The Regulatory Acceptance Board Report on revisions to judgment criteria used for the LLNA: DA

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): DA.[1] Having received the Skin Sensitization Test Peer Review Panel's report[2] on revisions to judgment criteria used for the LLNA: DA, we hereby report our evaluation of the following 10 items.

Discussion

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: DA 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 means of ^{3}H-methyl-thymidine (^{3}H-TdR), a radioactive substance, uptake into DNA. This test method is based on the same principle as the original LLNA, but ^{3}H-TdR is replaced by adenosine triphosphate (ATP), and ATP levels in the cells are measured as chemiluminescence induced by means of a luciferin-luciferase reaction.

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 44 test substances, including 14 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 1.8 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: DA 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 44 substances, including 14 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 valueof 3.0 or larger to one of 1.8 or grater, as verified by JaCVAM. 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 repeatability, interlaboratory reproducibility, and robustness. This test method involves the measurement of ATP levels in lymphocytes, and for this reason is not suitable for the testing of substances that affect the synthesis or the measurement of ATP.

Also, since ATP levels are likely to decrease across time following the removal of the lymph nodes, it is desirable that the time from the extraction of the lymph node to the measurement of chemiluminescence be as consistent as possible for each lymph node. Moreover, discrepancies can be expected in the quantity of luminescence and its attenuation produced by commercially available ATP assay kits. Nevertheless, as long as the latest protocol recommended by ICCVAM is adhered to, this test method can be considered robust against minor changes in 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?

Actual implementation of the conventional test method was subject to numerous restrictions due to the use of a radioactive to measure 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, it results 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 an alternative skin sensitization test method: Local Lymph Node Assy: DA (October, 2008; revised April, 2013)
2) Peer Review Panel report on an alternative skin sensitization test method: Local Lymph Node Assay: DA (July, 2012)
3) Takashi Omori, et al. Interlaboratory validation of the modified murine local lymph node assay based on adenosine triphosphate measurement. Journal of Pharmacological and Toxicological Methods. 58: 11-26 (2008)
4) ICCVAM (2010). ICCVAM Test Method Evaluation Report on the Murine Local Lymph Node Assay: DA, a Nonradioactive Alternative Test Method to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products. NIH Publication No. 10-7551. Research Triangle Park, NC: National Institute of Environmental Health Sciences