Internationally Harmonized Performance Standards for the Murine Local Lymph Node Assay (LLNA)

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Introduction

- United States and international regulatory authorities accept the murine local lymph node assay (LLNA) as an alternative test method for allergenic contact dermatitis testing (ICCVAM 1999, OECD 2002).
- The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), in conjunction with the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Centre for the Validation of Alternative Methods (JaCVAM), has developed LLNA performance standards (PS) that can be used to evaluate LLNA methods that are mechanistically and functionally similar to the LLNA.
- These performance standards consist of:
  - Essential test method components
  - Reference substances
  - The accuracy and reliability that should be achieved or exceeded by a modified test method

Essential Test Method Components

Essential test method components are structural, functional, and procedural elements of a validated test method, necessary for it to be evaluated using the PS. For the LLNA, these include:

- The test substance is applied topically to both ears of mice.
- Lymphocyte proliferation must be measured in the lymph nodes draining the site of test substance application.
- Lymphocyte proliferation must be measured during the induction phase of skin sensitization.
- The highest dose selected must be the maximum soluble concentration that does not induce systemic toxicity and/or excessive local irritation.
- A vehicle control must be included in each study and, where appropriate, a positive control should be used.
- A minimum of four animals per dose group is required.

The essential test method components have been internationally harmonized for the validation of modifications to the traditional LLNA. However, certain national regulatory authorities might have requirements that differ from the proposed use of a modified LLNA test method in support of regulatory submissions.

Performance Standard-Reference Substances for the LLNA

- 18 required substances and
- 4 “optional substances” that are either false positive or false negative in the LLNA when compared to either human or guinea pig responses.

Updated ICCVAM Recommended Test Method Protocols for LLNA

ICCVAM’s LLNA performance standards include the updated ICCVAM recommended test method protocols. (ICCVAM 2002)

Key Elements

- The highest dose tested should be the maximum soluble concentration that does not produce systemic toxicity and/or excessive local irritation.
- Individual animal data is collected.
- A concurrent positive control is included in each LLNA study.
- A minimum of four individual animals rather than five individual animals per group is required.

Criteria for Selection of Reference Substances

- Commercial availability
- Available LLNA, guinea pig, and (where possible) human data/experience
- Range of LLNA responses from negative to weak to strong
- Range of chemical and chemical classes
- Range of physical properties (e.g., solids vs. liquids)

Performance Standard-Reference Substances for the LLNA

- 18 required substances and
- 4 “optional substances” that are either false positive or false negative in the LLNA when compared to either human or guinea pig responses.

Test Method Performance Standard-Inter- and Intra-Method Variability

- Intra-method variability:
  - clerical ECI values for HCA on 4 separate occasions (minimum 1 week washout and 1 week washout between washout and test; 1 ECI per dose group per test, total of 4 ECIs per test, unless otherwise specified).
  - ECIs between 5 and 20% indicate acceptable intraindividual variability.

- Inter-method variability:
  - clerical ECI values for HCA and DNCB from at least one other laboratory.
  - ECIs between 5% and 20% for HCA and between 0.25% and 1% for DNCB indicate acceptable interlaboratory variability.

ICCVAM’s LLNA performance standards include the updated ICCVAM recommended test method protocols (ICCVAM 2002).