

# Technical Report No. 50 Alternative Methods for Mycoplasma Testing



2010

## **Alternative Mycoplasma Test Methods Task Force\***

---

### **Authors**

**David Asarnow**, PhD, Bayer HealthCare (Co-Chair)

**Kurt A. Brorson**, PhD, U.S. Food and Drug Administration (Co-Chair)

**Allen L. Burgenson**, Lonza Walkersville, Inc.

**Vladimir Chizhikov**, PhD, U.S. Food and Drug Administration

**Timothy A. Coleman**, Lonza Walkersville, Inc.

**Sven Deutschmann**, PhD, Roche Diagnostics GmbH

**Thomas E. Haemmerle**, PhD, Baxter (Co-Chair)

**Mihaela Z. Marian**, Amgen

**Brandye Michaels**, PhD, Pfizer Specialty Care/Biotechnology

**Barbara J. Potts**, PhD, Biologics Consulting Group\*

**Cynthia E. Romero-Arroyo**, OBI (Johnson & Johnson)

**Pranhitha Reddy**, Amgen

**Kathleen S. Souza**, Millipore Corporation

**Garry Takle**, PhD, WuXiAppTec

**Michele Tiraby**, Invivogen

**Helena M. Windsor**, Mycoplasma Experience

### **Contributors**

**Donna Chandler**, PhD, Chandler Vaccine Resources, LLC

**Maureen K. Davidson**, U.S. Food and Drug Administration

**Wang-Ting Hsieh**, SAIC-Frederick, Inc.

**Scott C. Lute**, U.S. Food and Drug Administration

**Jill Mariano**, PhD, Bionique Testing Laboratories, Inc.

**Jerold M. Martin**, Pall Corporation

**Catherine Milne**, PhD, EDQM Pharmacopeia

**Renate Rosengarten**, PhD, Mycosafe Diagnostics GmbH

**Radhakrishna S. Tirumalai**, PhD, United States Pharmacopeia

**Dmitriy Volokhov**, PhD, U.S. Food and Drug Administration

**Ann Warford**, DrPH, MedImmune, Inc.

\* The Alternative Mycoplasma Test Methods Task Force is a subgroup of the larger PDA Mycoplasma Task Force, which is chaired by Barbara Potts.

# Alternative Methods for Mycoplasma Testing

**Technical Report No. 50**

ISBN: 978-0-939459-31-5

© 2010 Parenteral Drug Association, Inc.

All rights reserved.



## Table of Contents

|  |           |   |           |
|--|-----------|---|-----------|
| <b>1.0 Introduction.....</b>   | <b>3</b>  | 4.7.1 Lab Set-up and Procedures.....  | 21        |
| 1.1 Purpose and Scope.....   | 3         | 4.7.2 Controls .....  | 21        |
| <b>2.0 Glossary of Terms .....</b>   | <b>4</b>  | 4.7.3 No Template Controls .....  | 21        |
| <b>3.0 Summary of Existing Assays and Compendial Methods.....</b>  | <b>7</b>  | 4.7.4 Negative Sample Controls .....  | 22        |
| 3.1 Limitations of Current Methods .....   | 8         | 4.7.5 Positive Controls.....  | 22        |
| 3.1.1 The Testing Time Period .....  | 8         | 4.7.7 Matrix Spike Controls .....   | 22        |
| 3.1.2 The Controlled Conditions .....  | 8         | 4.7.8 Interference Control.....   | 22        |
| 3.1.3 Mycoplasma Growth.....   | 8         | 4.7.9 Internal Amplification Controls.....  | 23        |
| 3.2 Background of Alternative Methods .....  | 8         | 4.7.10 Quantitative Standard.....   | 23        |
| 3.3 Applicability and Points of Use of Alternative Methods in Cell Culture Manufacturing and Development ..... | 9         | 4.7.11 Assay Noise and Non-Specific Amplification .....   | 23        |
| 3.3.1 In-process Testing of Cell Culture Harvest Prior to Release to Downstream Processing .....               | 9         | 4.8 Data Interpretation and Follow-up.....  | 23        |
| 3.3.2 Testing of Cell Lines and Research Cell Banks.....   | 9         | 4.9 Reference Materials.....  | 24        |
| 3.3.3 Use in Bioassay Development.....   | 10        | 4.10 Method Validation.....   | 26        |
| 3.3.4 Contamination Response and Corrective Actions.....   | 10        | 4.10.1 Existing Guidance.....   | 26        |
| 3.3.5 Raw Material Testing .....   | 10        | 4.10.2 Limit Tests .....  | 26        |
| 3.4 Targeted Species.....  | 10        | 4.10.3 Specificity .....  | 27        |
| <b>4.0 Nucleic Acid Amplification Technique (NAT) Methods.....</b>   | <b>13</b> | 4.10.4 Limit of Detection (LOD) .....   | 27        |
| 4.1 Polymerase Chain Reaction (PCR) Overview ..  | 13        | 4.10.5 Precision.....   | 27        |
| 4.1.1 Endpoint PCR .....   | 13        | 4.10.6 Robustness.....  | 28        |
| 4.1.2 Real Time PCR.....   | 13        | 4.10.7 Reproducibility.....   | 28        |
| 4.2 Primer and Probe Selection .....   | 14        | 4.10.8 System Suitability (Assay Qualification) .....   | 28        |
| 4.3 Available NAT Methods – Direct PCR.....  | 14        | 4.11 Comparability Studies to Previous Assays .....   | 28        |
| 4.3.1 DNA Endpoint PCR Assays .....  | 15        | 4.11.1 Preparation for and Conduct of a Comparability Study .....   | 29        |
| 4.3.2 Touchdown PCR Assays .....   | 15        | 4.11.2 Cross-industry Comparability .....   | 29        |
| 4.3.3 RNA Endpoint PCR Assays.....   | 16        | 4.11.3 Genome Copy: CFU Ratio in Genomic DNA Detection Based Methods.....   | 30        |
| 4.4 Available NAT Methods – Enhanced PCR and Other Methods .....   | 16        | <b>5.0 Non-NAT Alternative Methods.....</b>   | <b>32</b> |
| 4.4.1 Cases Where Low LOD Methods are Required.....  | 16        | 5.1 Immunologic Tests .....   | 32        |
| 4.4.2 Culture/NAT Methods of Biological Enrichment.....  | 17        | 5.2 Enzyme-Based Methods.....   | 32        |
| 4.4.3 Alternative RNA Based Methods (NASBA and TMA).....   | 18        | 5.2.1 ATP Generation by Mycoplasma .....  | 32        |
| 4.4.4 Sample Concentration Prior to NAT .....  | 18        | 5.2.2 Assay Mechanism .....   | 33        |
| 4.5 Sample Volume.....   | 19        | 5.2.3 Bioluminescent Detection System .....   | 33        |
| 4.6 Nucleic Acid Extraction and Purification.....  | 20        | 5.3 Mycoplasma Testing Using a Recombinant Cell Line .....  | 34        |
| 4.6.1 Lysis of Cells .....   | 20        | 5.4 Non-Amplification Nucleic Acid Hybridization Methods.....   | 34        |
| 4.6.2 Nucleic Acid Purification .....  | 20        | <b>6.0 Appendices.....</b>  | <b>35</b> |
| 4.6.3 Inactivation of Nucleases .....  | 21        | 6.1 Appendix 1: Comparison of Mycoplasma Methods in Regulations, Compendia and Guidance Documents Currently in Effect ..... | 35        |
| 4.7 Assay Set-up.....  | 21        | 6.2 Appendix 2: <i>In-House</i> Reference Strain Preparation Methods .....  | 41        |
|  |           | 6.2.1 Determination of the Titer of Delivered Mycoplasma Reference Strains.....   | 41        |

|  |    |                             |           |
|--|----|-----------------------------|-----------|
| 6.2.2 Test for Reference Preparation Purity ...                | 41 | <b>7.0 References .....</b> | <b>43</b> |
| 6.2.3 Preparation of Cryopreserved<br>Quantified Cultures..... | 41 |                             |           |

## FIGURES AND TABLES INDEX

|                     |   |           |                       |  |           |
|---------------------|---|-----------|-----------------------|--|-----------|
| <b>Table 3.0</b>    | <b>Taxonomy and Salient Characteristics of the Most Biopharmaceutically Relevant Genera of the Class Mollicutes ...</b> | <b>7</b>  | <b>Table 5.2.1</b>    | <b>Representative Mycoplasma Species and Their Energy-Generating Pathways.....</b>   | <b>32</b> |
| <b>Table 3.4</b>    | <b>Mycoplasma Species Associated with Different Source Material Types.....</b>  | <b>11</b> | <b>Figure 5.2.1-1</b> | <b>Endpoint of Mycoplasma-Specific Glucose Fermentation.....</b>   | <b>33</b> |
| <b>Figure 4.1.1</b> | <b>One Potential PCR Mycoplasma Detection Scheme .....</b>  | <b>13</b> | <b>Figure 5.2.1-2</b> | <b>Endpoint of Mycoplasma-Specific Arginine Lysis.....</b>   | <b>33</b> |
| <b>Table 4.3.2</b>  | <b>Comparison of PCR Methods.....</b>   | <b>16</b> | <b>Figure 5.2.3-1</b> | <b>Luciferase Bioluminescence Pathway for ATP Quantification ..</b>  | <b>33</b> |
| <b>Table 4.7.5</b>  | <b>Types of Standards for Use in Validation of a NAT Method for Mycoplasma Detection.....</b>                           | <b>25</b> | <b>Figure 5.2.3-2</b> | <b>Representative Ratio of Bioluminescence Signal to Background (B/A) of an Uninfected control and a Sample Containing <i>M. hyorhinis</i> .....</b> | <b>34</b> |

## 1.0 Introduction

### 1.1 Purpose and Scope

The scope of this Technical Report is the application of non-culture testing methodology, including Nucleic Acid Amplification Technique (NAT) assays and others (e.g., enzyme activity based), for detection of mycoplasmas, members of the class Mollicutes, in cell cultures and biologics. The purpose of the Technical Report is to describe assay procedures, assay validation, demonstration of comparability to reference methods, and potential applications for alternative mycoplasma testing methods.