Transfer of a two-tiered keratinocyte assay: IL-18 production by NCTC2544 to determine the skin sensitizing capacity and epidermal equivalent assay to determine sensitizer potency

At present, the identification of potentially sensitizing chemicals is carried out using animal models. However, it is very important from ethical, safety and economic point of view to have biological markers to discriminate allergy and irritation events, and to be able to classify sensitizers according to their potency, without the use of animals. Within the Sens-it-iv EU Frame Programme 6 funded Integrated Project (LSHB-CT-2005-018681), a number of in vitro, human cell based assays were developed which, when optimized and used in an integrated testing strategy, may be able to distinguish sensitizers from non-sensitizers. This study describes two of these assays, which when used in a tiered strategy, may be able to identify contact sensitizers and also to quantify sensitizer potency. Tier 1 is the human keratinocyte NCTC2544 IL-18 assay and tier 2 is the Epidermal Equivalent potency assay. The aim of this study is to show the transferability of the two-tiered approach with training chemicals: 3 sensitizers (DNCB, resorcinol, pPD) and 1 non sensitizer (lactic acid) in tier 1 and 2 sensitizers with different potency in tier 2 (DNCB: extreme and resorcinol; moderate). The chemicals were tested in a non-coded fashion. Here we describe the transferability to naïve laboratories, the establishment of the standard operating procedure, critical points, acceptance criteria and project management. Both assays were successfully transferred to laboratories that had not performed the assays previously. The two tiered approach may offer an unique opportunity to provide an alternative method to the Local Lymph Node Assay (LLNA). These assays are both based on the use of human keratinocytes, which have been shown over the last two decades, to play a key role in all phases of skin sensitization.