***Title of Research:***

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# The validation study of EpiSensA (Epidermal Sensitization Assay); the *in vitro* skin sensitization assay based on reconstructed human epidermis

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***Summary of Research:***

Lipophilic chemicals are difficult to correctly evaluate by existing in vitro tests because these tests employ aqueous-phase systems. To overcome the limitation, we focused on a reconstructed human epidermis (RhE) and developed the Epidermal Sensitization Assay (EpiSensA) based on the expression of four marker genes related to induction of skin sensitization. Based on the comparison with the results of animal test, we confirmed that EpiSensA has better predictive performance for a variety of chemicals including lipophilic chemicals than existing in vitro tests. Therefore, the validation study of EpiSensA was started from July 2018 at JaCVAM (Japanese Center for the Validation of Alternative Methods) to adopt it for OECD test guideline. At the last year, we reported that the validation management team (VMT) concluded the technical transfer from the lead laboratory to three participating laboratories (Food and Drug Safety Center, KOSÉ Corporation and LION Corporation) was successfully completed.

Therefore, For the evaluation of with-in laboratory reproducibility (WLR), the Phase I study was started from November 2018. The Phase I study using 15 coded test chemicals is divided into three parts (Phase I-A, I-B, and I-C) and each part includes three repetition tests of 5 chemicals. Regarding the Phase I-A, two out of three laboratories didn't meet the target criteria of WLR (>85%) established by the VMT. However, the following points of the protocol were revised and agreed by the VMT; i) statistically determined acceptance criteria of vehicle control, ii) clarified criteria of retesting at a low dilution rate and iii) retesting when internal control gene fails to meet the criteria. After reanalysis and retesting based on the revised protocol, all laboratories met the target criteria. In the subsequent Phase I-B, all laboratories showed 100% WLR according to the revised protocol. Finally, the mean WLR using 10 coded test chemicals was 93% and satisfied the target criteria. Currently, the Phase I-C study is under-evaluation.

***Timeline:***

March 1, 2019 – February 29, 2020

***Topics:***

Poster presentation at JCIA LRI Annual Workshop 2019 "The validation study of EpiSensA (Epidermal Sensitization Assay); the in vitro skin sensitization assay based on reconstructed human epidermis" (Tokyo, August 30th, 2019)