

## **Impact of Reducing Sample Size in the LLNA**

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Allergic contact dermatitis (ACD) is an adverse health effect that results in lost workdays and can significantly diminish quality of life. To minimize the occurrence of ACD, regulatory authorities require testing to identify substances that may cause skin sensitization. The local lymph node assay (LLNA) is an alternative test method that virtually eliminates pain and distress associated with testing of substances for skin sensitization potential. OECD Test Guideline 429, which describes the LLNA, includes a requirement of at least four mice per group if the lymph nodes from all mice in the treatment group are pooled and a minimum of five mice per group if the lymph nodes from each mouse are processed separately. To determine if data collected from four individual animals would suffice, NICEATM used data from 83 LLNA tests (275 treated groups) to empirically determine the impact on the LLNA outcome of reducing the number of mice in each group from five to four. The average likelihood of agreement (both stimulation index [SI] < 3 or both SI ≥ 3) between LLNA outcomes with either four or five mice per group was 97.5% for the 275 treated groups. When comparing results on a test-by-test basis, there was complete agreement between outcomes with four or five mice per group for 90% (75/83) of the tests. For the remaining eight tests, there were some differences in classification between five and four mice samples with the overall agreement averaging 83%. Much of the disagreement was due to the closeness of the SI to three, not to the reduction in sample size. The practical impact of reducing the sample size from five to four mice per group on the interpretation of experimental results appears to be minimal and, therefore, using four rather than five mice per group would not impact the overall performance of the LLNA for identifying potential skin sensitizers. NICEATM and ICCVAM have recently submitted a proposal to OECD to recommend that TG 429 be updated to include a requirement of a minimum of four animals per group. ILS supported by NIEHS contract N01-ES-35504.

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Categories: Regulatory/Policy (Hypersensitivity - 2nd choice)