MycoSEQ™ Mycoplasma Detection Assay

- Rapid time-to-results in less than 5 hours
- Detection of more than 90 Mycoplasma species
- Demonstrated sensitivity to detect less than 10 copies/reaction
- Proven specificity
- PrepSEQ™ sample preparation for high-efficiency DNA recovery
- Proprietary discriminatory positive/extraction control
- Externally validated
- Part of the Cell Culture Rapid Methods Program

Introduction

Mycoplasmas, the smallest known free-living organisms, are relatively common bacterial contaminants of mammalian cell cultures. Potential sources of infection include contaminated raw materials used for cell culture, laboratory staff, and exposure to contaminated cell cultures. Mycoplasmas present particular challenges because they are difficult to detect using traditional microbiological techniques. Figure 1 shows the different points in biopharmaceutical manufacturing where testing for Mycoplasma is typically performed.

Regulatory guidance requires that all products derived from mammalian cell culture be tested for the presence of Mycoplasma. In July 2007, the European Pharmacopoeia (5.8, Sec. 2.6.7) provided guidance on the validation requirements for nucleic acid amplification–based methods for detection of Mycoplasma.

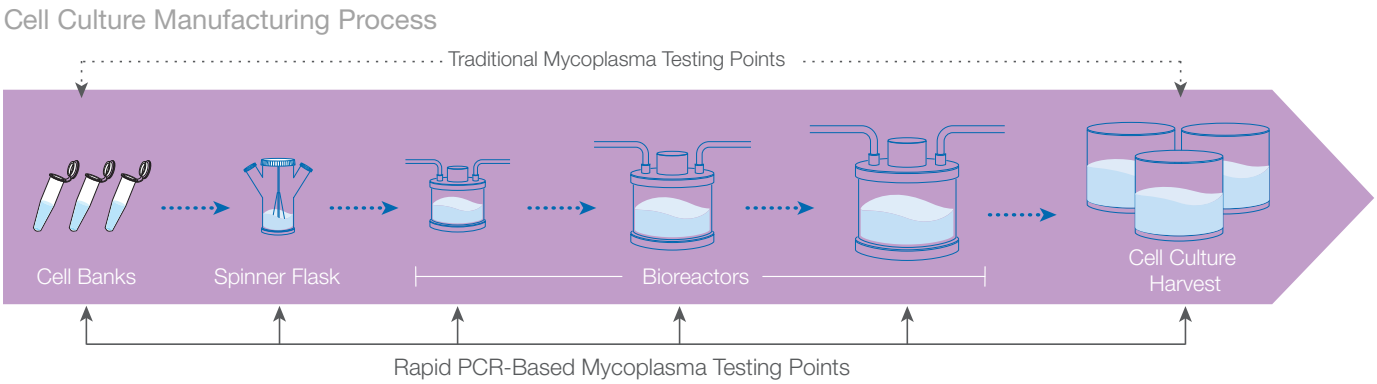

Figure 1. Sampling Points for Mycoplasma. Rapid PCR-based testing for Mycoplasma infection can be conducted throughout the cell culture manufacturing process, from inoculation through harvest.

Figure 2. Easy Workflow. Results are delivered in less than 5 hours, allowing for in-process testing.

MycoSEQ™ Mycoplasma Detection Assay

Applied Biosystems developed the MycoSEQ™ Mycoplasma Detection Assay, based on real-time PCR and *Power* SYBR® Green detection technology. Through intensive bioinformatics and highly optimized multiplexed primer design, the system allows for highly sensitive, specific, and comprehensive Mycoplasma species detection. The rapid time-to-results of less than 5 hours supports in-process monitoring for the presence of Mycoplasma during cell culture manufacturing, allowing for the earliest possible detection of a contamination event, providing protection against the spread of contamination into downstream equipment, processes, and media.

Components of the MycoSEQ™ Mycoplasma Detection Assay include:

- *Power* SYBR® Green Master Mix
- Assay mix
- Inhibition control
- Positive control
- Optimized PrepSEQ™ sample preparation
- Complete protocol for test setup and data analysis

Rapid Time-to-Results in Less Than 5 Hours

The MycoSEQ™ Mycoplasma Detection Assay has an easy workflow that can deliver results in less than 5 hours (Figure 2). This rapid time-to-results allows the earliest possible detection of Mycoplasma contamination.

Key features include:

- Variable test sample volumes, from 100 µL to 10 mL of cell culture containing up to 10^8 cells
- Closed-tube, single-step detection
- Load-and-run, walk-away automation during detection
- No gel electrophoresis, hybridization, or washing steps
- Minimal requirements on infrastructure and space
- Flexible throughput
- Optimized workflow to provide high sensitivity and specificity during routine testing

Multiparameter Analysis Using *Power* SYBR® Green Technology

The MycoSEQ™ assay uses highly optimized *Power* SYBR® Green detection technology, which utilizes multiple parameters, amplification plot (C_t), melting temperature (T_m), and derivative value (D.V.) for results interpretation. Multiparameter analysis provides highly sensitive and specific detection of fewer than 10 Mycoplasma genome copies per reaction (Figure 3). Numerical readouts for all parameters provide objective test result interpretation.

Detection of More Than 90 Mycoplasma Species

To help ensure the highest level of confidence in testing for the presence of Mycoplasma, an assay must have the ability to detect all known Mycoplasma species, not just the most common species. Intensive bioinformatics analysis was utilized to design amplification primers and reaction conditions to allow comprehensive detection of known Mycoplasma, Acholeplasma, and Spiroplasma species (Table 1), while avoiding detection of related bacterial species.

PrepSEQ™ Mycoplasma Sample Preparation Kit With Module M

Provided with the MycoSEQ™ assay, the PrepSEQ™ Mycoplasma Sample Preparation Kit with Module M is optimized for highly efficient DNA recovery for Mycoplasma detection. The PrepSEQ™ Kit with Module M uses proprietary magnetic bead–based separation technology to extract Mycoplasma DNA from mammalian cell culture samples with high efficiency.

The kit offers the flexibility to process from 100 µL to 10 mL of cell culture with a density as high as 10^8 cells. The PrepSEQ™ 1-2-3 is a small-scale protocol that can be used for rapid extraction of Mycoplasma genomic DNA. For larger volumes of up to 10 mL, a differential lysis protocol that captures DNA from both cell-associated and free Mycoplasma can be used for highly efficient extraction of the Mycoplasma DNA in the test sample.

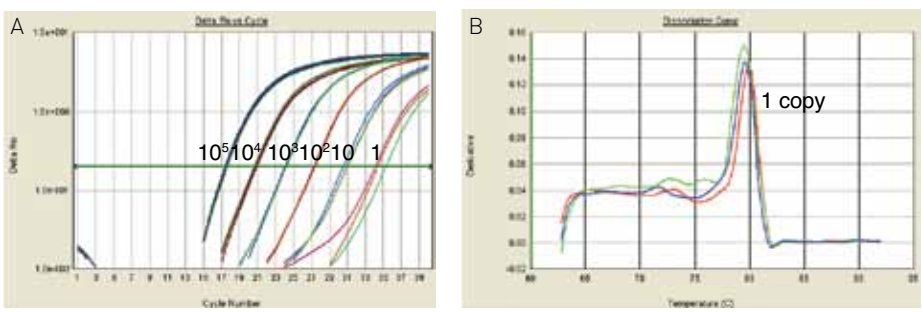

Figure 3. Sensitive Detection of Mycoplasma. (A) Analysis of a 10-fold dilution series of purified *M. arginini* DNA, 100,000 genome copies to 1 genome copy/reaction. (B) Melt curve analysis of the PCR reaction at 1 genome copy/reaction.

Table 1. Partial list of species detected by the MycoSEQ™ Mycoplasma Detection Assay. The kit detects over 90 *Mycoplasma* species, related *Acholeplasma laidlawii* and *Spiroplasma citri*, and other European Pharmacopeia species. Common isolated species recommended for testing and validation are in bold.

Inclusion Panel (Partial)		
Acholeplasma granularum	*Mycoplasma genitalium*	***Mycoplasma synoviae***
Acholeplasma laidlawii	*Mycoplasma gypis*	*Mycoplasma testudinis*
Acholeplasma pleciae	***Mycoplasma hominis***	*Mycoplasma timone*
Mycoplasma alkalescens	***Mycoplasma hyorhinis***	***Spiroplasma citri***
Mycoplasma alvi	*Mycoplasma imitans*	*Spiroplasma endosymbiont*
Mycoplasma anseris	*Mycoplasma indiense*	*Spiroplasma insolitum*
Mycoplasma arginini	*Mycoplasma lagogenitalium*	*Spiroplasma kunkelii*
Mycoplasma auris	*Mycoplasma lipofaciens*	*Spiroplasma melliferum*
Mycoplasma buccale	*Mycoplasmamobile*	*Spiroplasma mirum*
Mycoplasma californicum	*Mycoplasma molare*	*Spiroplasma phoeniceum*
Mycoplasma canadense	***Mycoplasma mycoides***	*Spiroplasma poulsonii*
Mycoplasma capricolum	*Mycoplasma neurolyticum*	*Spiroplasma sp.*
Mycoplasma caviae	***Mycoplasma orale***	*Mycoplasma bovirhinis*
Mycoplasma collis	*Mycoplasma phocidai*	*Mycoplasma bovis*
Mycoplasma cricetuli	***Mycoplasma pirum***	*Mycoplasma bovigenitalium*
Mycoplasma equirhinis	***Mycoplasma pneumoniae***	*Mycoplasma canis*
Mycoplasma fermentans	***Mycoplasma salivarium***	*Mycoplasma felis*
Mycoplasma gallinaceum	*Mycoplasma simbae*	*Mycoplasma fastidiosum*
Mycoplasma gallisepticum	*Mycoplasma sp.*	*Mycoplasma muris*
Mycoplasma gateae	*Mycoplasma spumans*	*Mycoplasma pulmonis*

The custom sample prep protocol design can accommodate a wide variety of sample types. We have tested the following samples:

- High titer CHO cultures from bioreactors
- High titer NS0 cultures from bioreactors
- Cell culture vaccine manufacturing harvest
- Transgenic milk
- Research cell cultures
- Bioassay cell lines
- Stem cell cultures
- Lymphocyte proliferation cultures for autologous transplantation
- Cell and tissue therapy cultures
- Serum
- Cell culture media

Discriminatory Positive Control

The MycoSEQ™ Mycoplasma Assay also includes the Discriminatory Positive/Extraction Control, a large plasmid containing a Mycoplasma DNA sequence. This control was designed to behave like Mycoplasma DNA in both the sample preparation and detection portions of the assay. Additionally, the DNA sequence has been modified so that the amplicon generated from this control has a melting temperature (T_m) of approximately 84°C, which is outside the range of amplicons generated from Mycoplasma with this assay (Figure 4). Thus, the T_m can be used to discriminate between a positive test result from Mycoplasma and the control DNA. This novel control enables risk-free DNA spike control testing protocol design, eliminating the possibility of a false positive test result due to accidental cross-contamination of a test sample with the positive control DNA.

AccuSEQ™ Real-Time PCR Software for Automated Mycoplasma Data Analysis

Automated presence/absence results from MycoSEQ™ Mycoplasma detection can be generated using AccuSEQ™ software. Advanced algorithms for this automated calling were developed using the data interpretation guidelines for the MycoSEQ™ Mycoplasma Detection Assay. Calls are made based on the T_m and derivative value of the test sample and the C_t value of the test sample and inhibition control. For in-depth review of the data, the AccuSEQ™ Software offers easy-to-use manual review tools, including a complete table of all T_m and C_t values, as well as amplification, multicomponent, and raw data plots.

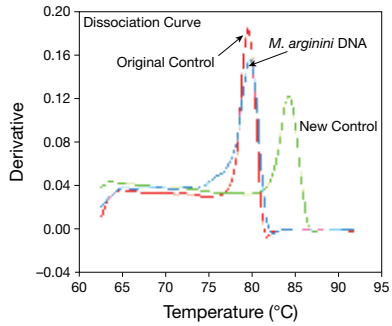

Figure 4. Melt Curve Analysis of Control vs. Mycoplasma DNA. The graph demonstrates the noticeable difference in melting point temperatures to enable clear differentiation between valid Mycoplasma DNA and control sample.

External Validation of PCR-Based Method

Experiments were executed by Mycosafe Diagnostics GmbH in Vienna, Austria, to evaluate and demonstrate assay performance and to help enable customers to design their internal validation studies. Study design followed guidance provided in E.P. 2.6.7, ICH Q2 (R1), and feedback gathered at the 2008 FDA-CBER Workshop on Rapid Mycoplasma Testing.

The study verified the level of detection (LOD) with both genome copies and live Mycoplasma stocks, using a test sample matrix of 10 mL of CHO cells. The study estimated the lowest LOD and analyzed the GC/CFU ratio for all 10 Mycoplasma species tested, and clearly demonstrates for the first time the sensitivity of a PCR-based test for Mycoplasma recovered from 10 mL samples of CHO cells.

Cell Culture Rapid Methods Program

The MycoSEQ™ Mycoplasma Detection Assay is part of the Cell Culture Rapid Methods Program, designed to streamline the detection of three common contaminants of mammalian cell culture–based biopharmaceutical manufacturing. The program sets new workflow standards in efficiency and product quality, combining one sample preparation step with real-time PCR–based assays for the detection of Mycoplasma, Vesivirus, and MMV on one instrument platform.

ORDERING INFORMATION

Description	Size	Part Number
MycoSEQ™ Mycoplasma Detection Assay *Includes PrepSEQ™ Sample Preparation with Module M, MycoSEQ™ Mycoplasma Real-Time qPCR Assay. Protocol and Quick Reference Card included.*	100 rxns	4407876
MycoSEQ™ Mycoplasma Detection Assay *Includes PrepSEQ™ Sample Preparation with Module M, MycoSEQ™ Mycoplasma Real-Time qPCR Assay. Protocol and Quick Reference Card not included.*	100 rxns	4409732
MycoSEQ™ Mycoplasma Detection Assay *Protocol and Quick Reference Card included.*	100 rxns	4399363
MycoSEQ™ Mycoplasma Detection Assay *Protocol and Quick Reference Card not included.*	100 rxns	4384772
MycoSEQ™ Discriminatory Positive/Extraction Control, 1,000 copies/µL	0.7 mL	4445000
Applied Biosystems® 7500 Fast Real-Time PCR System, with Notebook Computer	1 instrument	4365464
Applied Biosystems® 7500 Fast Real-Time PCR System, with PC Tower	1 instrument	4365463
AccuSEQ™ Real-Time PCR Detection Software v1.0 with Mycoplasma Software Analysis Module v1.0 *Includes AccuSEQ™ Software v1.0 and Mycoplasma Analysis Module v1.0, Getting Started Guide, and Quick Reference Card.*	1 license	4443421

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