ICCVAM Test Method Recommendations for the Reduced LLNA (rLLNA)

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Introduction

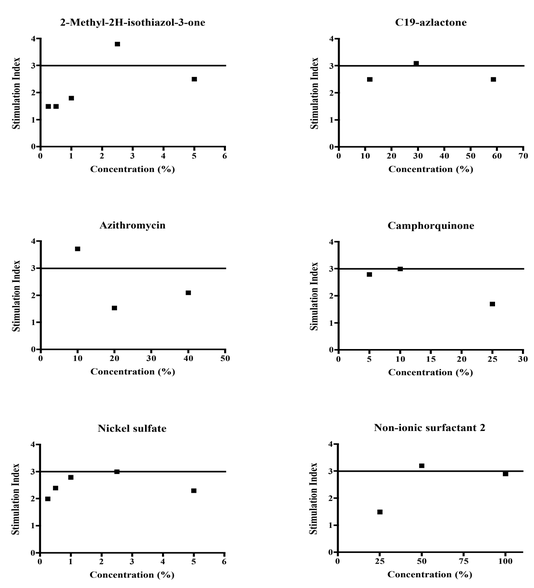
- The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is charged with evaluating the scientific validity of new, revised, and alternative toxicological test methods applicable to U.S. Federal agency safety testing requirements.
  - ICCVAM forwards recommendations to Federal agencies.
  - By law, the agencies must respond to ICCVAM within 180 days.

- ICCVAM recently evaluated the validation status of the reduced murine local lymph node assay (rLLNA), as an alternative to the traditional, multiple dose, murine local lymph node assay (LLNA) for identifying the potential of substances to cause allergic contact dermatitis (ACD).
- The rLLNA can reduce by 40% the number of animals used for each test compared to the LLNA.
- The ICCVAM Test Method Evaluation Report includes recommendations regarding:
  - Usefulness and limitations of the rLLNA
  - An rLLNA test method protocol
  - Future studies
- Final transmittal of the recommendations to agencies is currently in process.
- The rLLNA is included in an updated version of Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 429, which was circulated to member countries in July 2009.

Table 1. rLLNA Accuracy in Predicting Skin Sensitizers Compared to the LLNA

Data	N	Accuracy	Sensitivity	Specificity	False Positive Rate	False Negative Rate
Kimber et al. 2006	211	98.6% (208/211)	98.2% (166/169)	100% (42/42)	0% (0/42)	1.8% (3/169)
rLLNA ICCVAM 2009b	465	98.7% (459/465)	98.1% (309/315)	100% (150/150)	0% (0/150)	1.9% (6/315)

Figure 1. Dose-Response Curves for LLNA Positive Substances that Would Be Negative in the rLLNA



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rLLNA Accuracy and Reliability

Accuracy

- rLLNA results compared to the LLNA included 465 LLNA studies (315 positive, 150 negative)
- Six substances were positive in the 3-dose LLNA based on an SI ≥ 3 at a dose other than the highest dose (**Figure 1**).
  - Since the rLLNA only evaluates the highest dose tested, all six substances were incorrectly classified as nonsensitizers when compared to the LLNA.

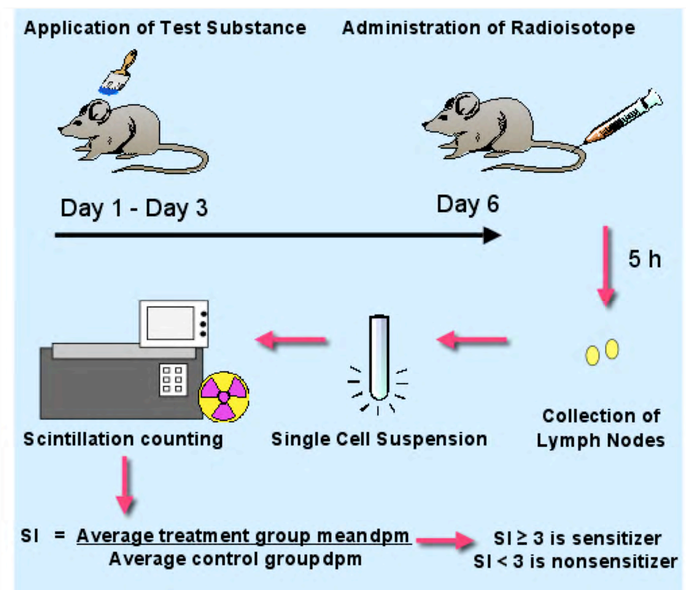
Reliability

- Because the rLLNA and LLNA use identical protocols and the data sets used to evaluate their accuracy are similar, the intra- and interlaboratory reliability of the rLLNA was deemed to be similar to that of the LLNA.

ICCVAM Recommendations: Test Method Usefulness and Limitations

- The rLLNA can be used to distinguish between skin sensitizers and nonsensitizers, if dose-response information is not needed.
- Compared to the traditional LLNA, the rLLNA will reduce animal use by 40%.
- The rLLNA should be used as the initial test for ACD, unless a substance is expected to produce ACD, and dose-response information is needed. In that case, test the substance initially in the LLNA, not the rLLNA.
- There is a small possibility of a false negative result (1.9% [6/318]) in the rLLNA compared to the LLNA.
  - Negative results should always prompt an integrated assessment of other available information (e.g., possibility of downturn in response at the high dose, ACD results with similar substances, peptide-binding activity, molecular weight, other testing data, human experience).
  - If false negative results are suggested, consider confirmatory testing in the 3-dose LLNA or another accepted skin-sensitization test method.

ICCVAM Recommendations: Test Method Protocol



- The only difference between the test method protocols for the LLNA (see **poster #580**) and the rLLNA is the number of dose levels tested for a test substance.
  - In the LLNA, at least three dose levels are tested for each substance.
  - Only the highest dose of a substance is tested in the rLLNA.
  - The highest dose should be based on maximum solubility and the avoidance of excessive local irritation and/or systemic toxicity.

ICCVAM Recommendations: Future Studies

- Additional research to understand the abnormal dose responses for the six false negative substances
- Individual animal data should always be collected
  - To allow for outlier analysis
  - To avoid false negative results
- Identification of opportunities to use fewer animals without compromising test method accuracy (e.g., concurrent positive control group)
- Further investigation of *in vitro* cell-based methods, peptide reactivity, and integrated approaches to further reduce animal use

ICCVAM Recommendations: Performance Standards

- The internationally harmonized performance standards for the LLNA (ICCVAM 2009a - see **poster #579**) may be used to evaluate the performance of modified test methods, including rLLNA versions, that are functionally and mechanistically similar to the LLNA.
- Modified protocols for the rLLNA that adhere to the LLNA performance standards would also be considered acceptable for hazard identification.

Independent Scientific Peer Review Panel

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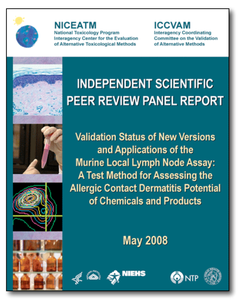

2008 LLNA Peer Review Panel Meeting

- A public meeting of an international independent scientific peer review panel organized by the ICCVAM and NICEATM was held at the Consumer Product Safety Commission in Bethesda, MD, on March 4-6, 2008.
- The Panel agreed that the available data supported the ICCVAM draft recommendations. Panel suggestions have been incorporated into the ICCVAM recommendations presented here.
- The Peer Review Panel Report is available at: http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf

International Acceptance

- A proposal to update OECD TG 429 (the LLNA) has been submitted to include recommendations for the rLLNA as described herein.
  - The draft revised TG 429 is currently under review by the OECD Test Guidelines Program.

Conclusions

- The accuracy and reliability of the rLLNA is considered adequate for ACD hazard classification purposes.
- The rLLNA should always be considered as the initial test for ACD, and used where determined appropriate.
- Compared to the LLNA, the rLLNA will reduce animal use by 40%.

References

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