

Table of Contents

List of Tables	D-8
List of Figures.....	D-9
List of Abbreviations and Acronyms	D-10
Interagency Coordinating Committee on the Validation Of Alternative Methods: Agency Representatives.....	D-13
Acknowledgements	D-15
Preface.....	D-21
Executive Summary.....	D-23
1.0 Introduction and Rationale for the Proposed Use of the Reduced Murine Local Lymph Node Assay (rLLNA) to Identify Skin Sensitizers	D-27
1.1 Introduction.....	D-27
1.1.1 Historical Background	D-27
1.1.2 Allergic Contact Dermatitis	D-27
1.1.3 U.S. Consumer Product Safety Commission (CPSC) Nomination	D-28
1.1.4 Description of the rLLNA	D-28
1.1.5 Results of an ECVAM Peer Review of the rLLNA	D-29
1.2 Regulatory Rationale and Applicability of the rLLNA	D-29
1.3 Scientific Basis for the rLLNA	D-29
1.3.1 Purpose and Mechanistic Basis	D-29
1.3.2 Applicability Domain.....	D-30
1.4 Test Method Validation	D-30
1.5 Selection of Citations for the rLLNA BRD	D-30
2.0 rLLNA Protocol Components	D-31
2.1 Overview.....	D-31
2.2 Basis for Test Method Selection.....	D-31
2.3 Proprietary Test Method Components	D-31
2.4 Basis for the Number of Mice per Dose Group	D-31
2.5 Study Acceptance Criteria	D-31
2.6 Basis for Selection of the Test Substance Dose.....	D-31

3.0 Substances Used for Validation of the rLLNA	D-33
3.1 Rationale for the Substances or Products Included in the Evaluation	D-33
3.2 Rationale for the Number of Substances Included in the Evaluation	D-33
3.3 Detailed Description of Substances Included in the Evaluation.....	D-33
3.4 Coding Procedures	D-35
4.0 Comparative <i>In Vivo</i> Reference Data – the Traditional LLNA.....	D-37
4.1 The Traditional LLNA Protocol Used to Generate Comparative <i>In Vivo</i> Reference Data.....	D-37
4.2 Comparative Traditional LLNA Reference Data Used	D-37
4.3 Availability of Original Records for Comparative Traditional LLNA Reference Data.....	D-37
4.4 Quality of Comparative Traditional LLNA Reference Data	D-37
4.5 Accuracy and Reliability of the Traditional LLNA	D-38
4.5.1 Accuracy	D-38
4.5.2 Reliability.....	D-38
5.0 rLLNA Test Method Data and Results	D-39
5.1 Description of the rLLNA Test Method Protocol Used to Generate Data ...	D-39
5.2 Availability of Original rLLNA Data Used to Evaluate Accuracy and Reliability.....	D-39
5.3 Description of the Statistical Procedure Used to Evaluate rLLNA Data	D-39
5.4 Summary of Results.....	D-39
5.5 Use of Coded Substances.....	D-40
5.6 Lot-to-Lot Consistency of Test Substances	D-40
5.7 Availability of Original Data for External Audit.....	D-40
6.0 Accuracy of the rLLNA	D-41
6.1 Performance Statistics	D-41
6.2 Discordant Results	D-43
7.0 Reliability of the rLLNA.....	D-47
8.0 rLLNA Data Quality	D-49
8.1 Adherence to National and International GLP Guidelines	D-49
8.2 Data Quality Audits	D-49
8.3 Impact of Deviations from GLP Guidelines	D-49
8.4 Availability of Laboratory Notebooks or Other Records	D-49

9.0	Other rLLNA Scientific Reports and Reviews.....	D-51
9.1	Reports in the Peer-Reviewed Literature.....	D-51
9.1.1	Ryan et al. (2008)	D-51
10.0	Animal Welfare Considerations.....	D-53
10.1	How the rLLNA will Refine, Reduce, or Replace Animal Use.....	D-53
10.2	Requirements for the Use of Animals.....	D-53
11.0	Practical Considerations.....	D-55
11.1	Transferability of the rLLNA.....	D-55
11.2	rLLNA Training Considerations.....	D-55
11.3	Cost Considerations	D-55
11.4	Time Considerations	D-55
12.0	References	D-57
13.0	Glossary	D-61
Annex I	ECVAM Scientific Advisory Committee Statement on the Validity of the rLLNA	D-65
Annex II	Physicochemical Properties of Substances Evaluated in the rLLNA .	D-71
Annex III	Traditional LLNA Data Used for the Performance Analysis of the rLLNA.....	D-109
Annex IV	Substances in the NICEATM LLNA Database for which an Initial Dose of 10% or Greater Elicited a Negative Result but a Subsequent Higher Dose Elicited a Positive Response	D-145