Second evaluation report on the LLNA : BrdU-ELISA for skin sensitization testing

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Summary

An LLNA-BrdU test method for assessing skin-sensitization potency without using radioisotope-labeled compounds, as proposed by Chemicals Evaluation and Research Institute, Japan, was the subject of a preliminary evaluation as an alternative to the local lymph node assay (LLNA) per H16-iyaku-005 (Development of Alternatives to Animal Testing for Assessment of Safety and Establishment of an Assessment System, Y. Ohno et al) issued by the Health Sciences Group in 2004. The LLNA-BrdU is quite similar to the LLNA-DA, which indexes changes in ATP, so the skin-sensitization potency test working group (WG) that was organized by the Peer Review Panel to evaluate the LLNA-DA was also asked to review the LLNA-BrdU. A preliminary review of the applicant's data by the working group determined the LLNA-BrdU to be beneficial in that it was based on the same principle as the original LLNA, incorporated bromodeoxyuridine (BrdU) into DNA to index cell proliferation rather than ^{3}H-methyl thymidine, but was equally efficacious in terms of identifying skin-sensitization, did so without using radioisotopes and was simple and convenient. In order to evaluate suitability as a regulatory test method, however, additional information about reliability of data and interlaboratory variation is needed. For that purpose the protocol was first modified in response to comments from the Peer Review Committee, and then the Validation Committee of the Japanese Society for Alternative to Animal Experiments (JSAAE) was requested to conduct a multi-laboratory validation. The Peer Review Committee organized an Executive Committee, chaired by Hajime Kojima, Ph.D., to oversee the implementation of this validation study. The Executive Committee implemented a first-stage validation of 12 substances (three doses per substance, based on the results of LLNA) at nine facilities. Final evaluation based on a stimulation index (SI is found by dividing the quantity of BrdU incorporated into the test substance group by the quantity of BrdU incorporated into the solvent control group) of the first-stage results, however, varied significantly from facility to facility. The likely cause of this problem was thought to be exaggerated SI values brought on by reduced absorbance in the solvent control group. After reviewing the first-stage results, a range of absorbance for the validation was established, and a pre-validation study looked at ways to ensure minimal variation of the SI value by maintaining absorbance within the specified range, which included determination of a final cell-suspension volume that ensured minimum absorbance as well as dilution of cell suspensions that exceeded maximum absorbance. This hypothesis was verified in a pilot validation at the nine facilities that participated in the first-stage validation, and a new test protocol was created, specifying an absorbance between 0.1 and 0.2 for the solvent control group. The Executive Committee then implemented a second-stage validation of 10 substances (three doses per substance, based on the LLNA results) at seven facilities. Results showed that roughly 60% of the validation tests satisfied the predetermined criteria for maintaining 0.1–0.2 absorbance needed for indicating the quantity of BrdU incorporated into the solvent control group.

This result was thought to indicate that the predetermined criteria were too strict. Also, numerous measurements suggested that further dilution of the cell suspension hindered uniform suspension of cells, which made it clear that variation would worsen. The Validation Executive Committee, after disclosing the code and reviewing results, relaxed the criteria, and without performing post-storage measurements of diluted solution, analyzed the second-stage validation results using an SI value of 2 or more in the positive control substance HCA at 50% concentration as a condition for validity. This analysis showed that, although there were some exceptions, the SI value was dependent on concentration, and gave overall low interlaboratory variation as well as good correspondence with the LLNA.
When these results were combined with the applicant's own in-house test results, a total of 30 substances showed a sensitivity of 83%, a specificity of 92%, positive predictivity of 94%, negative predictivity of 79%, correspondence of 87%, and an accuracy of 87%, which were high enough to result in the determination that the LLNA-BrdU is a useful alternative to the LLNA.