The Regulatory Acceptance Board Report on revisions to judgment criteria used for the LLNA: BrdU-ELISA

JaCVAM Regulatory Acceptance Board

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The JaCVAM Regulatory Acceptance Board previously evaluated the validity of an alternative test method for assessing skin sensitization, Local Lymph Noda Assay (LLNA): BrdU-ELISA.[1] Having received the Skin Sensitization Test Peer Review Panel's report[2] on revisions to judgment criteria used for the LLNA: BrdU-ELISA, we hereby report our evaluation of the following 10 items.

The Item Discussed

1. For which existing test methods is this test method an alternative and what kinds of toxicity will it be used to evaluate or predict?

LLNA: BrdU-ELISA is a modified form of the Mouse Local Lymph Node Assay, which is an alternative means of evaluating potential for skin sensitization of chemical substances conventionally performed using a guinea pig maximization test (GPMT) or a Buehler Test (BT). It is therefore used to predict potential for skin sensitization of the same chemical substances for which the LLNA is conventionally used.

2. What kind of scientific connection is there between this test method and existing test methods?

The LLNA is a test method for obtaining a quantitative measurement of sensitization-induced lymphocyte proliferation at the auricular lymph node by measuring uptake of ^{3}H-methyl-thymidine (^{3}H-TdR). This test method is based on the same principle as the original LLNA, but ^{3}H-TdR is replaced by bromodeoxyuridine (BrdU), and cell proliferation is measured as light absorbence by means of an enzyme-linked immunosorbent assay (ELISA).

3. Have this test method and the supporting validation data been subjected to a transparent and independent peer review process?

ICCVAM organized a Peer Review Panel to perform a retrospective analysis of test results for 43 test substances, including 10 test substances tested as part of a verification performed by JaCVAM,[3] and compared these results with results obtained from conventional test methods. This analysis showed that the use of a cutoff value of 1.6 or larger for skin-sensitization positive yields results equivalent to those of conventional test methods, with which the accuracy, sensitivity, and specificity of this test method were evaluated.[4] The organization and the evaluation results are available from the ICCVAM website.
In addition, a LLNA: BrdU-ELISA Skin Sensitization Test Peer Review Panel in Japan did a comparative review of a verification report on this test method prepared by JaCVAM with the above-mentioned evaluation published by ICCVAM.
We therefore consider the revisions to the judgment criteria for this test method to have been subjected to a transparent and independent peer review process.

4. As an alternative to an existing test, what substances or products will this test method be used to evaluate?

This test method will be used as an alternative test method to identify the potential for skin sensitization of any substances or products that are tested by conventional test methods including drugs used as external medicine for skin, quasi drugs for dermal application, cosmetics, agrochemicals, or medical devices.

5. Does this test method generate data useful for hazard or risk assessment purposes?

This test method is useful for hazard assessment of potential skin sensitization of the above-mentioned substances and products.

6. Is this test method capable of assessing toxicity of the subject substances and products? In which case, have the application parameters for this test method been clarified?

Supporting validation data for this test method test comprises results for 43 substances, including 10 substances tested as part of a verification performed by JaCVAM, including cosmetics, chemicals, agrochemicals, drugs, sanitizing and disinfecting agents, synthetic intermediates and raw materials, food additives, fragrances, or sanitary materials and solutions.
Accordingly, we consider this test method capable of assessing potential for skin sensitization in substances and products such as these.
As with the conventional test method, the maximum dosage used in this test method is one that does not produce excessive localized irritation or obvious systemic toxicity. In order to eliminate false negatives, positive judgment criteria for skin sensitization has been changed from a cutoff value from 2.0 or larger, as verified by JaCVAM, to 1.6 or larger. When using this revised judgment criteria, insofar as there exist substances that cause false positives, final determination of positive skin sensitization is to be made only after making reference to additional information about the test substance, such as dose-response information, evidence of systemic toxicity or excessive localized skin irritation, potential protein binding, molecular mass, or other records of related chemical substances.
The limits of applicability are the same as for the LLNA.

7. Is this test method robust against minor changes in protocol?

This test method is based on the same principle as the conventional test method and we expect it be identical in terms of accuracy, intralaboratory reproducibility and interlaboratory reproducibility, and robustness. Because measurement of BrdU is performed using a commercially available ELISA kit, it is likely that changes in how the measurement is performed (for example, washing and drying of plates or secondary antibody reaction times)

could result in variations in measured values. Thus it is necessary that each laboratory make reference to the ICCVAM report in establishing and following faithfully the test protocol.

8. Is this test method easily transferable among adequately trained and experienced personnel? Does this test method require special equipment?

This test method is easily learned by properly trained and experienced personnel. Compared with conventional test methods, there is no need for special equipment used at facilities where radioactive materials are handled.

9. Is this test method time and cost effective relative to conventional test methods?

Implementation of the conventional test method was subject to numerous restrictions due to the use of radioactive substances as a means of measuring induced lymphocyte proliferation, which required special facilities and equipment for the handling of radioactive substances and the disposal of radioactive waste. In contrast, this test method can be performed using ordinary laboratory equipment, and, because it does not use radioactive substances, does not require any special equipment or the disposal of radioactive waste, which means that it is considered both time and cost effective.

10. Is this test method likely to be useful in a regulatory context as an alternative means of evaluating the toxicity of subject substances and products from the perspective of both science and animal welfare?

This test method is not an alternative test method that does not use animals. Relative to the GPMT and other test methods used to predict potential for skin sensitization, is does result in less stress and discomfort for the test animals, which means that it is useful in terms of refinement. This test method will be used to identify the potential for skin sensitization of any substances or products that are contained in drugs used as external medicine for skin, quasi drugs for dermal application, cosmetics, agrochemicals, or medical devices, and insofar as it is possible to eliminate false negatives by lowering cutoff values used in judgment criteria as well as to obtain results similar to conventional test methods but without the use of radioactive substances, we consider this test method likely to be useful in a regulatory context.

Bibliography

1) Regulatory Acceptance Board report on the LLNA: BrdU skin sensitization test method, JaCVAM Regulatory Acceptance Board (May, 2010; revised April, 2011)
2) Peer Review Panel report on an alternative skin sensitization test method, Local Lymph Node Assay: BrdU-ELISA (July, 2012)
3) Hajime Kojima, et al. Inter-laboratory validation of the modified murine local lymph node assay based on 5-bromo-2'-deoxyuridine incorporation. J. Appl. Toxicol. 31: 63-74 (2011)

4) ICCVAM (2010). ICCVAM Test Method Evaluation Report on the Murine Local Lymph Node Assay: BrdU-ELISA, a Nonradioactive Alternative Test Method to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products. NIH Publication No. 10-7552. Research Triangle Park, NC: National Institute of Environmental Health Sciences.