ICCVAM Evaluation and Recommendations on the Nonradioactive LLNA: BrdU-ELISA for Evaluating Allergic Contact Dermatitis Hazards

A Jacobs[1], J Matheson[2], M Wind[2], V Malshet[1], J Toy[1], W Stokes[3]

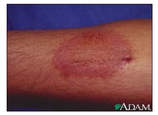
[1]U.S. FDA, Silver Spring, MD; [2]U.S. CPSC, Bethesda, MD; [3]NICEATM/NTP/NIEHS/NIH/DHHS, RTP, NC

Abstract

ICCVAM assessed the usefulness and limitations of the LLNA: BrdU-ELISA, a nonradioactive murine local lymph node assay (LLNA) that measures the amount of BrdU incorporation into the DNA of proliferating lymphocytes as an indicator of potential allergic contact dermatitis (ACD) hazards. Accuracy when compared to the traditional LLNA was assessed based on data generated with 43 substances and using several different stimulation indices (SI) as decision criteria. Optimal performance was achieved using SI ≥ 1.6; the LLNA: BrdU-ELISA correctly identified all 32 LLNA sensitizers (0% [0/32] false negatives) and 9/11 LLNA nonsensitizers (18% [2/11] false positives). The 2 false positives had maximum SI between 1.6 and 1.9. There were 18 substances with repeat tests. Results for 85% (11/13) of the LLNA sensitizers and 60% (3/5) of the LLNA nonsensitizers were 100% concordant among the repeat LLNA: BrdU-ELISA tests. ICCVAM concluded that the accuracy and reproducibility of the LLNA: BrdU-ELISA support its use to identify potential skin sensitizers and nonsensitizers. ICCVAM recommends SI ≥ 1.6 to identify potential sensitizers since there were no false negatives relative to the LLNA. In testing situations where dose-response information is not required or negative results are anticipated, ICCVAM recommends that the single-dose reduced LLNA: BrdU-ELISA should be considered and used, thereby reducing animal use by up to 40%. The ICCVAM-recommended protocol formed the basis of the recently adopted OECD Test Guideline 442B for the LLNA: BrdU-ELISA. Because the LLNA: BrdU-ELISA does not require radioactive reagents, more institutions can take advantage of the reduction and refinement benefits afforded by the LLNA compared to traditional guinea pig methods for ACD testing. The LLNA: BrdU-ELISA will also eliminate the environmental hazard associated with use and disposal of radioactive materials used in the LLNA.

Introduction

- The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is charged with evaluating the scientific validity of new, revised, and alternative toxicological test methods applicable to U.S. Federal agency safety testing requirements.[1]
  - ICCVAM forwards recommendations to Federal agencies.
  - Agencies must respond to ICCVAM within 180 days.[1]
- After a 2007 nomination by the U.S. Consumer Product Safety Commission (CPSC), ICCVAM evaluated the nonradioactive LLNA: BrdU-ELISA (**Figure 1**) to assess the allergic contact dermatitis (ACD) hazard potential of substances.
  - ACD is an allergic skin reaction characterized by redness, swelling, and itching that can result from repeat contact with a sensitizer.



ACD Rash

- Takeyoshi et al. developed the LLNA: BrdU-ELISA (Takeyoshi et al. 2001).
  - The LLNA: BrdU-ELISA measures BrdU incorporation in draining auricular lymph nodes as a measure of lymph node cell proliferation.
- This poster summarizes the ICCVAM evaluation and recommendations for the LLNA: BrdU-ELISA:
  - Usefulness and limitations
  - Test method protocol
  - Future studies
  - Performance standards

[1] ICCVAM Authorization Act. 2000. Public Law 106-545. 42 U.S.C. § 2851-2, 2851-5. Available: http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf.

Validation Status of the LLNA: BrdU-ELISA

Accuracy

- The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) analyzed the accuracy of the LLNA: BrdU-ELISA based on 43 substances with LLNA: BrdU-ELISA and traditional LLNA data.
  - Kojima et al. 2008 (interlaboratory validation study)
  - Takeyoshi et al. 2003; 2004a, b; 2005; 2006; 2007, and unpublished studies
- Several decision criteria for a positive LLNA: BrdU-ELISA response were evaluated to determine the optimum threshold for a positive LLNA: BrdU-ELISA response (**Figure 2**).
- A stimulation index (SI) ≥ 1.6 produced optimum results with high accuracy and no false negatives.
  - Accuracy = 95% (41/43)
  - False positive rate = 18% (2/11)
    - Hexane and lactic acid: 1.6 < SI < 1.9.
  - False negative rate = 0% (0/32)

Reliability

Intra- and interlaboratory reproducibility were assessed.

- Quantitative: NICEATM did a coefficient of variation (CV) analysis of SI values and estimated concentrations expected to produce an SI of 1.6 (EC1.6 values).
  - Intralaboratory CVs ranged from 1% to 80% for SI values of 13 substance/concentration combinations that were tested up to five times each.
  - Interlaboratory CV values for the EC1.6 values of seven sensitizers ranged from 31% to 93%.
- Qualitative: NICEATM analyzed concordance of sensitizer and nonsensitizer results.
  - **Table 1** shows multiple tests of 18 substances (13 LLNA sensitizers and 5 nonsensitizers).
  - Concordance for sensitizer outcomes for 83% (11/13) of the substances
    - Two discordant LLNA sensitizers, hydroxycitronellal and linalool, produced SI < 1.6 in one test and SI > 1.6 in another test.
  - Concordance for nonsensitizer outcomes for 60% (3/5) of the substances
    - Concordant results for one LLNA nonsensitizer, hexane, were false positive in two tests (2/2 tests had SI ≥ 1.6).
    - Concordance for the other two nonsensitizers was 71% (5/7) for isopropanol and 67% (2/3) for lactic acid.

Figure 1. LLNA: BrdU-ELISA Test Method Protocol

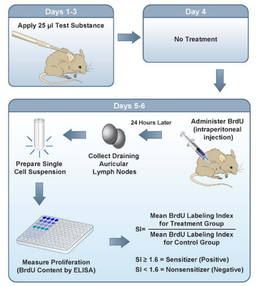


Table 1. Concordance of LLNA: BrdU-ELISA Tests Across Maximum SI Categories

Substance Name	LLNA: BrdU-ELISA Nonsensitizers (Maximum SI ≤ 1.6[1])	LLNA: BrdU-ELISA Sensitizers (Maximum SI ≥ 1.6)		Total Tests
		1.6 < Maximum SI < 1.9[1]	Maximum SI ≥ 1.9[1]	
***Sensitizers*[2]**				
Cyclamen aldehyde	0 (0%)	0 (0%)	0 (100%)	2
2,4-Dinitrochlorobenzene	0 (0%)	0 (0%)	9 (100%)	9
Diphenylcyclopropenone	0 (0%)	0 (0%)	3 (100%)	3
Eugenol	0 (0%)	0 (0%)	9 (100%)	9
Formaldehyde	0 (0%)	0 (0%)	3 (100%)	3
Glutaraldehyde	0 (0%)	0 (0%)	5 (100%)	5
Hexyl cinnamic aldehyde	0 (0%)	0 (0%)	12 (100%)	12
Hydroxycitronellal	1 (50%)	0 (0%)	1 (50%)	2
Isoeugenol	0 (0%)	0 (0%)	3 (100%)	3
Linalool	1 (50%)	0 (0%)	1 (50%)	2
Nickel sulfate	0 (0%)	0 (0%)	3 (100%)	3
1,4-Phenylenediamine	0 (0%)	0 (0%)	2 (100%)	2
trans-Cinnamaldehyde	0 (0%)	0 (0%)	4 (100%)	4
***Nonsensitizers*[2]**				
Hexane	0 (0%)	2 (100%)	0 (0%)	2
Isopropanol	5 (71%)	0 (0%)	2 (29%)	7
Lactic acid	0 (0%)	2 (67%)	1 (33%)	3
Methyl salicylate	3 (100%)	0 (0%)	0 (0%)	3
Propylene glycol	3 (100%)	0 (0%)	0 (0%)	3

Abbreviations: LLNA: BrdU-ELISA = murine local lymph node assay with enzyme-linked immunosorbent assay detection of bromodeoxyuridine; SI = stimulation index.

[1] Numbers shown reflect number of tests. Percentage in parentheses reflects percentage of the total number of tests for each substance.

[2] Categorization is based on traditional LLNA results.

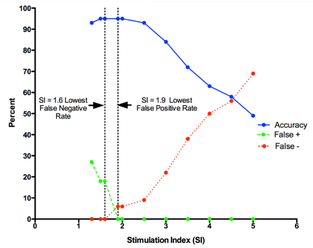
ICCVAM Recommendations: Test Method Usefulness and Limitations

- The LLNA: BrdU-ELISA can be used to identify potential skin sensitizers or nonsensitizers.
  - Use SI ≥ 1.6 to identify potential skin sensitizers.
  - SI ≥ 1.6 produced no false negatives, relative to the traditional LLNA.
- There is slight potential for false positives with borderline weak positive responses (1.6 < SI < 1.9).
  - Consider additional information such as the strength of the dose-response relationship, statistical significance, evidence of systemic toxicity and/or excessive skin irritation together with SI values.
- The LLNA: BrdU-ELISA might not be appropriate for testing certain classes of materials with properties that interfere with the assay.
  - Exception: Unlike the traditional LLNA, the LLNA: BrdU-ELISA can be used for testing nickel compounds based on its ability to correctly identify them as potential skin sensitizers.
- These limitations, as well as the expertise and equipment of the testing laboratory, should be considered when deciding whether this assay is appropriate for the intended use.

ICCVAM Recommendations: Test Method Protocol

- The LLNA: BrdU-ELISA protocol incorporates all aspects of the traditional LLNA protocol except for those procedures unique to the conduct of the LLNA: BrdU-ELISA.
- The LLNA: BrdU-ELISA protocol is the same as the traditional LLNA protocol except:
  - It measures BrdU instead of ^{3}H-thymidine incorporation into lymph node cells and uses ELISA to assess proliferation.
  - BrdU is injected intraperitoneally, instead of ^{3}H-thymidine intravenously through the tail vein.
- The reduced LLNA: BrdU-ELISA (rLLNA: BrdU-ELISA) should be used routinely to determine the ACD hazard potential of chemicals and products.
  - Like the reduced LLNA (Kimber et al. 2006; ESAC 2007; ICCVAM 2009a), the rLLNA: BrdU-ELISA protocol uses only the high dose further reducing animal use by 40%.
  - Using a high dose group only and SI ≥ 1.6 to identify skin sensitizers, the accuracy of the rLLNA: BrdU-ELISA was 95% (82/85), with a false positive rate of 0% (0/11) and a false negative rate of 4% (3/74). Three rLLNA: BrdU-ELISA studies were false negative
    - Two tests of 10%, 25% and 50% isopropanol produced a maximum SI = 2.04 and 2.22 at the lowest dose tested.
    - A test of 2-mercaptobenzothiazole produced a maximum SI = 1.62 at the lowest dose tested.
  - If existing information suggests a substance might have ACD hazard potential *and* dose-response information is needed, consider testing in the multidose LLNA: BrdU-ELISA.

Figure 2. SI Decision Criterion Performance of the LLNA: BrdU-ELISA Compared with the Traditional LLNA Using 43 Substances



Abbreviations: LLNA: BrdU-ELISA = murine local lymph node assay with enzyme-linked immunosorbent assay detection of bromodeoxyuridine; SI = stimulation index.

Compared to traditional LLNA results, the lines show the change in performance characteristics for the LLNA: BrdU-ELISA with the SI used to identify skin sensitizers. This analysis used LLNA results for 32 sensitizers and 11 nonsensitizers. For 18 substances with multiple LLNA: BrdU-ELISA test results, the most prevalent outcome was used.

ICCVAM Recommendations: Future Studies

- Efforts should be made to identify additional human data and experience for test substances to further assess the usefulness and limitations of this and other versions of the LLNA for identifying human skin sensitizers.
  - Post-marketing surveillance of consumers for allergic reactions
  - Occupational surveillance of potentially exposed workers
- Additional nonsensitizing skin irritants should be tested to determine their impact on the LLNA: BrdU-ELISA false positive rate.
- Efforts should be made to further characterize the ACD hazard potential of LLNA: BrdU-ELISA borderline weak positive substances (1.6 < SI < 1.9) to determine if such results might be false positives.
- Other available information could be considered to confirm that such borderline results are potential skin sensitizers, such as:
  - Dose-response data
  - Evidence of systemic toxicity or excessive local irritation
  - Statistical significance (where appropriate) together with SI values
  - Various properties of the test substance, including whether it is structurally similar to known skin sensitizers
- Decision criteria should be reassessed as additional discriminators and data become available.

ICCVAM Recommendations: Performance Standards

- ICCVAM-recommended performance standards (ICCVAM 2009b) for the traditional LLNA can be used to evaluate future modifications of the LLNA: BrdU-ELISA because it is functionally and mechanistically similar to the traditional LLNA.

ICCVAM Interagency Immunotoxicity Working Group (IWG)

Consumer Product Safety Commission
Joanna Matheson, Ph.D. (Working Group Co-chair)
Marilyn Wind, Ph.D. (through July 2010)

Environmental Protection Agency
Office of Pesticide Programs
Jonathan Chen, Ph.D.
John R. "Jack" Fowle III, Ph.D., DABT
Masih Hashim, D.V.M., Ph.D.
Marianne Lewis
Deborah McCall
Timothy McMahon, Ph.D.
John Redden
Jenny Tao, Ph.D.

Office of Pollution Prevention and Toxics
Elizabeth Margosches, Ph.D.
Ronald Ward, Ph.D.

Office of Research and Development
Marsha Ward, Ph.D.

Office of Science Coordination and Policy
Karen Hamernik, Ph.D.

Food and Drug Administration
Center for Devices and Radiological Health
Vasant G. Malshet, Ph.D., DABT
Jeffrey Toy, Ph.D.

Center for Drug Evaluation and Research
Ruth Barrett, Ph.D., D.V.M.
Paul Brown, Ph.D.
Abigail Jacobs, Ph.D. (Working Group Co-chair)
Jiaqin Yao, Ph.D.

Center for Food Safety and Applied Nutrition
Donnie Lowther
Neil Wilcox, D.V.M., M.P.H.

Office of the Commissioner
Suzanne Fitzpatrick, Ph.D., DABT

National Institute of Environmental Health Sciences
Warren Casey, Ph.D., DABT
Dori Germolec, Ph.D.
William Stokes, D.V.M., DACLAM

National Institute for Occupational Safety and Health
B. Jean Meade, D.V.M., Ph.D.
Paul D. Siegel, Ph.D.

National Library of Medicine
Pertti Hakkinen, Ph.D.

European Centre for the Validation of Alternative Methods - Liaison
Silvia Casati, Ph.D.
Alexandre Angers, Ph.D.

Japanese Center for the Validation of Alternative Methods - Liaison
Hajime Kojima, Ph.D.

Interagency Coordinating Committee on the Validation of Alternative Methods: *Designated Agency Representatives*

Agency for Toxic Substances and Disease Registry
*Moiz Mumtaz, Ph.D.
Bruce Fowler, Ph.D.
Edward Murray, Ph.D.
Eric Sampson, Ph.D.

Consumer Product Safety Commission
*Joanna Matheson, Ph.D. (Vice Chair)
+Kristina Hatfield, Ph.D., MPH

Department of Agriculture
*Jodie Kulpa-Eddy, D.V.M. (Chair)
+Elizabeth Goldentyer, D.V.M.

Department of Defense
*David Honey, Ph.D.
+Terry Besch, D.V.M., DACLAM, DACVPM
+Patty Decot

Department of Energy
*Michael Kuperberg, Ph.D.
+Marvin Stodolsky, Ph.D.

Department of the Interior
*Barnett A. Rattner, Ph.D.

Department of Transportation
+Steve Hwang, Ph.D.

Environmental Protection Agency
Office of Pesticide Programs
*John R. "Jack" Fowle III, Ph.D., DABT
+Vicki Dellarco, Ph.D.
+Tina Levine, Ph.D.
Christine Augustyniak, Ph.D.
Deborah McCall

Food and Drug Administration
Office of the Commissioner
*Suzanne Fitzpatrick, Ph.D., DABT

Center for Biologics Evaluation and Research
Ying Huang, Ph.D.
Richard McFarland, Ph.D., M.D.

Center for Devices and Radiological Health
Vasant G. Malshet, Ph.D., DABT

Center for Drug Evaluation and Research
+Abigail C. Jacobs, Ph.D.
Paul C. Brown, Ph.D.

Center for Food Safety and Applied Nutrition
David G. Hattan, Ph.D.
Neil L. Wilcox, D.V.M., MPH

Center for Veterinary Medicine
M. Cecilia Aguila, D.V.M.
Devaraya Jagannath, Ph.D.

National Center for Toxicological Research
Paul Howard, Ph.D.
Donna Mendrick, Ph.D.

National Cancer Institute
*T. Kevin Howcroft, Ph.D.
+Chand Khanna, D.V.M., Ph.D.

National Institute of Environmental Health Sciences
*William S. Stokes, D.V.M., DACLAM
+Warren Casey, Ph.D.
Rajendra S. Chhabra, Ph.D., DABT
Jerrold J. Heindel, Ph.D.

National Institute for Occupational Safety and Health
*Paul Nicolaysen, V.M.D.

National Institutes of Health
*Margaret D. Snyder, Ph.D.

National Library of Medicine
*Pertti (Bert) Hakkinen, Ph.D.
+Jeanne Goshorn, M.S.

Occupational Safety and Health Administration
*Surender Ahir, Ph.D.

*Principal agency representative
+Alternate principal agency representative

LLNA Peer Review Panel Meetings

- NICEATM and ICCVAM organized public meetings of an international independent scientific peer review panel (Panel) at the CPSC in Bethesda, MD, on March 4-6, 2008, and at the National Institutes of Health in Bethesda, MD, on April 28-29, 2009 (see **Figure 3**).

Independent Scientific Peer Review Panel

Left to right: Back row: Takahiko Yoshida, M.D., Ph.D., Asahikawa Medical College, Hokkaido, Japan; Michael Olson, Ph.D., A.T.S., GlaxoSmithKline, Research Triangle Park, NC; Kim Headrick, B.Admin., B.Sc., Health Canada, Ottawa, Ontario, Canada; Thomas Gebel, Ph.D., Federal Institute for Occupational Safety & Health, Dortmund, Germany; James McDougal, Ph.D., Wright State University, Dayton, OH; Michael Woolhiser, Ph.D., Dow Chemical, Midland, MI; Howard Maibach, M.D., University of California–San Francisco, San Francisco, CA; Steven Ullrich, Ph.D., M.D. Anderson Cancer Center, Houston, TX

Middle row: William Stokes, D.V.M., D.A.C.L.A.M., National Institute of Environmental Health Sciences, Research Triangle Park, NC (ICCVAM Executive Director, NICEATM Director); Peter Theran, V.M.D., Consultant, Massachusetts Society for the Prevention of Cruelty to Animals, Novato, CA; Dagmar Jirová, M.D., Ph.D., National Institute of Public Health, Prague, Czech Republic; Jean Regal, Ph.D., University of Minnesota Medical School, Duluth, MN; Michael Luster, Ph.D., Senior Consultant to the National Institute for Occupational Safety and Health, Morgantown, WV (Panel Chair); Raymond Pieters, Ph.D., Utrecht University, Utrecht, The Netherlands

Front row: Nathalie Alépée, Ph.D., L'Oréal Research and Development, Aulnay sous Bois, France; Marilyn Wind, Ph.D., U.S. Consumer Product Safety Commission, Bethesda, MD (ICCVAM Chair through July 2010); Nancy Flournoy, M.S., Ph.D., University of Missouri–Columbia, Columbia, MO; Anne Marie Api, Ph.D., Research Institute for Fragrance Materials, Woodcliff Lake, NJ; David Lovell, Ph.D., FBiol, CStat, CBiol. University of Surrey, Guildford, Surrey, U.K.

Not pictured: Sidney Green, Ph.D., Howard University, Washington, DC; Jonathan Richmond, MB ChB, FRCSEd, Home Office, London, U.K.

Charge to the Peer Review Panel

- Review the draft background review document (BRD) for errors and omissions
- Provide conclusions and recommendations on the current validation status of the LLNA: BrdU-ELISA
- Comment on whether the draft BRD supports ICCVAM's draft test method recommendations

Peer Review Panel Conclusions

- Agreed that available data and test method performance supported the use of the LLNA: BrdU-ELISA to identify substances as potential skin sensitizers and nonsensitizers, with certain limitations
- Noted that the analysis supported using two SI decision criteria (i.e., one to identify skin sensitizers and one to identify nonsensitizers); however, the Panel questioned how indeterminate results between two criteria would be useful for regulatory purposes and emphasized that additional guidance would be needed on how to classify substances with such results
- Concurred with ICCVAM that validation studies indicated that the standardized protocol was sufficiently transferable and reproducible
- Concurred with ICCVAM's recommendations for future studies
- The complete LLNA Peer Review Panel Reports can be accessed at:
  - http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf
  - http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2009.pdf

International Acceptance of LLNA: BrdU-ELISA

- After the Panel review, ICCVAM agreed with the OECD Expert Consultation group (see **Figure 3**) that a single SI ≥ 1.6 to classify substances as skin sensitizers would avoid false negative and indeterminate results, which are not useful for regulatory purposes.
- OECD Test Guideline 442B Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA, which includes the SI ≥ 1.6 to classify substances as skin sensitizers, was adopted on July 22, 2010 (OECD 2010).
- OECD Test Guideline 442B can be accessed at http://www.oecd-ilibrary.org/environment/test-no-442b-skin-sensitization_9789264090996-en
- International acceptance of the LLNA: BrdU-ELISA is expected to result in broader use of LLNA tests, which will further reduce and refine animal use for ACD hazard assessments on a global basis, while ensuring human safety.

Figure 3. Timeline for Evaluation of the LLNA: BrdU-ELISA

January 10, 2007	ICCVAM receives nomination from CPSC for several LLNA review activities[1], including evaluation of the LLNA: BrdU-ELISA.
March 4-6, 2008	Independent Peer Review Panel Meeting on LLNA review activities; public meeting with opportunity for oral public comments.[2]
April 28–29, 2009	Independent Peer Review Panel Meeting on LLNA review activities; public meeting with opportunity for oral public comments.[3] • Agreed with the draft ICCVAM recommendations that the LLNA: BrdU-ELISA may be useful for identifying substances as potential skin sensitizers and nonsensitizers. • Indicated that more information and data were needed before definitive conclusions on the usefulness and limitations of the LLNA: BrdU-ELISA could be made. – Detailed test method protocol – Individual animal data for the validation database – Evaluation of interlaboratory reproducibility
October 20-22, 2009	OECD Expert Consultation Meeting on proposed updates to TG 429 and two new TG proposals for nonradioactive LLNA test methods (includes the LLNA: BrdU-ELISA).
March 23-25, 2010	Working Group of National Co-ordinators of the Test Guidelines Programme approves the proposed updates to TG 429 and two new TG proposals for nonradioactive LLNA test methods, which includes the LLNA: BrdU-ELISA.
June 29, 2010	*Federal Register* (75 FR 37443) notice: Announces availability of ICCVAM TMER for the LLNA: BrdU-ELISA.
July 22, 2010	OECD Council adopts TG 442B Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA, and the updated TG 429.

Abbreviations: CPSC = U.S. Consumer Product Safety Commission; ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods; LLNA = murine local lymph node assay; LLNA: BrdU-ELISA = murine local lymph node assay based on bromodeoxyuridine detection by enzyme-linked immunosorbent assay; OECD = Organisation for Economic Co-operation and Development; TG = Test Guideline; TMER = test method evaluation report

[1]The CPSC nomination may be viewed on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/immunotox/llnadocs/CPSC_LLNA_nom.pdf

[2]The report of the 2008 Peer Review Panel meeting is available at: http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf

[3]The report of the 2009 Peer Review Panel meeting is available at: http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2009.pdf

References

ESAC. 2007. ESAC statement on the reduced local lymph node assay. Ispra, Italy, European Commission Directorate General, Joint Research Centre, Institute for Health and Consumer Protection, European Centre for the Validation of Alternative Methods.

ICCVAM. 2009a. ICCVAM Test Method Evaluation Report. The Reduced Murine Local Lymph Node Assay: An Alternative Test Method Using Fewer Animals to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products. NIH Publication 09-5439. RTP, NC: NIEHS. Available at http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-LDTMER.htm.

ICCVAM. 2009b. Recommended Performance Standards: Murine Local Lymph Node Assay. NIH Publication 09-7357. RTP, NC: NIEHS. Available at http://iccvam.niehs.nih.gov/methods/immunotox/llna_PerfStds.htm.

Kimber I, et al. 2006. The local lymph node assay and skin sensitization: a cut-down screen to reduce animal requirements? Contact Dermatitis 54: 181-185.

Kojima H, et al. 2008. Inter-laboratory validation study on LLNA-BrdU. In: Society of Toxicology Annual Meeting. Seattle, WA.

Takeyoshi M, et al. 2001. Development of non-radio isotopic endpoint of murine local lymph node assay based on 5-bromo-2'-deoxyuridine (BrdU) incorporation. Toxicol Lett 119: 203-208.

Takeyoshi M, et al. 2003. Assessment of stastistic analysis in non-radioisotopic local lymph node assay (non-RI-LLNA) with alpha-hexylcinnamic aldehyde as an example. Toxicology 191: 259-263.

Takeyoshi M, et al. 2004a. Assessment of the skin sensitization potency of eugenol and its dimers using a non-radioisotopic modification of the local lymph node assay. J Appl Toxicol 24: 77-81.

Takeyoshi M, et al. 2004b. Differences in responsiveness of mouse strain against p-benzoquinone as assessed by non-radioisotopic murine local lymph node assay. Exp Anim 53: 171-173.

Takeyoshi M, et al. 2005. Novel approach for classifying chemicals according to skin sensitizing potency by non-radioisotopic modification of the local lymph node assay. J Appl Toxicol 25: 129-134.

Takeyoshi M, et al. 2006. Advantage of using CBA/N strain mice in a non-radioisotopic modification of the local lymph node assay. J Appl Toxicol 26: 5-9.

Takeyoshi M. 2007. Promising Endpoint And Assay Performance of Non-radioisotopic Local Lymph Node Assay Based on BrdU Incorporation. In: 6th World Congress on Alternatives and Animal Use in the Life Sciences. Tokyo, Japan. 21-25 August 2007.

Acknowledgements

The Intramural Research Program of the NIH, National Institute of Environmental Health Sciences supported this poster. Technical support was provided by ILS, Inc. under NIEHS contract N01-ES 35504.

The views expressed above do not necessarily represent the official positions of any Federal agency. This poster reflects the views of the authors. Since the poster was written as part of the official duties of the authors, it can be freely copied.

ICCVAM and NICEATM gratefully acknowledge the following individuals and institutions for submitting data to NICEATM for the LLNA: BrdU-ELISA evaluation.

Masahiro Takeyoshi, Ph.D.
Chemicals Evaluation and Research Institute
Saitama, Japan

Hajime Kojima, Ph.D.
Japanese Center for the Validation of Alternative Methods
National Institute of Health Sciences
Ministry of Health, Labour and Welfare
Tokyo, Japan

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

NIEHS National Institute of Environmental Health Sciences

NTP National Toxicology Program
U.S. Department of Health and Human Services