent a multilaboratory validation study with the support of the Japanese Center for the Validation of Alternative Methods (JaCVAM) and an international group of experts in skin sensitization and method validation. JaCVAM subsequently convened a peer review panel to review the validation process and performance of the EpiSensA. The outcome of the validation study demonstrated the predictivity, transferability, between-laboratory reproducibility (BLR), and within-laboratory reproducibility (WLR) of the assay. The study also assessed whether the approach could be used for categorization according to the United Nations Globally Harmonized System of Classification and Labelling of Chemicals. The peer review panel met virtually twice and held a face-to-face meeting to complete the review of the validation study. The panel assessed the applicability and rationale behind the assay itself, the completeness of the validation study, and the logic behind in-process changes to the standard operating procedure (SOP). The panel also assessed reproducibility of the assay based on concordance with murine local lymph node assay results. The assay had 86.7-93.3% WLR and 88.9% BLR, both exceeding the prespecified minimum goals of the study, specifically 85% WLR and 80% BLR. Individual labs assessed a total of 27 chemicals for skin sensitization, while the lead lab tested a total of 136 chemicals. The rationale for use of an RhE model for the EpiSensA was to increase the applicability domain beyond that of existing KE2 assays, with the RhE model having greater ability to handle both lipophilic chemicals and pre-/pro-haptens. The EpiSensA correctly predicted 35/37 pre-/pro-haptens, with good scientific rationale behind the two that were not correctly predicted. Additionally, with 69 lipophilic compounds ($LogK_{OW}$ >3.5), the assay had better sensitivity (83%), specificity (65%), and accuracy (78%) than other internationally accepted methods. Transferability to three other facilities was successfully demonstrated. Justified SOP alterations included altering the positive control to provide a more stable alternative and adjusting endogenous control gene criteria and tissue viability acceptance criteria. The peer review panel felt that the EpiSensA is a good method to assess a wide range of chemicals for skin sensitization and supported assessment for inclusion at the Organisation for Economic Cooperation and Development as a test guideline, where it is on the 2022 workplan. The panel recommended that the assay developers clarify how to assess borderline calls and provide performance criteria for development of other similar RhE-based assays. ENR's time on this project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

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