

Table of Contents

	Page Number
List of Tables	vi
List of Abbreviations and Acronyms	vii
Interagency Coordinating Committee on the Validation of Alternative Methods: Agency Representatives	x
Acknowledgements	xi
Preface	xiii
Executive Summary	xvii
1.0 Introduction and Rationale for the Proposed Use of <i>In Vitro</i> Pyrogen Test Methods	1-1
1.1 Introduction	1-1
1.1.1 Historical Background of <i>In Vitro</i> Pyrogen Test Methods and the Rationale for Their Development.....	1-1
1.1.2 Peer Reviews of <i>In Vitro</i> Pyrogen Test Method Validation Studies	1-3
1.2 Regulatory Rationale and Applicability.....	1-3
1.2.1 Current Regulatory Testing Requirements and ICCVAM Prioritization Criteria.....	1-3
1.2.2 Intended Uses of the Proposed <i>In Vitro</i> Pyrogen Test Methods	1-7
1.2.3 Similarities and Differences in the Endpoints Measured by the Proposed Test Methods and the <i>In Vivo</i> Reference Test Method	1-7
1.2.4 Use of the Proposed Test Methods in an Overall Strategy of Hazard or Safety Assessment.....	1-7
1.3 Scientific Basis for the <i>In Vitro</i> Pyrogen Test Methods.....	1-8
1.3.1 Purpose and Mechanistic Basis of the <i>In Vitro</i> Pyrogen Test Methods.....	1-8
1.3.2 Similarities and Differences of Modes of Action Between the <i>In Vitro</i> Pyrogen Test Methods and the Fever Response in Humans and/or Rabbits.....	1-8
1.3.3 Range of Substances Amenable to the <i>In Vitro</i> Pyrogen Test Methods and Limits of These Methods	1-9
1.4 Validation of the <i>In Vitro</i> Pyrogen Test Methods.....	1-9
1.5 Search Strategies and Selection of Citations for the ICCVAM BRD.....	1-10
2.0 <i>In Vitro</i> Pyrogen Test Method Protocol Components	2-1
2.1 Overview of How the <i>In Vitro</i> Pyrogen Test Methods Are Conducted	2-1
2.2 Description and Rationale for the Test Method Components for Proposed Standardized Protocols.....	2-1
2.2.1 Methods Used to Analyze the Data, Including Methods to Analyze for Interference with the Assay	2-7
2.2.2 Decision Criteria and the Basis for the Prediction Model Used to Identify a Pyrogenic Substance	2-7

2.2.3	Information and Data to be Included in the Study Report and Availability of Standard Forms for Data Collection and Submission.....	2-8
2.3	Basis for Selection of the Test Method Systems	2-9
2.4	Proprietary Components.....	2-10
2.5	Number of Replicates	2-11
2.5.1	Number of Donors.....	2-11
2.5.2	Number of Assay Replicates.....	2-11
2.6	Modifications to the Test Method Protocols Based on ECVAM Validation Study Results.....	2-11
2.7	Differences Between Comparable Validated Test Methods with Established Performance Standards.....	2-11
3.0	Substances Used for the Validation of <i>In Vitro</i> Pyrogen Test Methods	3-1
3.1	Rationale for the Substances or Products Selected for Testing.....	3-1
3.2	Number of Substances	3-1
3.3	Identification and Description of Substances Tested.....	3-1
3.4	Sample Coding Procedure.....	3-2
3.5	Rationale for the Selection of the Recommended Reference Substances	3-3
4.0	<i>In Vivo</i> Reference Data for the Assessment of Test Method Accuracy	4-1
4.1	Description of the Protocol Used to Generate <i>In Vivo</i> Data	4-1
4.1.1	The Rabbit Pyrogen Test (RPT).....	4-1
4.1.2	Current <i>In Vivo</i> Pyrogen Test Method Protocols	4-5
4.2	Reference Data Used to Assess <i>In Vitro</i> Test Method Accuracy	4-6
4.3	Availability of Original Records for the <i>In Vivo</i> Reference Data	4-6
4.4	<i>In Vivo</i> Data Quality	4-6
4.5	Availability and Use of Toxicity Information from the Species of Interest.....	4-7
4.6	Information on the Accuracy and Reliability of the <i>In Vivo</i> Test Method.....	4-7
5.0	Test Method Data and Results.....	5-1
5.1	Test Method Protocol.....	5-1
5.2	Availability of Copies of Original Data Used to Evaluate Test Method Performance.....	5-1
5.3	Description of the Statistical Approaches Used to Evaluate the Resulting Data	5-1
5.4	Summary of Results.....	5-2
5.5	Use of Coded Chemicals and Compliance with GLP Guidelines	5-3
5.6	Lot-to-Lot Consistency of Test Substances	5-3
5.7	Availability of Data for External Audit	5-3
6.0	Relevance of the <i>In Vitro</i> Pyrogen Test Methods	6-1
6.1	Accuracy of <i>In Vitro</i> Pyrogen Test Methods.....	6-1
6.1.1	Relevance of the Cryo WB/IL-1 β Test Method.....	6-2
6.1.2	Relevance of the MM6/IL-6 Test Method.....	6-2

6.1.3	Relevance of the PBMC/IL-6 Test Method	6-3
6.1.3.1	Relevance of the PBMC/IL-6 Method When Using Cryo PBMCs.....	6-3
6.1.4	Relevance of the WB/IL-6 Test Method.....	6-3
6.1.5	Relevance of the WB/IL-1 β Test Method	6-3
6.1.5.1	Relevance of the WB/IL-1 β Test Method When Using 96-Well Plates.....	6-3
6.2	Summary of the Performance Statistics for <i>In Vitro</i> Pyrogen Test Methods	6-4
6.2.1	Discordant Results.....	6-4
6.2.2	Strengths and Limitations of <i>In Vitro</i> Pyrogen Test Methods	6-4
7.0	Reliability of the <i>In Vitro</i> Pyrogen Test Methods	7-1
7.1	Selection Rationale for the Substances Used to Evaluate the Reliability of <i>In Vitro</i> Pyrogen Test Methods	7-1
7.2	Analysis of Intralaboratory Repeatability and Reproducibility.....	7-1
7.2.1	Intralaboratory Repeatability	7-2
7.2.2	Intralaboratory Reproducibility.....	7-3
7.2.3	Interlaboratory Reproducibility.....	7-7
7.3	Historical Positive and Negative Control Data.....	7-8
8.0	Test Method Data Quality.....	8-1
8.1	Adherence to National and International GLP Guidelines.....	8-1
8.2	Data Quality Audits	8-1
8.3	Impact of Deviations from GLP Guidelines.....	8-1
8.4	Availability of Laboratory Notebooks or Other Records.....	8-1
8.5	Need for Data Quality	8-1
9.0	Other Scientific Reports and Reviews	9-1
9.1	Summaries of <i>In Vitro</i> Pyrogen Test Methods and Data from Published and Unpublished Studies	9-1
9.1.1	Andrade et al. (2003).....	9-1
9.1.2	Bleeker et al. (1994)	9-4
9.1.3	Carlin and Viitanen (2003)	9-4
9.1.4	Carlin and Viitanen (2005)	9-7
9.1.5	Daneshian et al. (2006).....	9-7
9.1.6	Eperon et al. (1996, 1997).....	9-8
9.1.7	Marth and Kleinhappl (2002).....	9-10
9.1.8	Martis et al. (2005)	9-11
9.1.9	Pool et al. (1998)	9-11
9.1.10	Taktak et al. (1991).....	9-13
9.2	Conclusions from Scientific Literature Based on Independent Peer-Reviewed Reports and/or Reviews	9-13
9.2.1	De Groote et al. (1992)	9-14
9.2.2	Fennrich et al. (1999).....	9-14
9.2.3	Hansen and Christensen (1990).....	9-15
9.2.4	Hartung and Wendel (1996).....	9-15
9.2.5	Moesby et al. (1999).....	9-15

9.2.6	Nakagawa et al. (2002).....	9-16
9.2.7	Pool et al. (1999).....	9-16
9.2.8	Poole et al. (2003).....	9-16
9.2.9	Schindler et al. (2004).....	9-17
10.0	Animal Welfare Considerations (Refinement, Reduction, and Replacement).....	10-1
10.1	How the Five <i>In Vitro</i> Test Methods Will Refine, Reduce, or Replace Animal Use	10-1
10.2	Requirement for the Use of Animals	10-2
10.2.1	Rationale for the Use of Animals.....	10-2
11.0	Practical Considerations	11-1
11.1	Transferability of the <i>In Vitro</i> Pyrogen Test Methods.....	11-1
11.1.1	Facilities and Major Fixed Equipment	11-1
11.1.2	General Availability of Other Necessary Equipment and Supplies.....	11-1
11.2	Personnel Training Considerations.....	11-1
11.2.1	Required Training and Expertise Needed to Conduct the <i>In Vitro</i> Pyrogen Test Methods	11-2
11.3	Cost Considerations	11-2
11.4	Time Considerations.....	11-2
12.0	References.....	12-1
13.0	Glossary	13-1
Appendix A	ECVAM BRDs and Standard Operating Procedures	A-1
A1	The Human Whole Blood (WB)/Interleukin (IL)-1 β <i>In Vitro</i> Pyrogen Test.....	A-3
A2	The Human WB/IL-1 β <i>In Vitro</i> Pyrogen Test: Application of Cryopreserved (Cryo) Human WB	A-133
A3	The Human WB/IL-6 <i>In Vitro</i> Pyrogen Test.....	A-237
A4	The Human Peripheral Blood Mononuclear Cell (PBMC)/IL-6 <i>In Vitro</i> Pyrogen Test.....	A-335
A5	The Monocytoid Cell Line Mono Mac 6 (MM6)/IL-6 <i>In Vitro</i> Pyrogen Test.....	A-459
Appendix B	ECVAM Response to ICCVAM Questions.....	B-1
Appendix C	Additional Information Requested by the Panel	C-1
C1	ESAC Statement on the Validity of <i>In Vitro</i> Pyrogen Tests.....	C-3
C2	Press Release: "Fewer Tests on Animals and Safer Drugs: New EU Tests Save 200,000 Rabbits per Year"	C-9
C3	ECVAM Replies to Questions of ICCVAM Pyrogenicity Peer Review Panel	C-15
C4	Rationale for the Selection of the 10 Substances Tested in the Validation/Catch-Up Validation Study of <i>In Vitro</i> Assays for Pyrogen Testing	C-21
C5	Comparison and Validation of Novel Pyrogen Tests Based on the	

	Human Fever Reaction: Trial Data Report.....	C-25
C6	List of Drugs for the Catch-Up Validation Study.....	C-41
C7	Analytical Procedure to Identify and Eliminate Outlying Observations	C-45