1. The submitted test method and supporting validation data should have been subjected to a transparent and independent peer review process.

   The results of performance studies at participating laboratories can be found in published academic research. The results of inter-laboratory validation have been published in an academic journal.[3]

   The content of these publications was reviewed by the Japanese Committee for the Academic Validation of Alternative Methods, an independent committee of experts, chaired by Yasuo Ono, and the results of that review have been presented in a *Report on First- and Second-Phase Validation of the LLNA: BrdU Test Method for Assessing Skin-Sensitization Potency as an Alternative to Animal Testing as Proposed by the Chemicals Evaluation and Research Institute, Japan*.

   Some modifications to the protocol were made during the validation process.

2. Data generated by the test method should adequately measure or predict the endpoint of interest. For replacement test methods, the data should show a linkage between the proposed test method and an existing test method, and/or the proposed test method and effects in the target or model species.

   This test method is considered an alternative to the LLNA[4] test method for immunotoxic skin sensitization.

   This test method has been sufficiently validated against guinea-pig maximization tests (GPMT) and murine local lymph node assays (LLNA) without uncovering any significant issues.

   This test method is identical to the LLNA with the exception that bromodeoxyuridine (BrdU), rather than a radioisotope such as 3H-methyl-thymidine, is used as a marker.

   The LLNA provides a very high sensitivity and specificity of 80% of better for the endpoint of interest, and this test method also achieves a similar standard.

   Based on the results of validation at multiple laboratories, both intra- and inter-laboratory reproducibility are equivalent to the LLNA.

3. The test method should generate data useful for hazard/risk assessment purposes.

Although we consider usefulness for hazard assessment to be equivalent to the LLNA, this test method is not suitable for risk assessment.

4. The submitted test method and supporting validation data should adequately cover a spectrum of chemicals and products representative of those administered by the regulatory program or agency for which the test method is proposed, and the applicability and limitations of the test method should be clearly described.

This test method is designed for soluble substances and is not suitable for testing solids or substances of low solubility.

The assessment of irritating or sensitizing substances is difficulty and lies outside the applicability of this test method.

The limits of applicability are the same as for the LLNA.

5. The test method should be sufficiently robust (relatively insensitive to minor changes in protocol) and transferable among properly-equipped laboratories with adequately-trained staff.

Test methods that utilize the incorporation of tritiated methyl-thymidine to DNA as a means of labeling cells involve no particular processing of the cells but rather measure the activity of the radioisotope. The LLNA: BrdU, on the other hand, requires preprocessing of the cells and compared with the LLNA, involves more factors that could potentially contribute to variance and inter-laboratory discrepancies.

This test method requires strict conformity with the test protocol in order to obtain a valid assessment of test substances.

Compared with the LLNA, a relatively high level of technical expertise is required to obtain favorable performance.

6. The test method should be both time and cost effective as well as likely to be used in a regulatory context.

This test method does not utilize radioisotopes, and compared with the LLNA, reduces time and cost both in a regulatory context and for participating laboratories.

Operating time is equivalent to that for the LLNA.

Cost is less than for the LLNA.

Therefore, this test is suitable for use as a regulatory test method.

7. Justification should be provided (scientific, ethical, and economical) for the new or updated test method in light of already existing test methods.

Scientifically, this test method utilizes the same mechanism as the LLNA in that both tests are based on observing proliferation of lymphocytes.

There is little ethical difference with the LLNA.

There are no restrictions placed on laboratories or test technicians, and reductions in the cost of waste disposal provide economic justification.

Use can be justified as a modification of the LLNA.

8. The test method should be acceptable for use in regulatory documentation on safety assessment.

This test method is designed to assess skin sensitization, and is capable of detection on a par with existing LLNA test methods. It is useful in the assessment of potential skin sensitization by drugs, quasi drugs, cosmetic products, cosmetic ingredients, and chemical substances.

Based on the above, the JaCVAM Regulatory Acceptance Board has determined that correct application in accordance with all precautions stipulated by the LLNA: BrdU test method for assessing skin-sensitization potency is a scientifically-valid means of assessing the skin-sensitization potency of chemical substances.