Evaluation report on the Local Lymph Node Assay (LLNA): BrdU-ELISA for skin sensitization assay

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Summary

The Local Lymph Node Assay (LLNA) in mice is a test method for measuring objectively and quantitatively lymphocyte proliferation induced by sensitization at the auricular lymph node by means of radioactive 3H-methyl]-thymidine (3H-TdR) uptake. LLNA: BrdU-ELISA is based on the same principle as the original LLNA, but the radioactive 3H-TdR is replaced by bromodeoxyuridine (BrdU), and cell proliferation is measured as absorbance by means of an enzyme-linked immunosorbent assay (ELISA). The principle of this test method together with its simplicity and convenience has been recognized worldwide. Between Japan and overseas, however, there is a difference in cutoff values [that is, the stimulation index (SI), or the ratio of BrdU labeling index for the test substance-treated group to that for the solvent-treated group (the negative control group)] to evaluate whether or not a test substance is positive for skin-sensitization..

In this report, the difference in cutoff values (SI values) was investigated by comparing the validation report on LLNA-BrdU-ELISA (2008) issued by JaCVAM with the LLNA: BrdU-ELISA Evaluation Report (2010) issued by ICCVAM. As a result of this comparison, it was concluded that it would be reasonable to adopt a cutoff value of 1.6, which would result in a judgment of positive for all 32 substances validated as positive for skin-sensitization by ICCVAM. The OECD guideline for LLNA: BrdU-ELISA (2010) also gives 1.6 as a cutoff value for skin-sensitization positive. On the basis of these circumstances, it was considered to be reasonable to adopt the cutoff value of 1.6 even in Japan. In the meanwhile, as far as there are compounds that exhibit false positive for skin sensitization at SI values between 1.6 and 2.0, it is necessary for the final determination of a potential for skin-sensitization in a compound to reference collateral information, such as dose-response information, evidence of systemic toxicity or significant localized skin irritation, as well as statistical comparison of the treated and solvent reference groups, peptide reactivity, molecular weight, results of related substances and other data, as recommended by ICCVAM.